Integrated management of Pythium root disease complexes to improve sustainability and productivity of crop rotations

Summary

Pythium is often described as the common cold of cropping systems, due to its prevalence in soil and capacity to infect all grain crops and pastures. The incidence of pythium root rot is much greater than previously thought, causing significant reductions in productivity. Only partial disease control has produced yield increase in cereals (2-12%), canola (5-25%) and pulses (5-35%), the scale of the responses depending on the season and previous cropping history. Disease management strategies have integrated fungicides and bio-control strains during highly susceptible rotation phases and identified rotation sequences to avoid severe disease and high levels of inoculum carryover into the next season.

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Conclusions
This research, with its strong emphases on understanding the biology of the target pathogens, integration of disease control options and pathways to enable adoption of management strategies (e.g. agribusiness and agronomy networks), has made a significant contribution to managing pythium root disease complexes and improving the productivity of southern Australian cropping systems.

Research conclusions:

- Root pathogenic *Pythium* species are widely distributed throughout cropping soils in the mid-higher rainfall (>350mm) cropping zone. *P. irregulare* is the dominant species in canola-cereal-legume cropping systems, comprising more than 90% of all strains isolated.

- Incidence and impacts of pythium root rots tend to be greater under minimum tillage cropping systems in higher fertility acidic rather than alkaline soils.

- Pythium-selective seed dressings (metalaxyl-M®) significantly reduced soil-borne inoculum and root infection, increasing yields of canola, cereals and pulses. Yield benefits occurred with no significant improvements in crop emergence, indicating that pythium root rot is the major disease expressed not seedling ‘damping-off’.

- The limited albeit significant disease control achieved using metalaxyl seed dressings was significantly improved by post-emergent applications of pythium-selective phosphate®-based fungicides, resulting in further yield increases.

- Bio-control trials with a novel *Trichoderma* inoculant were as effective as metalaxyl seed dressings in controlling pythium root rot in wheat. The inoculant was compatible with the chemical treatment and their integration produced a synergistic effect, further reducing soil inoculum (-38%) and increasing wheat yields (12%).

- Crop species and varieties showed significant differences in their susceptibility to *Pythium* infection and capacity to ‘carry over’ inoculum, indicating that crop rotation and selection of disease tolerant varieties can be used to manage pythium root rot. Lupins were ranked the most susceptible followed in order by canola, peas, wheat and barley.

- Inoculum pressure and disease incidence were significantly higher in the less diverse (i.e. 2-year wheat-canola), compared with more diverse 3-4 year rotation cycles (i.e. those that included a pulse). Rotations that included two cereal phases (i.e. wheat and barley) showed more dramatic fluctuations in inoculum levels across the cycle, resulting in lower disease incidence in the cereal phases and improvements in grain yield.

- Pathogenicity tests indicated crop-preference among genotypes of *P. irregulare*, suggesting host-adaptation of pathogen genotypes. Isolates with extended (4-year) exposure to the same crop had higher overall levels of pathogenicity than those from crops in annual rotation.

- Genetic composition of *Pythium* populations shifts in response to crop rotation, as a result of differential host-mediated selection pressures. *Pythium* populations appear to consist of a complex of crop-adapted genotypes that vary in abundance depending on the rotation sequence.
Epidemiological, ecological and genetic analyses of Pythium populations have delivered crucial information to underpin and interpret Pythium molecular diagnostics (under development) required to accurately predict root disease incidence. This knowledge forms the basis to develop and deliver disease management packages that integrate fungicide and biological (inoculant) treatments within appropriate rotation sequences.

