Hort Innovation

Final Report

Effective Management of Parsley Summer Root Rot

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Effective Management of Parsley Summer Root Rot – VG13101

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Summary

Parsley Summer Root Rot (SRR) is a disease complex affecting all parsley growing regions of Australia. The condition causes severe rot and stunting of root systems coupled with collar rot, leaf chlorosis and plant collapse. Crop losses can be as high as 100%, a concerning figure for the parsley industry which has an estimated value of $34M annually (Australian Horticulture Statistics Handbook, 2015-16). Crop surveys and grower consultation was conducted in NSW, Queensland, Victoria and Western Australia. Curly leaf parsley varieties were observed to be particularly susceptible in contrast to continental (flat-leaf) varieties. Disease occurrence was sporadic in both soil and soilless systems.

Previous studies of parsley root rot disease led to speculation that certain species or strains of the fungus *Fusarium* was responsible for SRR while various species of the oomycete *Pythium* were shown to be the causal pathogens for a similar disease occurring during cooler months in Victoria. In Queensland there was speculation that a bacterium was a potential cause of SRR in the Stanthorpe area. During the course of this project a range of fungi, oomycetes and bacteria were investigated to resolve the disease etiology. Potential environmental or cultural practices that may be contributing factors to SRR disease outbreaks were also studied.

Extensive diagnostic and pathogenicity testing resolved the oomycete *Pythium sulcatum* to be the primary causal agent for SRR. However SRR can have a complex etiology; in some cases other pathogens including *Rhizoctonia solani* and *Pythium mastophorum* were observed to elicit similar disease expression. A number of other oomycete and fungal species (including *Pythium irregulare, P. ultimum* var. *ultimum, Fusarium oxysporum, F. solani, Plectosphaerella cucumerina,* and *Colletotrichum gloeosporioides*) were commonly associated with plants with SRR symptoms but they failed to consistently reproduce disease symptoms in subsequent pathogenicity tests. Soft rot bacteria (*Pectobacterium* spp.) and some pseudomonads such as *P. syringae* and *P. pseudoalcaligenes* were commonly isolated from plants suffering advanced stages of SRR however they were likely to be secondary pathogens as they were unable to elicit SRR symptoms when applied to healthy plants under experimental conditions.

A number of environmental factors were also considered in an effort to identify those that either enhanced or suppressed disease expression in the field. The use of pre-emergent herbicides, a range nitrogen based fertilisers, soil moisture content and temperature were examined in a number of greenhouse and field-based experiments. SRR was successfully induced in parsley in all growing environments but we didn't find any significant effects on disease expression from pre-emergent herbicides, organic or inorganic fertilisers, or compost soil amendments. Similarly microbial biocontrols and a range of agrichemicals were of no significant value. All these findings point to a more integrated approach to SRR disease management. Improved farm biosecurity and crop hygiene would be a basic strategy to minimize risks. Crop rotations without other apiaceous crops need to extend over a two year interval. Fumigants or biofumigants could be applied to lower initial pathogen potential in soils and microbial biocontrols and/or agrichemicals may be applied to suppress disease development. Ultimately, improved production technologies such as protected cropping in soilless systems could be applied to avoid the one major environmental predisposition; excess soil moisture.

Keywords

Parsley

Summer Root Rot

Pythium

Soil borne disease

Introduction

Parsley Summer Root Rot (SRR) is a soil-borne disease occurring in all major Australian growing regions. The condition leads to root rot in developing parsley plants that can cause up to 100% crop losses. Both immature and mature plants can be affected with characteristic symptoms including leaf chlorosis, plant stunting, browning and rotting roots, loss of secondary roots and in some instances a spongy soft rot developing around the crown. Plants eventually wilt and collapse if the crown rot and root damage is severe.

SRR appears to vary both temporally and geographically with disease expression typically occurring during the summer months in Queensland, whereas in Victoria diseased plants can be found in summer and winter. Whilst the disease has been previously recorded in NSW it is less prevalent. Disease expression can vary greatly within a single farm and affected plants have been found in both soil and soilless systems.

An extensive literature review was conducted at the commencement of this project (Appendix 1) to identify the potential causes and possible management options that required further investigation. Previous investigations (Minchinton et al., 2007 & 2013; Grigg, 2013) failed to completely resolve the disease etiology however fungal pathogens such as *Fusarium oxysporum* and various species of the oomycete *Pythium* were suspected as prime candidates. The scoping study by Grigg (2013) nominated the fungus *Fusarium* as the most likely cause based upon its consistent association with SRR affected plants in Victoria; albeit from a limited sample size. Literature reports of *Fusarium* spp. causing parsley root diseases are sketchy and there is no definitive study that has reported a Fusarium vascular wilt disease on parsley despite such diseases known on the botanically related plants, celery, coriander and cumin. Minchinton et al. (2013) were more circumspect in defining a causal pathogen for SRR and also presented some data supporting a bacterium as the possible cause. This suggestion was largely based upon earlier observations of bacterial infections associated with SRR of hydroponic parsley crops grown in Stanthorpe, Queensland. A limited pathogenicity study conducted at high temperature (35^{o}C) confirmed an isolate of a *Pseudomonas* sp. was capable of causing disease symptoms on parsley. There are reports in the international literature of various *Pseudomonas* spp. causing leaf spot disease in parsley however there are no known records of a bacterium inducing the symptoms consistent with SRR. Soft rot bacteria are known to be associated with field and post-harvest rots of parsley but there are no reports suggesting these bacteria are primary pathogens; they are generally associated with opportunistic entry through damaged tissue.

The key objectives of this study were to determine the causal pathogen(s) of SRR and understand it’s disease epidemiology, particularly in relation to predisposing environmental conditions and practices. Finally, the project aimed to develop robust and integrated disease management strategies.

Methodology

Surveys & Laboratory Diagnostics

Nineteen farms across four states were surveyed during the course of the project to collect plant samples affected by SRR, interview growers about their experiences with SRR, and audit farm biosecurity and crop hygiene. The criteria used to assess exposure to biosecurity risks and the potential for pathogens to spread on-farm were based on a checklist published in the factsheet *"Best Practice for Farm and Crop Hygiene"* which was developed in this project (refer Appendix 2).

In order to increase the chances of isolating causal pathogens from plant samples, local laboratories were used in Victoria (assisted by Dr E Minchinton, Biosecurity Officer Agriculture Victoria at Knoxfield and Cranbourne) and in Queensland (assisted by Dr C Gambley, Principal Plant Pathologist, Queensland DAFF, Applethorpe Research Station). Plants were washed free of dirt and selected diseased tissue was dissected. Sub-samples were surface-sterilised while other segments were cultured directly to various agar media. Plates were incubated and regularly viewed under a light microscope for development of fungi, oomycetes and bacteria. Sub-cultures were made and used for morphological determinations using standard taxonomic keys. Selected fungal and oomycete isolates were further characterised by amplifying and sequencing rDNA ITS regions and comparing them with GenBank accessions. Selected bacterial isolates were further characterised by comparing their fatty acid profiles and 16S rDNA sequences with reference libraries.

Pathogenicity experiments

Pathogenicity experiments were conducted under controlled environment conditions at the NSW DPI Central Coast Primary Industries Centre greenhouse facility, Ourimbah. Several isolates of each taxon were tested in different substrates (1:1:1 sand/vermiculite/perlite, coir bags, Grodan® (rockwool) blocks or hydroponic channels (Nutrient Film Technique [NFT]). Candidate pathogens are listed in Table 1. Each experiment was established in replicated complete blocks or Latin Square designs. Inocula were grown on agar media and macerated just prior to application to the bases of plants or to nutrient solutions. Concentrations of fungal and oomycete spore suspensions were standardized after estimates were made under a light microscope using a haemocytometer. Bacterial isolates were applied by different methods across a number of experiments: as atomized sprays to the foliage; aqueous suspensions drenched around the stem bases; or injected into the crown tissue with a syringe. Parsley seedlings (cv. Limerick) were grown from seed using standard seedling media, however in some instances seeds were directly germinated into *Grodan®* blocks. Parsley plants were irrigated with a complete nutrient solution as required while nutrient recirculated continuously through the root zone of plants in NFT hydroponic channels. Growing conditions such as temperature, water availability and humidity were manipulated depending on the experiment. Some trials utilized macerated diseased plant material as inoculum in an effort to reproduce SRR given ambiguous results in earlier experiments with *Fusarium* spp. isolates and the potential for a cryptic causal pathogen. Agrichemicals with different activities were also used to segregate discrete pathogen groups within treatment effects. The broad-spectrum antibiotic, Streptomycin was used in one experiment to help elucidate if a pseudomonad taxon was involved with SRR.