**Recommendations**

- **Continue to communicate research outcomes.** Growers need to be aware that pathogenic Pythium species are widely distributed in cropping soils. They are a major factor reducing the crop productivity throughout the mid-higher rainfall (>350mm) zone of southern Australia.
- **Minimum tillage cropping in higher fertility, acidic to neutral soils is more at risk of pythium root rot than lower rainfall regions with alkaline soils.** Growers would be advised to seek advice on assessing Pythium inoculum levels and use metalaxyl-M® as crop protectants.
- **Growers to note that all crops used in rotations are susceptible to Pythium infection and that high rainfall or cold soils subject to waterlogging are not necessarily a pre-requisite for pythium root rot.** For example, yield benefits in our trials occurred during periods of severe drought (2002 and 2005 in particular), conditions not generally considered conducive for Pythium diseases.
- **Crops experiencing reduced early vigour and consecutive yield decline with no obvious above-ground symptoms are likely to be suffering pythium root rot.** Growers would be advised to seek advice on Pythium incidence and consider using metalaxyl-M seed dressings and post-emergent applications of other pythium-selective fungicide (e.g. phosphite®) in subsequent cropping phases.
- **Where feasible, growers should consider using more diverse crop rotations to manage pythium root rot.** Less diverse cropping rotations, in particular repetitive wheat-canola are at significantly higher risk of disease than more diverse 3-4 year rotation cycles. Rotations with a 4-year cycle that included two cereal phases (wheat and barley) had the lowest incidence of pythium root rot in wheat.
- **Continuous cereal (wheat) cropping needs to be considered with caution, as Pythium is a likely contributor to yield decline reported in repetitive wheat cropping (i.e. wheat-on wheat effect).** This research documented significant increases in Pythium inoculum and root infection when growing the same crop consecutively, compared when grown in annual rotation.
- **Further research needed to commercialise the Trichoderma strain as an inoculant for bio-control of pythium root rot in wheat and extend it to other crops.** Growers to continue to be made aware of the benefits of using the inoculant and its ease of application during seeding.
- **Communicate that disease management strategies for pythium root rot are likely to be durable in the longer-term, because of limited capacity of the pathogen to inherit pathogenicity or fungicide resistance characteristics (limited sexual recombination) and the limited migration (dispersal) of Pythium genotypes between locations.**

**Outcomes**

This research has defined the distribution of Pythium in southern Australian cropping systems and increased industry awareness of the growing incidence and adverse impacts of the pythium root rot. The research has changed the industry perception of Pythium, so that it is now recognised as an increasingly important primary root rot pathogen with significant detrimental impacts on crop productivity. The realisation that even partial pythium disease control will provide significant benefits to growers will assist adoption of strategies to manage the disease, especially given that they can be readily incorporated into current systems.

Economic benefits of improving management of pythium root disease complexes. The extensive distribution of Pythium in southern Australia and the impacts of pythium root diseases on the productivity of crop rotations are often underestimated or overlooked. However, this research clearly showed significant yield increases in canola (5-25%), cereals (1-12%) and pulses (2-35%) from using Pythium-selective fungicides.

The current target cropping areas consist of the portion of the southern Australian grain belt with a mean annual rainfall greater than or equal to 350mm. Based on yield results from Pythium-selective fungicide and bio-control field trials, limiting high incidences of pythium root rot and ‘damping-off’ diseases could conservatively increase grain production in these regions by at least 5%.

Pythium disease incidence over the target grain growing area:
Assuming that inoculum levels at or above the threshold level capable of causing significant disease occur over 20% of the target grain growing area, control strategies could increase production by:
5% yield increase: $351.8 million x 0.2 (20% of target area) = $70.3 million.

Given the widespread occurrence of Pythium in southern Australia and the generally high soil-borne inoculum levels recorded in the target growing area, it is assumed inoculum levels at or above the damage threshold in at least 60% of the target growing area. Consequently, control strategies could increase production by:
5% yield increase: $351.8 million x 0.6 (60% of target area) = $211 million.

Environmental benefits
In addition to increasing production, improved pythium disease control has a number of environmental benefits including more sustainable use of on-farm resources (nutrients) and increased adoption of conservation farming practices, thereby reducing soil erosion and atmospheric pollution. Improved root disease management therefore has potential to reduce the input-costs (fertilisers, fuel) and off-site impacts associated with annual cropping systems. Furthermore, increased productivity and profitability will provide a greater range of land management options to growers, providing greater flexibility and opportunities to diversify their agricultural practices.

Achievements/Benefits
The project has defined the distribution of Pythium in southern Australian cropping systems and increased grower and industry awareness of the growing incidence and adverse impacts of pythium root rot. The research has changed the industry perception of Pythium, such that it is now recognised as an increasingly important primary root rot pathogen with significant detrimental impacts on crop productivity. Furthermore, its harmful effects increase when it forms a disease complex with other root pathogenic fungi. The realisation that even partial control of Pythium will provide significant economic benefits to growers will assist adoption of research recommendations to manage the disease, especially given that it can be achieved with only minimal changes to current practices.

This research aimed to provide robust epidemiological and genetic information necessary for the development of an integrated disease management package for Pythium, soil-borne root pathogens that cause seedling damping-off and root rots in all phases of canola-cereal-legume rotations. A primary goal was to quantify the effects of pythium diseases, thereby increasing grower awareness of their adverse impacts and defining the scale of the problem in southern Australian cropping systems.

Pythium has been described as the common cold of cropping systems, with disease impacts on cropping productivity in southern Australia generally being underestimated or overlooked. Until recently Pythium was primarily regarded as a seedling ‘damping-off’ disease that reduced crop emergence and early establishment. This research has shown however, that the incidence of pythium root rot is much greater than previously thought, causing significant reductions in productivity often in the absence of ‘damping-off’.

Pythium is fast growing and can infect seeds as soon as they start to germinate. Pythium produces large numbers of spores (oospores) that enable it to rapidly and continuously re-infect growing roots. Consequently, crops can experience repeated ‘waves’ of Pythium infection throughout the growth season, rather than the slower inoculum build up attributed to other fungal root diseases such as take-all and rhizoctonia. The weakened plant is then more vulnerable to infection by these other fungal pathogens. While Pythium is an important primary root rot pathogen, its harmful effects can be increased significantly when it forms a disease complex with other pathogens.

In recent years pythium root rots have been reported with increasing frequency and severity in southern Australian cropping systems. The main reasons appear to be related to changes in the farming systems associated with increased adoption of reduced tillage systems and the switch to less diverse, potentially higher risk crop rotations (e.g. repetitive canola-wheat rotations). Both of these trends are here to stay because of their economic and environmental benefits. Consequently, there is an urgent need to develop management strategies for this emerging disease. Project research (CSO000016 and CSO000032) has identified pythium root rots as a critical issue and that improved management will continue to deliver significant economic benefits to the grains industry.

Managing pythium diseases in current minimum tillage, high intensity cropping systems represents a significant challenge. The broad host range of the pathogen indicates that inoculum can build up over consecutive seasons, causing poor early growth and yield declines in rotations. This research has however, provided evidence of host-adaptation within Pythium, with
different cropping sequences favouring different *Pythium* genotypes. Consequently, by developing a thorough knowledge of *Pythium* inoculum levels in cropping soils, how they shift across phases of the rotation and what crops are most susceptible to different genotypes of *Pythium*, targeted rotation strategies to minimise crop losses could be developed.

Summary of project outcomes and achievements:

Fungicide and rotation trials: disease incidence and control efficacy.

Integrated fungicide, rotation and bio-control trials (total of 66 at four sites, 2002-2006) defined the widespread distribution of *Pythium* and high incidences of root rot were responsible for significant reductions in crop productivity *Pythium*-selective seed dressings (4-year averages, all crops) significantly decreased soil inoculum (-25%) and root infection (-23%). Despite only partial disease control, average grain yields (4-year averages) increased in cereals (1-4%), canola (12%) and pulses (11-26%). Notably, these yield benefits occurred during periods of severe drought (2002 and 2005 in particular), conditions not considered conducive for pythium diseases.