SRR management experiments

Greenhouse trials were conducted using candidate pathogens or macerated roots from diseased plants that were collected from affected farms. Various treatments included potential chemical controls, microbial biocontrols and

nutrient supplements. Another experiment compared susceptibility of several different commercial varieties of parsley to SRR. Further details of these experimental methods are described in Appendix 3.

Field experiments were conducted at the NSW DPI field station at Somersby and on a commercial farm at Clyde, Victoria. Chemical products were chosen with efficacy to key potential fungal and oomycete pathogens associated with SRR. They were: metalaxyl-M which has known efficacy to oomycetes such as *Pythium* spp.; fludioxonil which has efficacy to *Fusarium* and *Rhizoctonia* spp.; and cyprodinil which has efficacy to a range of fungal pathogens.

Statistical analyses

GenStat 18 was used for data analysis. ANOVA was used where appropriate and assumptions underlying ANOVA were checked graphically. Data was normalized by appropriate transformations as required. REML analysis was also used for analysing data. Treatment means were separated using the least significant difference procedure at the 5% significance level.

Outputs

Farm Surveys, Audits & Biosecurity

Seven parsley farms had experienced severe SRR within the past year of the crop surveys whilst several other growers reported SRR had occurred in previous years. SRR was reported by growers as more prevalent during and after wet weather.

Farm audits highlighted a lack of grower understanding for sound biosecurity practices on-farm. Hygiene standards were generally low in soil-based production enterprises with very few protocols in place to reduce the risk of new pathogens being introduced to sites. It was observed that several hydroponic enterprises in Stanthorpe, Queensland had better crop and farm biosecurity and interestingly they also had fewer SRR issues during the audit period. These enterprises utilized run-to-waste hydroponic systems and did not crop through the cooler months. Run-to-waste systems reduce the risk of pathogens spreading via recirculating nutrients. However such systems allow pathogens to move into the surrounding soil and environment with the waste water. Based on these observations sanitation of hydroponic troughs between crops and management of grassy and weed-free lawns around hydroponic tables appear to be effective cultural controls of SRR. Only one hydroponic farm with observable poorer hygiene standards had persistent SRR issues. On this farm volunteer parsley plants were observed growing in soil surrounding hydroponic tables and fungus gnats and their larvae were found associated with sawdust substrate and plant roots. These insects are known to spread *Pythium* species (Goldberg and Stanghellini 1990; Gardiner et al. 1990) as well as cause root feeding damage which would allow pathogens an easy entry.

Whilst a majority of farms had designated parking areas and displayed signage for visitors to report to the site office, most farms had no designated wash down facilities (either foot baths or for vehicles) and there was largely unrestricted vehicular access directly to growing areas. Hand-washing stations for workers were generally well provided but disinfection practices for tools and farm machinery were often not employed. Addressing these issues would help to reduce disease transmission between sites, aid in the prevention of new pathogens successfully establishing on-farm and reduce risks of pathogen re-entry after fumigant or chemical intervention.

As part of the extension material developed from this project a biosecurity manual, *"Managing Farm Biosecurity in the Parsley Industry"* was produced and distributed to provide growers with clear and comprehensive directions on how to best manage biosecurity issues within their commercial operation. Due to its large size this document has not been included in this report. A factsheet (*Effective management of Parsley Summer Root Rot - Best Practice for Farm and Crop Hygiene*) was also developed to provide growers with a checklist to manage farm hygiene as well as background information on SRR (Appendix 2).

Laboratory diagnostics and characterization of candidate causal organisms

Several fungi, oomycetes and bacteria known to be pathogens on parsley or other vegetable hosts were isolated and characterised from SRR affected plant samples. Table 1 below lists these organisms and their geographical incidence. Several of these organisms were frequently isolated together from individual plants, particularly as disease symptoms became more pronounced. *Fusarium solani* was frequently isolated from internal tissue at the top of the tap root while *Rhizoctonia solani* and *Rhizoctonia* sp. (= *Ceratobasidium* sp.) were mostly found associated with petiole bases and crown tissue on plants with collar rot symptoms. Other fungi and oomycetes were distributed widely across all subterranean tissue and virtually all secondary roots yielded one or more *Pythium* species.

Table 1: Incidence of fungi, oomycetes and bacteria associated with parsley root rot diseases in this study

Organism	Victoria	NSW	Queensland	Western Australia
Fusarium solani	+	+	+	+
Fusarium oxysporum	+	+	+	+
Pythium sulcatum	+	+	+	+
Pythium mastophorum	+	+	-	+
Pythium irregulare	+	+	+	+
Pythium ultimum	+	+	+	+
Pythium dissotochum	-	+	-	-
Plectosphaerella cucumerina	+	+	+	+
Rhizoctonia solani	+	-	-	+
Rhizoctonia sp.	+	-	-	-
Thielaviopsis basicola	-	+	-	-
Macrophomina phaseolina	+	-	-	+
Colletotrichum gloeosporioides	-	-	-	+
Pseudomonas syringae	+	+	+	-
Pseudomonas spp.	+	+	+	+
Pectobacterium spp.	+	-	+	+

Pathogenicity testing of Candidate Pathogens & Varietal Susceptibility to SRR

Several trials assessed the pathogenicity of a bacterial, fungal and oomycete species. Given the null hypothesis that a *Fusarium* species was the cause of SRR there was initial consternation as none of the isolates tested yielded pathogenic effects on parsley or they produced inconsistent disease effects on treatment replicates. It was later revealed that there were confounding issues with cross-contamination of pots likely due to the presence of fungus gnats in the greenhouse. This problem was ameliorated in latter experiments through the use of yellow sticky traps that were monitored regularly and insecticides applied as appropriate. Pathogenicity trials were run under different environmental conditions and combinations of pathogens were applied as treatments given they commonly occurred together on SRR affected plants. No *Fusarium* spp. isolates or other species of fungi resulted in consistent disease symptoms under any environmental conditions or in any substrate tested. Similarly none of the other fungi (excepting *Rhizoctonia solani*) or bacterial isolates tested reproduced disease symptoms on parsley. Alternatively *Pythium sulcatum*, *P. mastophorum* and *Rhizoctonia solani* did cause disease symptoms that were consistent with SRR. Table 2 outlines a brief summary of the trial results. Appendix 3 provides more detail of the experimental trials conducted in this project.

Table 2: Experimental results of pathogenicity trials for Parsley Summer Root Rot

Candidate Pathogens	Number of trials	Outcome
Bacteria *Pseudomonas syringae* *Pseudomonas* spp. *Pectobacterium* spp.	8	Root or collar rot disease was not induced by any single or combination of bacterial isolates
Fungi *F. oxysporum, F. solani*	8	Root rot or SRR disease was not consistently induced by any single or combination of isolates
Fungi *Plectosphaerella cucumerina, Macrophomina phaseolina, Colletotrichum gloeosporioides*	4	Root rot disease was not induced by isolates of these fungal species
Fungi *Rhizoctonia solani* *Rhizoctonia* sp.	3	Two isolates of *R. solani* were associated with root rot and plant collapse. These isolates were from WA and Victoria and caused significant root rot and plant collapse. Isolates of a *Rhizoctonia* sp. (binucleate isolates consistent with a *Ceratobasidium* sp.) were found associated with crown rot symptoms in Victoria but were not confirmed as causal pathogens.
Oomycetes *P. sulcatum, P. irregulare, P. mastophorum, P. ultimum, P. dissotochum*	11	Root rot disease symptoms were consistently induced by *P. sulcatum* and *P. mastophorum*

Figure 1 below illustrates the strong disease effect of three *P. sulcatum* isolates on parsley growing in NFT hydroponic units. Mean wet weights were indicative of plant health; plants with low vegetative yields also had severely rotted roots and collapsed.