During the project, numerous requests were received from agronomists to determine *Pythium* incidence and inoculum from 60 farms and cropping trials (2002-2005) was quantified. This resulted in 13 extra fungicide trials (four in Western Australia (WA), nine in South Australia (SA)) funded by agribusiness. Results of *Pythium* inoculum dynamics, disease incidence (CSIRO) and crop performance (external) were comparable to and supported those from our GRDC-funded trials. Collectively, this research indicated that pythium root rots are a major factor reducing the productivity of rotations throughout the mid-higher rainfall (>350mm) cropping regions across southern Australia (evidenced from the South Australian, Victorian, Western Australian and New South Wales trials).

Previously, disease risk ratings have only considered pythium as a ‘damping-off’ disease that reduces crop emergence and have not taken into account infections throughout the season that result in root rots. In these trials, however, diseased crops showed no obvious ‘above-ground’ symptoms and yield benefits occurred with no significant improvements in emergence. The limited disease control using metalaxyl seed dressings may be due to its reported short window of activity (4-8 weeks). This, combined with the pathogen’s ability to rapidly produce large numbers of infective spores, indicates that *Pythium* numbers can quickly recover as the season progresses. Consequently, the yield benefits are primarily associated with improved early crop growth and more effective, sustained disease control throughout the season should lead to further improvements in grain yields.

The 2005 rotation trials were modified to include ‘in crop’ fungicide treatments (i.e. phosphite spray and metalaxyl soil drench) at six weeks post-emergence to follow up the initial metalaxyl seed dressing. Phosphite alone or in combination with the seed dressing improved yields of all crops, significant increases observed in canola (13-25%) and wheat (12%). The metalaxyl soil drench was inconsistent and less effective. While resources were not available to monitor inoculum levels and root infection, the yield benefits imply that these additional treatments provided more effective pythium control than the seed dressings alone.

In addition to the original proposal, research was conducted to develop a biological control agent for *Pythium*. Three years of bio-control trials against root rot in wheat (2004-2006) compared pythium-selective metalaxyl-M with a *Trichoderma* fungal inoculant. Seed and ‘in furrow’ *Trichoderma* treatments (3-year averages, two sites) decreased soil-borne inoculum (-16%) and root infection (-55%) and increased wheat emergence (9%) and grain yield (4%), disease bio-control being as effective as the fungicide. Integration of the treatments (2006) indicated a synergistic effect, further reducing soil inoculum (-38%) and increasing wheat yield (12%). Research continues on developing the fungus for commercial release through independent testing in Australia and overseas via the GRDC-Philot Bios joint venture.

Integration of a *Pythium*-selective metalaxyl-M in a range of low (wheat-canola) and high (wheat-canola-barley-pea) species diversity rotations defined the relative capacities of chemical and crop selection to manage pythium root rot. Inoculum pressure and disease incidence were significantly higher in the less diverse (i.e. 2-year), compared with more diverse 3-4 year rotation cycles (i.e. those that included a pulse). In particular, rotations that included two cereal phases (i.e. wheat and barley) showed more dramatic fluctuations in *Pythium* inoculum levels across the cycle, resulting in lower disease incidence in the cereal phases and improvements in grain yield. Similarly, when crops were treated with metalaxyl, the fungicide was more effective at decreasing *Pythium* soil inoculum and root infection in the higher diversity rotations compared to the repetitive wheat-canola rotations, thereby further increasing grain yield.

Despite the generally accepted view that all crops are equally susceptible to *Pythium*, project results indicate differential susceptibility to infection and possibly tolerance to root rot. Analyses comparing root infection, soil-borne inoculum survival,
crop emergence and grain yield (four years, 2002-05) were used to rank crops for their susceptibility to pythium disease. The rankings from highest to lowest susceptibility were as follows: lupins > canola > peas > wheat > barley. Furthermore, crop varieties (two tested per crop at each of the four core trial sites) exhibited significant differences in root infection, emergence and yield in the absence of the *Pythium*-selective fungicides. These variety-based differences were more apparent in the higher compared with the lower diversity rotations and are likely to be related to the lower inoculum and disease pressures in the diverse rotations. These findings are important as they open up the possibility of using targeted crop rotations and selection of disease tolerant varieties to better manage pythium damping-off and root rot.

Molecular ecology and population genetics of *Pythium* in crop rotations
Throughout this research pathogenicity tests and DNA markers (species-specific and host-related) were developed and applied to strains and populations of *P. irregulare* isolated from the rotation trials. Analyses indicated that certain pathogen genotypes were differentially adapted to infecting crops and that the size and genetic composition of *Pythium* populations can change abruptly in response to different crop species.

Pathogenicity tests indicated crop-preference among genotypes of *P. irregulare*, suggesting that different crops may select out host-adapted genotypes of these pathogens. Furthermore, strains with longer periods of exposure to the same crop species (i.e. four years continuous cropping) had higher overall levels of pathogenicity and host-adaptation than strains isolated from crops in annual rotation.

Summary of *P. irregulare* population genetics:
· Of the *Pythium* species isolated from crop roots (cereals, legumes and canola), *P. irregulare* was identified as the dominant pathogen (>90% of all isolations).
· 2002-05 population genetic analyses (660 isolates from four cropping species) were completed using five co-dominant and two multilocus (fingerprinting) Restriction Fragment Length Polymorphism (RFLP) probes. In addition to the original proposal, eight populations (i.e. seven from 2004 and one from 2005) were analysed with four multilocus Amplified Fragment Length Polymorphism (AFLP) markers.
· *P. irregulare* exhibited significant intra- (between crops within locations) and inter- (between locations) population genetic differentiation.
· Levels of genetic diversity significantly different between crop-based populations (inter-population), implying preferential selection of pathogen genotypes (i.e. evidence of host-adaptation).
· Genetic differentiation significantly greater between 2004 populations (compared with 2003 and 2002), indicating differential pathogen responses to rotation sequences.
· Low heterozygosity detected within host-and location- based populations indicates outcrossing is rare (i.e. low levels of sexual recombination).

Overall, significant levels of genetic differentiation among *P. irregulare* populations and low genetic identities between geographically close populations implied low levels of inter-population gene flow. Significant levels of differentiation between populations from different crop species within locations, suggesting a sub-division of *P. irregulare* populations into host-based groups, presumably resulting from crop-mediated selection and infrequent sexual recombination. These analyses suggest that random genetic drift, combined with shifts in the intensities of crop-mediated selection pressures caused the genetic differentiation. Consequently, *Pythium* populations may be comprised of a series of crop-adapted genotypes that vary in abundance and distribution in response to different crop rotation sequences.

These analyses have described how the balance of these different *Pythium* strains changes in response to different crop rotation and fungicide strategies. This is important in determining the numbers and types of strains that survive as inoculum to infect next season's crops, the rates at which they spread within and between fields and how quickly the pathogen can respond to overcome disease control strategies.

Information to develop pythium disease prediction and management frameworks
The research has provided industry with independent, field-validated epidemiological and population genetic information on pythium diseases in crop rotations. In summary, this included:
· Identifying the target *Pythium* species (DNA sequence) and providing type cultures, purified DNA and soil samples (pythium-quantified) to South Australian Research & Development Institute (SARDI) to calibrate *Pythium* diagnostic test (GRDC/Meat and Livestock Australia (MLA) soil biology alliance).
· Defining the relative disease susceptibilities (infection) and abilities of crops to ‘carry-over’ inoculum within rotations and the effect of cropping diversity on disease incidence.
Determining the potential rates of genetic change (sexual recombination), rotation-induced genetic differentiation and host-adaptation within *Pythium* populations.