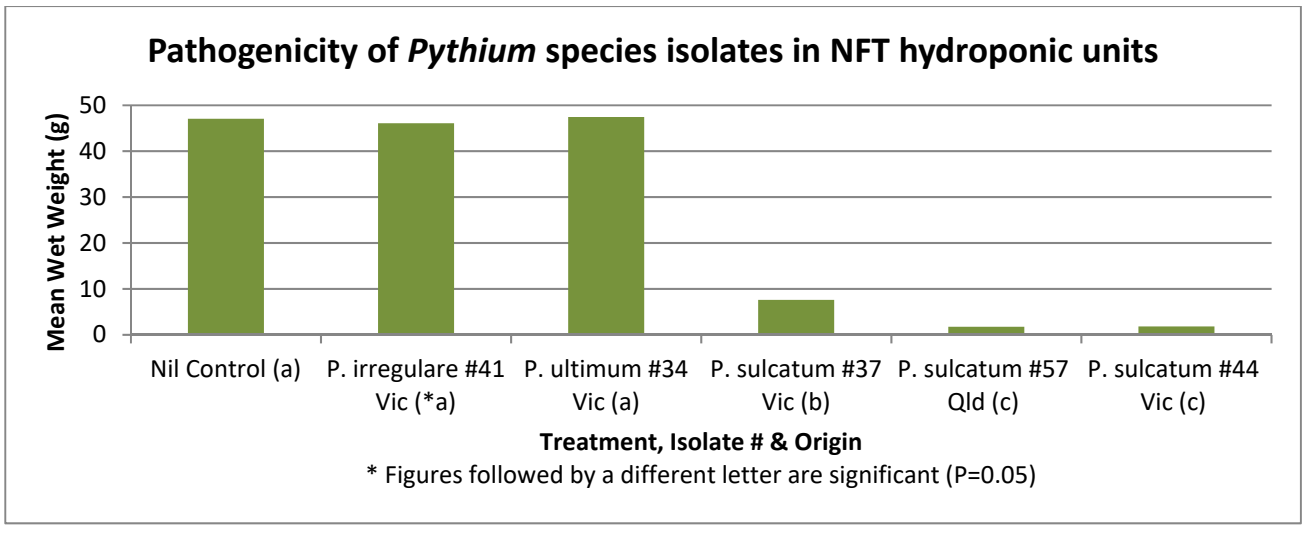

Figure 1: Pathogenicity of *Pythium* species isolates in NFT hydroponic units

SRR management experiments

Only drenching plants with metalaxyl-M tested in greenhouse experiments gave significant control of *Pythium sulcatum* or combinations of pathogens that included this species. This result is illustrated below in Table 3 which also shows disease control failure by drenches of a combination of newer chemicals with activity to oomycetes (oxathiapiprolin and mandipropamid).

Table 3: Agrichemical drench efficacy to *P. sulcatum* on parsley (cv. Limerick) in rockwool cubes

Treatment	Disease rating (0-5 scale)	Shoot dry weight (g)
Nil pathogen, no agrichemical	0.125 d*	1.991 a
P. sulcatum	2.625 a	0.458 d
Nil pathogen + oxathiapiprolin + mandipropamid	0.542 cd	1.436 b
P. sulcatum + oxathiapiprolin + mandipropamid	2.208 a	0.729 cd
Nil pathogen + metalaxyl-M	1.167 b	1.025 c
P. sulcatum + metalaxyl-M	1.000 bc	1.005 c

*Figures followed by a different letter are significant (P=0.05)

Efficacy of metalaxyl-M was not confirmed in field experiments in NSW and Victoria despite 3 repeat applications. Similarly use of compost or chicken manure soil amendments did not influence disease expression in two trials in Victoria. Furthermore, pre-emergent herbicides tested in one field trial had no effect on disease severity.

One hydroponic trial testing efficacy of a *Bacillus subtilis* formulation to *Pythium sulcatum* did show some disease suppression but this was only a temporary effect and the disease eventually overtook plants. Results are presented in Table 4 below. Another *B. subtilis* formulation did not influence disease incidence or severity in a Melbourne field experiment.

A fertilizer product (Maxstim®) also failed to ameliorate disease in a hydroponic trial (Table 4). A variety of nitrogen based fertilisers were tested on the hypothesis that there may be an interaction between the ammonium form of nitrogen and the pathogen which may exacerbate disease symptoms. This phenomenon is well known for Fusarium wilt (Huber & Thompson, 2007). Pelleted urea and composted chicken manure were trialed in the field at the Somersby research facility with no influence on parsley root rot disease.

Table 4: Efficacy of Microbial Biocontrol and Fertiliser Efficacy to *P. sulcatum* on parsley (cv. Limerick) NFT hydroponic units

Treatment	*Pythium* isolate # & origin	Mean wet weight g)
Nil Control	-	79.2 a*
P. sulcatum	#17-42 Victoria	1.9 c
P. sulcatum + *Bacillus subtilis*	#17-42 Victoria	8.0 b
P. sulcatum + Maxstim® fertiliser	#17-42 Victoria	1.8 c

*Figures followed by a different letter are significant (P=0.05)

All curly leaf parsley varieties tested in pot trials were similarly susceptible to SRR while flat leaf (continental) varieties were far less so. A factsheet describing this finding was produced *"Parsley Summer Root Rot - Varietal Susceptibility"* and can be found in Appendix 4.

Presentation of findings at meetings/conferences

Project progress and general parsley disease recognition and management information (including SRR) was presented to NSW growers at three workshops in October 2016 (Leppington & Richmond) and April 2017 (Sydney Markets). A table listing chemical product permits for use on parsley was distributed and discussed at these events. An exert pertaining to Pythium root rot control is presented in Appendix 5. Workshops were organized in conjunction with the National Vegetable Extension Network through the NSW Local Land Services and a translator (into Cantonese) was used at two of those events. Parsley growers in Stanthorpe, Queensland were given project updates individually during farm surveys and audits. Victorian growers were also given project updates individually and to a wider industry network at the East Gippsland Vegetable Innovation Days held at Lindenow in May 2017.

Related presentations that included parsley SRR information were delivered in two Soil-borne Disease Master Classes delivered in 2015 (Mornington, Victoria) and 2016 (Ipswich, Queensland) through project VG15010. A webinar entitled '*Understanding and Managing Pythium Diseases of Vegetables'* was delivered in March 2016, again in conjunction with project VG15010. A conference abstract has been accepted for 'Science Protecting Plant Health 2017' Conference, Brisbane Queensland (refer Appendix 6). Finally we also produced a factsheet (Appendix 7) summarizing all of the project findings and recommended SRR management options.

Outcomes

Tools & information to improve farm hygiene

The project publications *Managing Farm Biosecurity in the Parsley Industry* and a related factsheet have useful checklists and protocols for growers to improve their farm and crop hygiene. This is particularly important for parsley SRR management since the causal oomycete *Pythium sulcatum* can be spread with water, machinery and certain insects. Education and engagement activities with growers and allied industries have provided a useful platform to discuss improving farm biosecurity. Ongoing engagement is needed to ensure growers successfully adopt risk mitigation protocols and integrate them into their overall farm management plan. Successful control of parsley SRR has largely been achieved in hydroponic production in Stanthorpe through sound crop hygiene and sanitation.

Disease management implications from determining the cause for parsley *Summer Root Rot*

Research from this project confirmed that *Pythium sulcatum* is the most important cause of parsley SRR. This knowledge means growers can now apply more appropriate and effective management strategies. *P. sulcatum* is an aggressive pathogen of apiaceous plants and survives for more than two years in soils. It can also contaminate water reservoirs. *P. sulcatum* can grow in a wide temperature range so it can infect roots at any time of the year. It should be noted that this pathogen is also the primary cause of cavity spot and root forking diseases in Australian carrot production. There have been several previous research projects that have focused on this host and pathogen combination and some effective management options developed for cavity spot control may be useful for parsley growers. Key options are discussed below:

- Longer crop rotations are clearly needed in soil-based parsley production systems, particularly on farms where other apiaceous crops are grown. This strategy may be challenging on some intensive production enterprises with limited land. Brassicas and alliums are not susceptible to *P. sulcatum* so they are suitable rotation crops. Both types of plants also release sulphurous and cyanogenic chemicals from their decomposing roots that are suppressive to soil-borne pathogens.

- Chemical interventions need to be carefully considered given our negative field efficacy results and those from previous studies on parsley and carrots. Successful control with specific fungicides is challenging given the typically long production period for parsley. Plants are continuously exposed to infection from seeding through to their final harvest. There is evidence from carrot cavity spot research that certain fumigants, fertilisers (notably Perkla) and biofumigants can decrease initial soil populations of *P. sulcatum*. Sustained chemical control was not demonstrated in trials from this project but it could potentially be achieved with judicious applications of biological and/or chemical controls, particularly if used after reducing the pathogen population prior to sowing (either by longer crop rotations, fumigants or biofumigant applications). However, this approach needs extensive field validation for parsley SRR.

- In a wet environment *P. sulcatum* produces masses of motile spores (zoospores) that have sensors to detect chemo-electric fields around roots. This field literally guides zoospores to the root surface. The sudden explosion in the pathogen population also greatly increases the likelihood of multiple infections on roots and subsequently more severe disease expression. Improved soil drainage and irrigation scheduling are therefore important cultural management options. Over-irrigation or periodically saturating soils favour zoospore production and root infection. Consideration could also be given to avoid wet weather by using protected cropping systems. Protective structures with a retractable roof would be ideal given plants can be protected

during wet weather. Recirculated hydroponic systems have a distinct disadvantage as roots are constantly exposed to zoospores. We have previously shown that water disinfection treatments can be effective (scarlett et al, 2015). One option that doesn't rely on chemical intervention is microfiltration, which we successfully demonstrated (in project VG13052) could remove *Pythium* from nutrient solutions.

- We saw no improvement in SRR control with a compost soil amendment prior to planting. This failure does not mean that this approach has no value given there are extensive reports in the world literature of improved general disease suppression with compost amendments.

Evaluation and discussion

A number of questions regarding SRR disease etiology were answered by this research project. *Pythium sulcatum* was determined to be the primary pathogen responsible for SRR. Other significant but less common pathogens associated with SRR are *Pythium mastophorum* and *Rhizoctonia solani*. Experimental results refuted the contention that either *Fusarium oxysporum, F. solani* or various bacteria were causal agents for SRR. This conclusion was reached after several experiments that included treatments consisting of individual isolates, some that were applied as combinations of similar or different species. Data supporting this contention was obtained in experiments where no disease control was achieved with chemical treatments that had known efficacy to *Fusarium* species or suspect bacteria. The bacteria species isolated from a soft collar rot symptoms on plants are likely to be secondary invaders since they can easily enter plants via wounds created by insects or mechanical damage during typical farming operations. Repeated harvesting is common on parsley and the remaining petiole bases are vulnerable to bacterial infections which could easily move down the hollow piths and into the tap root.

Environmental variables such as humidity and root zone moisture were included into experimental designs in an effort to establish if there were any particular conditions that initiate SRR. Several growers had reported onset of SRR after periods of wet weather. It is worth noting however that there may be environmental factors not identified or tested in this study which could influence symptom expression (Marthe et al, 2003). For instance, there was some anecdotal evidence from Victoria where one soil-based grower with a low SRR disease incidence who was using dam water. In contrast other nearby growers with higher incidence of SRR were using treated municipal waste water. This suggests that there may be an interaction between SRR and increased salinity or other constituents in wastewater which was not tested in our project.

Whilst SRR was found to affect parsley growing in both soil and hydroponic production, the latter has a distinct advantage as growers could undertake decontamination and sanitation during the ley periods. This approach was largely successful in Stanthorpe. Managing soil-borne pathogens on farms engaged in intensive vegetable production poses a significant challenge. In particular, growers engaged in soil-based mixed vegetable production (such as the Victorian growers surveyed in this project) have rotation crops from the *Apiaceae* family such as coriander, flat-leaf parsley, Dutch carrots and celery that are susceptible to *Pythium sulcatum* and *P. mastophorum*. In fact both of these species have relatively restricted host ranges to the Apiaceae (Davison & McKay 2003; Plaats-Niterink 1981). Our study also confirmed that all parsley cultivars grown in Australia are susceptible to SRR however curly varieties are far more susceptible. Similarly SRR was observed on coriander plants growing on one Victorian property but with much less severity than on neighbouring blocks sown to curly parsley.

None of the chemical and biological control products evaluated in this project gave robust control of SRR. Metalaxyl-M was effective in greenhouse trials but this was not reproduced in the field. Poor field control of SRR with metalaxyl-M is consistent with an earlier study of this disease (Minchinton et al 2007). One product formulation of a *Bacillus subtilis* did hold root rot caused by *Pythium sulcatum* temporarily but it was not sustained. This and other products may be more efficacious on-farm given our experimental hydroponic units recirculate nutrients (and pathogens) while the systems in Stanthorpe are run-to-waste.

Effective management of this disease will require further studies employing a range of strategies including crop rotation (noting *P. sulcatum* affects related plants such as coriander and potentially other apiaceous plants) and possibly newer chemical control options. Meanwhile there needs to be a sustained focus on farm hygiene and biosecurity.

Recommendations

There is a need for continuing education of parsley (and more broadly vegetable) industry about sound on-farm biosecurity practices. This is being addressed in current activities delivered by the national vegetable extension network and other industry project initiatives such as Enviroveg. The adoption of better site control and provision of wash down facilities for decontaminating vehicles, machinery and tools would significantly contribute to the managing biosecurity risks.

Farm trials are needed for soil-based growers to confirm suitable rotation crops for parsley that are not hosts of *P. sulcatum*. Use of a molecular detection assay developed by SARDI through HIA project VG15009 could provide quantitative data to monitor pathogen levels and perhaps set economic thresholds.

There are a number of potential alternative agrichemicals and microbial biocontrols for management of Parsley SRR. Table 5 lists products that were not tested in this project but might be valuable in integrated management of this disease. A future study should incorporate some of these products. Surplus funds from this project could be utilized for such field trials.

Table 5: Potential Agrichemical and Microbial Biocontrols for Parsley Summer Root Rot Control

Chemical name	Trade names & Company	FRAC Activity Group	Comment
Azoxystrobin	Amistar (Syngenta)	Gp 11 Strobilurin	Used overseas High risk of resistance developing
Cyazofamid	Ranman	Gp 21	Used overseas Medium to high risk of resistance developing
Propamocarb + Fluopicolide	Infinito (Bayer)	Gps 28 + 43	Low to medium risk of resistance developing
Calcium cyanimide	Perlka	Fertiliser	Varying results for Pythium disease control in other crops
Beneficial bacteria: *Bacillus amyloliquifaciens*, *B. subtilis*	Various	Gp 44	Induce host resistance; produce lipopeptide antibiotics Resistance not known
Beneficial bacteria: *Streptomyces lydicus*	Various	Not listed	Used in the USA by organic producers Resistance not known
Beneficial bacteria: *Pseudomonas fluorescens*	Sudo-Shield (Nutri-tech Solutions)	Not listed	Used in the USA by organic producers Resistance not known

Scientific refereed publications

No publications generated at the time of this report

Intellectual property/commercialisation

No commercial IP generated

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Appendices

Appendix 1: Literature Review

Introduction

An extensive review of the international literature of parsley root rot diseases was included as a chapter in a recent HAL final report for project VG08026 (Minchinton et al, 2013). It would be superfluous to reproduce the contents of that review here. Therefore, this review aims to provide supplementary literature that addresses potential causes and management options for the disease described as *summer root rot* of parsley. Much of the discussion regarding the cause of this disease that follows will remain speculative until it has been fully researched later in this project. Therefore, this review will attempt to build upon what knowledge and opinions have been gleaned from previous Australian research and industry consultation, together with further international literature on similar diseases affecting parsley and related plants in the family *Apiaceae*. The format adopted here will be to pose questions pertinent to our current understanding the etiology and epidemiology of parsley *summer root rot* and then attempt to answer them with what evidence is currently available and highlight what gaps remain.

What are the typical symptoms of summer root rot of parsley – and where does it occur?

Following are some general findings from previous studies in Australia (Minchinton et al, 2006; 2007; 2013; Grigg, 2013). There is no specific set of symptoms that distinguishes *summer root rot* of parsley. The above-ground symptoms described include yellowing, wilting, a brown collar rot and death of plants. Meanwhile, symptoms below ground include soft-rotting of the crown, and various brown or red-brown rot symptoms of the primary, secondary or fine root systems. Interestingly, none of the previous research has associated *summer root rot* of parsley with browning of the vascular tissue; a symptom that is typical of true Fusarium Wilt diseases. Given the rosette architecture of parsley plants in their vegetative state this symptom could be easily overlooked. However, if it is a Fusarium Wilt, there should still be some evidence of browning in the root stellar tissue and in the vascular ring in the collar region. Furthermore, browning and stem blight would be evident in plants running to seed.

The disease appears to be quite variable in both temporal and geographic occurrence, even on the same farm. For instance, no disease appeared in a trial that followed directly after a severely affected crop on one Victorian farm (Minchinton, pers. comm.). It is likely that the disease affects parsley crops in all Australian production areas although it has only been recognised in the three Eastern mainland states during previous projects noted above. Anecdotal evidence cited by Grigg (2013) suggests that it does not affect botanically related crops, coriander and carrots, at least on one Victorian farm.

What causes parsley summer root rot?