- Identifying integrated chemical (seed dressing and post-emergent) and bio-control treatments for pythium control in southern Australian crop rotations.

This information is crucial to interpret the molecular diagnostics for pythium disease prediction and the development of sustainable integrated disease management strategies focused on appropriate crop selection, targeted applications of fungicides and bio-control inoculants. These strategies will limit the impacts of *Pythium* and reduce its capacity to interact with other root pathogens, thereby avoiding severe disease incidence in the current cropping phase and high levels of inoculum carryover into the next season.

**Other research**

Significant opportunities have emerged to integrate ecological and genetic studies of root pathogenic fungi and introduced antagonists (inoculants) on crop hosts to achieve and enhance disease suppression. The focus is on broad host-range root pathogens, for which no consistent control measures are currently available (e.g. *Pythium* and *Rhizoctonia*) and an elite bio-control (e.g. *Trichoderma*) strain.

1. Development of pythium disease prediction and management model.

Epidemiological and genetic analyses of *Pythium* populations have delivered crucial information to underpin and interpret *Pythium* molecular diagnostics (being developed). This knowledge forms the basis to predict root disease incidence and develop integrated disease management packages. Information is now available to integrate fungicides and bio-control strains during highly susceptible rotation phases (e.g. legumes and canola) and diversify the rotation sequence to avoid severe disease and high levels of inoculum carryover. Collaborative research between CSIRO and SARDI is required to develop a pythium disease incidence model and management framework for grain growers.

2. Integrated management of rhizoctonia-pythium root disease complexes.

While the impacts of *Rhizoctonia AG8* on cereal production are well known, there is little information in Australia on the incidence of AG2 and AG4 on canola and legumes. Research has shown that *Rhizoctonia* and *Pythium* have pathogenic interactions in rotations and that controlling pythium may also significantly reduce rhizoctonia disease. The aim is to define management strategies to limit rhizoctonia-pythium-crop interactions and in turn, quantify the impacts of diseases on beneficial rhizosphere functions (e.g. microbial nitrogen fixation). Identifying the primary drivers of these plant-microbe interactions will indicate how to enhance beneficial microbial activity related to root disease suppression.


Bio-control with a *Trichoderma* inoculant was as effective as fungicides in controlling pythium root rot in wheat and the treatments were synergistic, further reducing inoculum and increasing wheat yield. Previous research has shown that this inoculant also has disease control efficacy against take-all and rhizoctonia. The aim is to develop and release a *Trichoderma* bio-control inoculant for the grains industry. Firstly, commercialisation relies on identification and genetic differentiation from existing products. Secondly, ecological understanding of interactions with crop roots, other soil microbiota and the impacts of management practices on inoculant survival will define how best to achieve consistent performance. Thirdly, identifying the bio-control mechanism(s) (e.g. antibiotic, parasitism) is essential for patent protection and product registration.

4. Identifying mechanisms of *Pythium* host adaptation and disease tolerance in crop plants.

Crop species and varieties were identified to vary significantly in their susceptibility to *Pythium*. Crop germplasm with increased infection tolerance can be used in resistance breeding programs and provides opportunities to begin to understand mechanisms of disease expression. Defining the molecular basis of pathogenicity may identify novel means of pathogen control and induce disease tolerance in the crop. Useful approaches include studying plant root-*Pythium* gene expression and gene knockout of pathogenic strains to identify characters responsible for disease expression.

**Intellectual property summary**

CSIRO Entomology and the GRDC jointly own the intellectual property rights for the *Pythium* DNA probes used in this research. Other species- (DNA sequence) or isolate- (AFLP) specific DNA markers have been developed via modification of techniques and information currently in the public domain.
These technologies and related interpretive IP are being used to develop disease prediction models and management strategies for controlling pythium root diseases. CSIRO and the GRDC will jointly hold further IP arising from this research and jointly share in the benefits of IP as a result of investing in this and future research associated with the development of pythium management strategies.

Additional information