Evidence for the cause(s) of *summer root rot* of parsley in Australia from previous HAL projects (Minchinton et al., 2006; 2007; 2013 and Grigg, 2013) is inconclusive and sometimes contradictory. Most of Minchinton's research focused upon *Pythium* spp. that were clearly important pathogens in cooler months. The *Pythium* species associated with a root rot complex of winter-grown parsley and parsnips included *P. sulcatum*, *P. mastophorum* and *P. tracheiphilum* which caused severe root rot of parsley seedlings in pathogenicity experiments (Petkowski et al., 2013). Tesoriero (Chapter 3 in Minchinton et al., 2006) confirmed eight *Fusarium* sp. isolates were pathogenic to parsley, causing stunting, leaf chlorosis and root rot symptoms. Similarly, Martin (Chapter 4 in Minchinton et al., 2007) also confirmed pathogenicity of two *F. oxysporum* isolates from parsley in Queensland. It was noted that symptoms tended to be more severe at

higher temperatures.

In the more recent scoping study, Grigg (2013) interviewed eight growers, an agronomist and a seed company representative from Victoria and listed a number of factors thought to either contribute to the disease or thought to successfully manage it. A limited set of pathology testing (from three growers) was conducted and, despite the isolation of the fungus *Fusarium* sp. (putatively *F. oxysporum;* Tesoriero, pers. comm.) that was commonly associated with diseased field plants from each sample, it would be difficult to conclude that this was the causal organism without at least completing Koch's postulates. Earlier chemical and biological control efficacy trials conducted on commercial parsley crops in Victoria through summer months of 2003-7 provided little evidence supporting *Fusarium* sp. as a potential cause of this disease. In fact, despite a relatively high incidence of dry, cracked, red-brown lesions on roots, there were no disease symptoms visible above the ground (Minchinton et al, 2007; Appendix 2 in Minchinton et al, 2013). As noted above, the lack of typical vascular browning symptoms diminishes the likelihood that *summer root rot* being caused by a true Fusarium Wilt pathogen. In fact, there is no recognised Fusarium Wilt described for parsley in the international literature (Summerell et al., 2011) except for a single non-refereed report from Japan (Kasuyama & Inoue, 2007) based on work done a decade earlier. Alternatively, there are distinct Fusarium Wilt diseases described for other members of the family *Apiaceae* such as celery (Schneider, 1984), coriander (Srivastava, 1969) and cumin (Patel & Prasad, 1963) that are caused by separate *F. oxysporum formae speciales*.

However, there are reports from the international literature of *F. oxysporum* associated with root rot and leaf chlorosis diseases of parsley. Studies in Germany (Marthe et al, 2003) demonstrated *F. oxysporum* caused leaf chlorosis of parsley. However, in spite of its relatively high incidence, damage was low when compared with losses due to *Alternaria radicina* (the cause of Black Leaf Spot) and *Erysiphe heraclei* (the cause of Powdery Mildew) These authors state that symptom expression resulting from natural infections generally starts late in the vegetative period and that plant physiological stress factors may contribute to their development. This latter statement is consistent with observations of *summer root rot* of parsley in Australia (Grigg, 2013).

F. oxysporum has been found commonly associated with parsley seeds and diseased field seedlings in Poland (Nawrocki, 2005; Nawrocki & Mazur, 2004), however glasshouse trials demonstrated that *Alternaria alternata* and *F. avanaceum* were the main causes of damping-off.

In the USA, *F. oxysporum* and *F. solani* were shown to cause root rot and post-emergence damping-off of parsley (Walker, 1944). However, in a more recent study by Hershman et al. (1986), isolates of these species were either non-pathogenic or weakly pathogenic compared with isolates of *Pythium* spp. and *Rhizoctonia solani*. Interestingly, these latter pathogens caused greater disease incidence and severity at higher temperatures. The authors speculated that physiological stress of the host plants at higher temperatures and/or adverse effects of these conditions on soil microflora that is antagonistic to *Pythium* spp. and *R. solani* may be more important for disease development than direct temperature effects on these pathogens.

One further report from the international literature described a Phytophthora root rot of parsley in California (Davis, 1994). The pathogen was identified as *P. cryptogea* and it caused wilting, stunting and general yellowing of the foliage. These symptoms were reproduced in pathogenicity experiments as well as plant death within three weeks of inoculation.

Could summer root rot of parsley be caused by a bacterial pathogen?

It has been postulated that summer root rot in Queensland is caused by a bacterial pathogen belonging to the genus *Pseudomonas* (Gamley, pers. comm.). However, there are no international reports of a disease caused by this bacterial

genus with symptoms similar to the ones described for *summer root rot* of parsley. There are two bacterial pathogens causing leaf spot disease on parsley in California and they were recently genetically characterised as *P. syringae* pv. *apii* and *P. syringae* pv. *coriandricola* (Bull et al., 2011). These bacteria have previously been shown to cause leaf spot and stem blight on celery and coriander respectively. So it is possible that either of these organisms or a related bacterial strain could cause, or contribute to, *summer root rot* of parsley in Australia.

Alternatively, soft rot bacteria (*Pectobacterium* spp.) commonly affect a wide range of plant species and would be capable of causing *summer root rot* of parsley if there was mechanical injury to roots or crown tissue such as damage caused by insects or through scars following destructive harvests. A disease was reported from Brazil on coriander and parsley causing symptoms similar to *summer root rot* of parsley in Australia. They described a bacterium closely related to *Erwinia carotovora* subsp. *betavasculorum* causing leaf chlorosis and plant death within a few days. Basal parts of the petiole and the root system showed symptoms of a soft rot and had an unpleasant odour. *Erwinia carotovora* has been since been reclassified as *Pectobacterium carotovorum.*

Could *summer root rot* be due to an emerging plant pathogen?

Emergence of a new disease is also possible and they are regularly described in the international plant pathology literature. For instance, Japanese research has recently described Plectosphaerella rot of hydroponically cultured lettuce, coriander and chervil caused by the fungus, *P. pauciseptata* (Usami et al., 2012). Fungal species from this genus have similar appearance to the fungus, *Fusarium*, but are more closely related to another fungal genus, *Verticillium*, of which there are species capable of causing vascular wilt diseases on a range of crops. Similarly, other species of this fungus have recently been described causing sudden wilt diseases on horticultural crops in Europe (Carlucci et al., 2012). Isolates of this fungus have been isolated from vegetable crops in Australia, but have not been fully characterised not researched.

Conclusions

From the information presented above it is unlikely that parsley *summer root rot* is caused by a single pathogen, but could be a disease complex that might involve a suite of pathogens that possibly interact with other biotic and/or abiotic factors.

Many workers have considered the formation of disease complexes by *Pythium* with other organisms. Zogg (1950) studied mixed infections in foot rots of cereals. He concluded that it was difficult to forecast the virulence of a mixed infection which assumes the character of a new disease. Kerr (1963) reported a foot rot and Fusarium Wilt complex of peas in South Australia. He found that *P. ultimum* alone caused initial stunting from which the plants recovered. Similarly, *F. oxysporum* alone also caused little damage. Together, the fungi caused initial stunting followed by severe wilting. Tesoriero (2011) determined that *Pythium* spp. and *F. oxysporum* formed a synergistic relationship in Australian greenhouse cucumbers.

A number of disease complexes between plant parasitic nematodes and soil-borne pathogens were reviewed by Back *et al*. (2002). There are several examples where this interaction has been shown to be synergistic, such as in the potato ‘early dying’ complex that is caused by species of *Pratylenchus* and *Verticillium dahliae*. They note some disparities exist between different studies that have been attributed to different fungal isolates (for example, representing different *V. dahliae* VCG groups) and nematode pathotypes having different aggressiveness. A range of other factors such as fluctuating environmental parameters, soil pH and soil type are also cited as affecting these interactions. Similar

phenomena could be responsible for the sometimes conflicting observations of parsley *summer root rot* in Australia.

Despite previous studies describing *Pythium* spp. and *F. oxysporum* as both being present in root systems of diseased parsley plants, there has been no formal study to determine if they are involved in a disease complex.

The key aim of systematic crop surveys and diagnostics in the next phase of this project will be to resolve the etiology of this disease. This will require careful examination of diseased plants and detailed isolation and characterisation of the organisms associated with affected tissue. Monitoring for insect pests, prevailing weather, cultural practices and soil chemistry will also be necessary to determine if any of these factors correlate with disease incidence or severity. This will be integral to developing robust disease management options.

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NSW GOVERNMENT Department of Primary Industries

Best Practice for Farm and Crop Hygiene

Michelle Smith and Len Tesoriero

Farm Biosecurity

- Establish high and low risk farm zones to manage movement of people and machinery
- Erect signs to notify people of your farm biosecurity policy
- Record visitors and restrict access to high security zones
- Implement ‘come clean – go clean’ practice
- Provide boot cleaning, foot baths and hand washing stations for staff and visitors
- Set up wash-down bay and tyre wash facilities for machinery and vehicles
- Disinfect dam water

Crop Biosecurity

- Buy planting material from reputable suppliers and retain all documentation such as seed batch numbers
- Inspect crops regularly for early signs of diseases and pest infestations
- Rogue diseased plants
- Seek laboratory diagnosis for unknown crop health problems
- Control weeds
- Dispose of crop waste away from growing areas
- Disinfect tools such as knives at regular intervals while cutting parsley (allow up to 10 minutes soaking time and change disinfectant regularly)
- Clean picking and packing boxes or crates

More information

Contact your local crop consultant, reseller or state Department of Primary Industries

Appendix 3: Experimental Trial Summary

Trial	Bacterial Trials 1, 2, 3
Pathogen tested	Bacteria isolates obtained from diseased plants - 8, 19 and 28 isolates respectively
Trial Design	All trials consisted of a randomised block design with at least 1 control Trial 1: 9 treatments x 5 replicates Trial 2: 20 treatments x 2 replicates Trial 3: 29 treatments x 4 replicates
Outcome	None of the bacteria trials produced a significant result, indicating that the bacteria isolates tested were not causal agents for SRR in parsley

Trial	Greenhouse Trial 1
Pathogen tested	*Fusarium* spp. (18)[1], *F. oxysporum* (3), *F. solani* (2), *Plectospharella cucumerina* (1)
Trial Design	The trial consisted of a randomised block design with 4 controls 28 treatments x 3 replicates
Outcome	None of the isolates tested produced a significant disease symptoms, indicating that they were not causal agents for SRR in parsley

Trial	Greenhouse Trial 2
Pathogen tested	*Fusarium* spp. (12), *F. oxysporum* (4), *F. solani* (3), *Plectospharella cucumerina* (4), *Macrophomina phaseolina* (2), *Pseudomonas* sp. (1), *Rhizoctonia solani* (1), *Colletotrichum gloeosporioides* (1)
Trial Design	The trial consisted of a randomised block design with 1 control. 140mm pots were filled with potting mix (coir:vermiculite:perlite; 4:1:1 volumes respectively) and 2 x seedling plugs (10 plants in each) transplanted into each pot 29 treatments x 4 replicates
Outcome	None of the isolates tested consistently produced significant disease symptoms, indicating that they were unlikely to be causal agents for SRR in parsley. There was some significant variation in treatment effect between replicates.

Trial	Greenhouse Trial 3
Pathogen tested	*F. oxysporum* (3), *F. solani* (4), *Pythium sulcatum* (1)
Trial Design	The trial consisted of a randomised block design with 3 controls

[1] Number refers to the number of isolates tested

	12 treatments x 4 replicates
Outcome	Only the *P. sulcatum* isolate tested was shown to significantly affect plant health. Mean shoot weights were significantly lower than those from fungal and control treatments. Symptoms were consistent with SRR.

Trial	Greenhouse Trial 4
Pathogen tested	*F. oxysporum* (2), *F. solani* (2), *Plectosphaerella cucumerina* (1), *P. sulcatum* (1), *P. mastophorum* (1)
Trial Design	The trial consisted of a randomised block design with 2 controls 20 treatments x 4 replicates This trial tested each isolate individually then combinations of isolates together
Outcome	Six treatments were shown to have a significant detrimental effect on plant health consistent with SRR. The common pathogen in all of these treatments was *P. mastophorum*

Trial	Greenhouse Trial 5
Pathogen tested	*F. solani* (14), *F. oxysporum* (9), *Fusarium* spp. (5)
Trial Design	The trial consisted of a randomised block design with 1 control. 29 treatments x 4 replicates 140mm pots were filled with potting mix (coir:vermiculite:perlite; 4:1:1 volumes respectively) and 2 x seedling plugs (2 plants in each) transplanted into each pot
Outcome	None of the isolates tested significantly affected plant health, indicating that they were not causal agents for SRR in parsley

Trial	Greenhouse Trial 6
Pathogen tested	Diseased plant material, *F. solani, F. oxysporum, P. sulcatum,* Bacterial spp. +/- chemical products
Trial Design	The trial consisted of a randomised block design with 5 controls 15 treatments x 10 replicates This trial was testing 4 different control products against SRR in three different scenarios: no pathogen, diseased plant material (macerated and poured over healthy plants) and a pathogen blend of a range of *Fusarium* and *Pythium* isolates previously obtained from diseased plant material. Against each pathogen group 5 treatments were applied: 4 chemical product combinations were tested plus a “control” where no product was added. Chemical products tested included Switch® (active ingredient cyprodinil + fludioxonil (rate 0.1g/m^2), used to suppress *Fusarium* and *Rhizoctonia* spp.), Switch®+Ridomil® (active ingredient metalaxyl-M (rate 0.1ml/m^2), used to suppress *Pythium* spp.), Streptomycin (antibiotic used to suppress bacterial pathogens (rate 200mg/1L)), Urea (rate 10g/1L) + Switch®+ Ridomil®.

	Urea was incorporated into certain treatments since higher ammonium nitrogen levels are known to exacerbate Fusarium wilt disease severity. This also mirrored a common grower practice in Victoria to top-dress fowl manure on parsley beds. Fowl manure is also known to release ammonium nitrogen into the soil environment.
Outcome	Results involving the urea treatment showed a trend for higher plant weights indicating, not surprisingly, that the urea may have had a growth promoting effect. Clearly there was no synergistic effect between increased ammonium levels and the presence of *Fusarium oxysporum* suggesting that Fusarium wilt was not associated with SRR. The pathogen blend disease scenario scored poorly overall indicating the pathogens used were highly virulent isolates and produced symptoms consistent with SRR. Treatments involving Ridomil® expressed better root scores compared to the other control products, however this trend was only significant for the no pathogen controls and diseased plant material disease scenarios.
Photos *Clockwise from top left:* *1) Typical symptoms of SRR;* *2) Rep 1 showing from left>right Control, DPM, Pathogen blend plants, all without control products;* *3) Plant roots (Treatment 6) of DPM inoculated parsley showing root rot and browning symptoms of SRR;* *4) Healthy plant roots of an uninoculated parsley plant*	   

Trial	Hydroponic trial 1
Pathogen tested	*P. ultimum* (1), *P. sulcatum* (3), *P. irregulare* (1)
Trial Design	The trial consisted of a randomised block design with 1 control 6 treatments x 4 replicates
Outcome	The three *P. sulcatum* isolates significantly affected plant health consistent with SRR and confirming the results obtained in Greenhouse trial 3. Neither the *P. ultimum* nor *P. irregulare* isolates successfully induced symptoms of SRR.

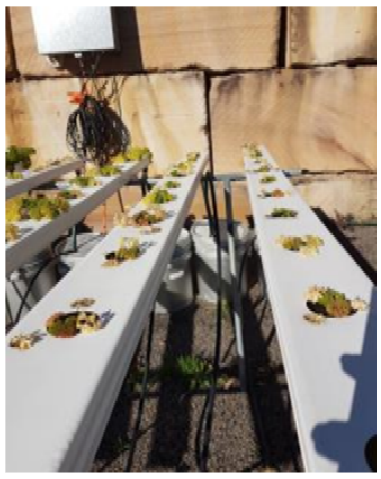
Trial	Hydroponic Trial 2
Pathogen tested	*P. irregulare* (1), *P. ultimum* (1), *P. sulcatum* (2), *P. mastophorum* (1)
Trial Design	The trial consisted of a randomised block design with 1 control 6 treatments x 4 replicates
Outcome	There was very high variability in the results and an issue encountered with cross-contamination possibly due to inclement weather at the time of the experiment. Both *P. sulcatum* isolates reduced plant growth dramatically and caused disease symptoms but It was resolved to run this experiment again.

Trial	Hydroponic Trial 3
Pathogen tested	*P. irregulare* (1), *P. ultimum* (1), *P. sulcatum* (2), *P. mastophorum* (1)
Trial Design	The trial consisted of a randomised block design with 1 control 6 treatments x 4 replicates
Outcome	Again high variability, and controls affected by disease. There was very high variability in the results and an issue encountered with cross-contamination due to inclement weather at the time of the experiment. It was resolved to run this experiment again and make improvements to the hydroponic channels to reduce interference by wind.

Trial	Hydroponic Trial 4
Pathogen tested	*P. sulcatum* (3) + control products (2)
Trial Design	The trial consisted of a randomised block design with 1 control 4 treatments x 6 replicates This trial was testing 2 disease control products against the suspected candidate of SRR *P. sulcatum*. Maxstim® is a micronutrient supplement promoted to stimulate plant growth and reduce disease susceptibility (rate 1.25ml/1L). Fulzyme® is a biological containing *Bacillus subtilis* (rate 2ml/1L).

Outcome	*P. sulcatum* was shown to have a significant detrimental effect on plant health consistent with SRR. Effective control of SRR was not obtained with Maxstim® in this instance. Initial results with Fulzyme® seemed promising however the effect seemed to wear off over time.
Photos *Showing plant/root health vs in situ in hydroponic channels for all 4 treatments, left > right: 1) Control, 2) P. sulcatum only, 3) P. sulcatum + Fulzyme®, 4) P. sulcatum + Maxstim®*	 

Trial	Hydroponic Trial 5
Pathogen tested	*P. sulcatum* (3) + control products (4)
Trial Design	The trial consisted of a randomised block design with 1 control 4 treatments x 6 replicates This trial was testing a number of disease control products against the suspected candidate of SRR - *P. sulcatum*. Fulzyme®, a biological containing *Bacillus subtilis* (rate 3ml/1L), was tested alone and in combination with 3 other products: Sudo-shield® (biological containing *Pseudomonas fluorescens* (rate 0.25g/1L)), Serenade® (biological containing *B. subtilis* (rate 0.5ml/1L)), Rhizovital® (biological containing *B. amyloliquefaciens* (rate 0.4ml/1L)).
Outcome	*P. sulcatum* was shown to have a significant detrimental effect on plant health consistent with SRR. Effective control of SRR was not obtained with either of the control product combinations. Further investigation may be required to determine an appropriate dosage rate/application schedule for hydroponic situations.

Photos *Left > right: Control plants vs chemical treatments and P. sulcatum infected plants*	 

Trial	Rockwool Trial 1
Pathogen tested	*P. sulcatum* (1), *F. solani* (2), *F. oxysporum* (1), and a mixed bacterial inoculum (*Pseudomonas* and *Pectobacterium* spp).
Trial Design	The trial consisted of a randomised block design with 1 control 6 treatments x 30 replicates
Outcome	*P. sulcatum* was shown to have a significant detrimental effect on plant health consistent with SRR.

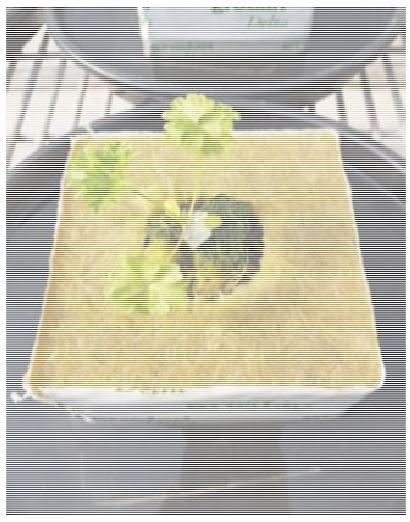
Trial	Rockwool Trial 3
Pathogen tested	*P. sulcatum* and a mixed bacterial inoculum (*Pseudomonas* and *Pectobacterium* spp).
Trial Design	The trial consisted of a 4x4 Latin square design with 1 control 4 treatments x 24 replicates This trial was conducted within polypropylene tunnels within a greenhouse to maximise both temperature and humidity. Observations by farmers indicated disease expression was worse in warm and wet conditions so this trial aimed to explore if bacterial infection (in conjunction with *P. sulcatum*) was exacerbated in high humidity to produce the collar rot symptom occasionally encountered in SRR
Outcome	Only treatments with *P. sulcatum* exhibited significant disease symptoms suggesting the bacteria were not pathogens of parsley

Trial	Rockwool Trial 4
Pathogen tested	*P. sulcatum* (1) + control products (3)
Trial Design	The trial consisted of a 6x6 latin square design with 1 control 6 treatments x 24 replicates

	Three control products were tested against *P. sulcatum* to effect control of SRR. Ridomil® (active ingredient Metalaxyl-M (rate 0.1ml/m^2), used to suppress *Pythium* spp.) was tested alone and a combination of Orondis® (active ingredient oxathiapiprolin (rate of 1.4ml/1L), used to suppress *Pythium* spp.) + Revus® (active ingredient mandipropamid (rate of 3ml/1L), used to suppress *Pythium* spp.) was tested.
Outcome	*P. sulcatum* was shown to have a significant detrimental effect on plant health consistent with SRR. Only treatments that included metalaxyl-M gave significant disease control.

Trial	Rockwool Trial 6
Pathogen tested	*F. solani* (1) + *Pseudomonas* (1) + mixed bacterial inoculum (*Pseudomonas* and *Pectobacterium* spp).
Trial Design	4 treatments x 2 replicates (including 1 control) This trial was a simple pathogenicity test to assess whether these particular pathogens could induce a vascular wilt when directly injected into the stem of a healthy parsley plant. Each treatment was injected into the stem approximately 1cm above the crown. Following the conclusion of the trial the plants were destructively harvested by slicing them longitudinally down the stem to assess presence of vascular wilt and browning within the vascular tissue.
Outcome	Aside from some small brown marks at the injection site no trace of vascular damage was noted nor was any root rot or crown rot symptoms consistent with SRR detected.
Photos *Left > right: Pseudomonas treatment; Fusarium solani treatment*	

Trial	Rockwool Trial 7
Pathogen tested	*P. sulcatum* (1) + *Rhizoctonia sp.* (1), Bacteria spp. (1)
Trial Design	The trial consisted of a randomised block design with 1 control 5 treatments x 20 replicates Four pathogen combinations were tested in an effort to induce the collar rot symptoms consistent with SRR: *Rhizoctonia* alone, *Pythium* alone, *Rhizoctonia + Pythium*, and *Rhizoctonia + Pythium +* bacteria. *Rhizoctonia* was applied as small cubes of agar sandwiched around the parsley stem. *Pythium* cultures were macerated then poured over the parsley plants. Bacteria cultures were diluted with water and poured over the parsley plants.
Outcome	*P. sulcatum* was shown to have a significant detrimental effect on plant health consistent with SRR. The bacterial spp. and *Rhizoctonia* did not induce collar rot or root rot symptoms consistent

	with SRR.
Photos *Left > right: Inoculation with Rhizoctonia culture; Pythium infected plant*	 

Trial	NSW DPI field station Somersby, NSW
Pathogen tested	Each bed was inoculated with macerated disease plant material from farms in Victoria and Queensland. The aim was to determine Fusarium was a significant pathogen and if it could be influenced by fertiliser or agrichemical treatments.
Trial Design	The commercial fungicide Switch® was applied as a drench treatment (rate 0.1g/m^2) (APVMA Permit 7250) and plots were split to include an added ammonium fertiliser treatments using a randomized block design (6 treatments x 6 replicates).
Outcome	There were no significant effects of chemical or fertiliser treatments on SRR

Trial	Melbourne Farm Trial 1
Pathogen tested	Naturally infected soils on farm + control products (2)
Trial Design	Trial consisted of 3x3 Latin square design with 1 control 3 treatments x 3 replicates Two control products were tested against parsley naturally infected with SRR. Switch® (active ingredient Cyprodinil + Fludioxinil (rate 0.1g/m^2), used to suppress Fusarium) was tested alone and in combination with Ridomil® (active ingredient Metalaxyl-M (rate 0.1ml/m^2), used to suppress *Pythium* spp.).
Outcome	Neither treatment successfully suppressed SRR.

Trial	Melbourne Farm Trial 2 - Bay 2
Pathogen tested	Naturally infected soils on farm + control products (4) tested with and without compost
Trial Design	Trial consisted of 2 randomised block designs side by side with and without soil amelioration

	5 treatments x 5 replicates > soil ameliorated with compost 5 treatments x 5 replicates > soil ameliorated without compost 4 control product combinations (plus 1 control) were tested against naturally SRR infected parsley in the field. The addition of compost to one block was to test if this exacerbated disease expression or aided plant defence by enhancing beneficial microbes in the soil. One row of parsley in each bed had been previously treated with a pre-emergent herbicide. Treatment 1 - Control; Treatment 2 - Switch® (rate 0.1g/m^2); Treatment 3 - Switch® + Ridomil® (rate 0.1ml/m^2); Treatment 4 - Serenade® (active ingredient *Bacillus subtilis (*rate 19ml/1L)) ; Treatment 5 - Switch® + Ridomil® + Serenade®
Outcome	Soil amendment of compost had no effect on SRR disease expression. None of the chemical and biological control products suppressed SRR.

Trial	Melbourne Farm Trial 3 - Bay 1
Pathogen tested	Naturally infected soils on farm with chicken manure incorporated into the soil or compost incorporated into the soil with chicken manure spread on top
Trial Design	2 treatments x 3 replicates Three beds of parsley were sown into soil where only chicken manure was incorporated and a further 3 beds were sown into soil where compost was incorporated into the soil plus chicken manure spread on top.
Outcome	Soil amended with compost had no significant effect on SRR disease expression.

Appendix 4: Varietal Susceptibility - Project Factsheet #2

Department of Primary Industries

Parsley Summer Root Rot

– Varietal Susceptibility

Cause:

Parsley Summer Root Rot (*SRR*) is a disease complex involving a combination of several different pathogenic fungi, oomycetes and bacteria. The primary pathogen is the oomycete *Pythium sulcatum* which can attack a range of plants botanically related to parsley such as parsnips, celery and carrots. It is also the primary cause of the important disease Cavity Rot of carrots. Another oomycete *Pythium mastophorum* causes a similar seedling root rot disease while common strains of the fungi *Fusarium oxysporum* and *Fusarium solani* are commonly associated with *SRR* although not able to initiate the disease alone. The fungus *Rhizoctonia solani* can also be associated with *SRR* and can also cause damping off and collar rot diseases of parsley. A number of soft rot bacteria are also commonly associated with plants affected by *SRR*, particularly in the advanced stages of the disease.

Symptoms:

SRR causes plants to become stunted, the older leaves and petioles turn yellow or purple and wilt, and eventually the whole plant wilts and dies. The tap root develops an orange-brown rot, particularly at the collar which becomes soft and mushy as secondary bacteria invade tissue. Other parts of the tap root can also display striped brown lesions and finer feeder roots have a brown rot. Infection may extend into the lower stem of seedlings, causing damping-off.

Varietal Susceptibility:

Both flat-leaf and curly parsley varieties can be affected but curly varieties are far more susceptible. There does not appear to be any varietal resistance which is understandable given the number of pathogens associated with *SRR*.

SRR affected plants wilting with purpling of petioles

Roots with watery brown rot and stripy marks on tap root

Appendix 5: Exert from parsley chemical table provided at grower meetings

Registered fungicide	Some common trade names ®	Rate per		Withholding period (days)	Critical Use Comments/Restraints
		100 L	ha		
ROOT ROT (*Phytophthora* spp.) (*Pythium* spp.)					
Phosphorous (Phosphonic) Acid present as mono-dipotassium phosphanate (200, 400; 600 or 620 g/L) (FRAC Gp 33)	Sprayphos 400; Sprayfos 620; Agri-Fos 600 Dominator 600 Phosic 600 plus others	Refer to label		1	• Apply a maximum of 4 sprays per crop • Apply sprays more than 7 days apart • Test for possible phytotoxicity on a few plants before full application **PERMIT NUMBER – PER13698** **Expires 30 September 2017**
Metalaxyl (480 g/kg) (FRAC Gp 4)	Ridomil Gold 480	625 mL/100 m row	40 kg		• Apply to soil surface in 30 cm band of planting • Irrigate to incorporate • Temporary MRL set at 0.3 mg/kg **PERMIT NUMBER – PER83797** **Expires 31 March 2022**

Appendix 6: Abstract submitted for the 'Science Protecting Plant Health 2017' Conference

Etiology of Parsley Summer Root Rot in Australia

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Parsley production in Australia is valued at $34M per annum. A root and collar rot over the summer months can decimate crops causing plants to wilt and collapse. Curly leaf cultivars are particularly susceptible. Crop surveys in NSW, Queensland, Victoria and Western Australia determined that disease occurrence is widespread in both soil and soilless production systems. Previous studies of parsley root rot postulated that this summer disease was distinct from those occurring in cooler months that were attributed to species of *Pythium*. Those studies identified *Fusarium oxysporum* as a potential causal pathogen and/or an uncharacterised *Pseudomonas* sp. Diagnostic pathology on samples collected during this project produced an array of *F. oxysporum*, *F. solani*, *Pythium* spp, and bacterial isolates. These were partially characterised and used in pathogenicity experiments. Several experiments were conducted in potted parsley plants as well as soilless systems using the nutrient film technique or rockwool blocks. Inocula were applied to plants as single isolates or as combinations of different isolates or species. Only treatments that included isolates of either of two *Pythium* species were capable of inducing disease symptoms. The more commonly occurring of these was *P. sulcatum* which is an aggressive pathogen of apiaceous plants. It is known to cause the diseases cavity rot and forking of carrots as well as root rots of parsley and coriander. It has a wide temperature range for growth so could be solely responsible for parsley root rots throughout the year. However, there are some unexplained issues from our results and observations. Often early disease symptoms appear at the plant collar. Why do coriander and flat leaf parsley crops appear to be less affected? Could there be other contributing factors such as residual herbicides or fertiliser interactions? Is there a cryptic pathogen that we have not identified?

Department of Primary Industries

Appendix 7: Parsley Summer Root Rot – Integrated Disease Management: Project Factsheet #3

Parsley Summer Root Rot

Integrated Disease Management

Cause:

Parsley Summer Root Rot (*SRR*) is a disease complex involving combinations of several different pathogenic fungi, oomycetes and bacteria. The primary pathogen is the oomycete *Pythium sulcatum* which can attack a range of plants botanically related to parsley such as parsnips, celery and carrots. It is also the primary cause of the important disease Cavity Rot of carrots. Another oomycete *Pythium mastophorum* causes a similar root rot disease while common strains of the fungi *Fusarium oxysporum* and *Fusarium solani* are commonly associated with *SRR* although not able to initiate the disease alone. The fungus *Rhizoctonia solani* can also be associated with *SRR* and can also cause damping off and collar rot diseases of parsley. A number of soft rot bacteria are also commonly associated with plants affected by *SRR*, particularly in the advanced stages of the disease.

Figure 1: SRR affected parsley plant

Symptoms:

SRR causes plants to become stunted, the older leaves and petioles turn yellow or purple and wilt, and eventually the whole plant wilts and dies. The tap root develops an orange-brown rot, particularly at the collar which becomes soft and mushy as secondary bacteria invade tissue. Other parts of the tap root can also display striped brown lesions and finer feeder roots have a brown rot. Infection may extend into the lower stem of seedlings, causing damping-off. Both flat-leaf and curly parsley varieties can be affected but curly varieties are far more susceptible.

Integrated management strategies:

Following are key management options that need to be applied for effective SRR disease control:

- Apply farm biosecurity and crop hygiene practices to minimise risks of introduction or re-introduction of *P. sulcatum* and other pathogens to production systems.
- Longer crop rotations are clearly needed in soil-based parsley production systems, particularly on farms where other apiaceous crops are grown. *P. sulcatum* is known to survive in soils for up to two years. This strategy may be challenging on some intensive production enterprises with limited land. Brassicas and alliums are not susceptible to *P. sulcatum* so they are suitable rotation crops. Both types of plants also release sulphurous and

cyanogenic chemicals from their decomposing roots that are suppressive to soil-borne pathogens.

- Chemical interventions need to be carefully considered given our negative field efficacy results and those from previous studies on parsley and carrots. Successful control with specific fungicides is challenging given the typically long production period for parsley. Plants are continuously exposed to infection from seeding through to their final harvest. There is evidence from carrot cavity spot research that certain fumigants, fertilisers (notably Perkla) and biofumigants can decrease initial soil populations of *P. sulcatum*. Sustained chemical control was not demonstrated in trials from this project but it could potentially be achieved with judicious applications of biological and/or chemical controls, particularly if used after reducing the pathogen population prior to sowing. However, this approach needs extensive field validation for parsley SRR.
- In a wet environment *P. sulcatum* produces masses of motile spores (zoospores) that have sensors to detect chemo-electric fields around roots. This literally guides zoospores to the root surface. The sudden explosion in the pathogen population also greatly increases the likelihood of multiple infections on roots and subsequently more severe disease expression. Improved soil drainage and irrigation scheduling are therefore important cultural management options. Over-irrigation or periodically saturating soils favours zoospore production and root infection. Consideration could also be given to avoid wet weather by using protected cropping systems. Protective structures with a retractable roof would be ideal given plants can be protected during wet weather. Recirculated hydroponic systems have a distinct disadvantage as roots are constantly exposed to zoospores. Water disinfection treatments (radiation, chemical or filtration) can be very effective at removing pathogens such as *Pythium* species from nutrient solutions.
- We saw no improvement in SRR control with a compost soil amendment prior to planting. This failure does not mean that this approach has no value given there are extensive reports in the world literature of improved general disease suppression with compost amendments.

Figure 2: Advanced SRR symptoms with watery brown rot of tap root

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