Hort Innovation

Final Report

Extension of the PREDICTA Pt Potato Diagnostic Service

Project leader:

Michael Rettke

Delivery partner:

South Australian Research and Development Institute

Project code:

PT15008

Project:

Extension of the PREDICTA Pt Potato Diagnostic Service (PT15008)

Disclaimer:

Funding statement:

This project has been funded by Hort Innovation, using the potato – fresh and potato – processing research and development levy and contributions from the Australian Government. Hort Innovation is the grower-owned, not-for-profit research and development corporation for Australian horticulture.

Publishing details:

ISBN 978-0-7341-4700-4

Published and distributed by: Hort Innovation

Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

Content

Summary

This project has enhanced the value of PREDICTA Pt to growers by expanding the range of tests with disease risk categories, adding new tests and developing a new service to test seed tubers. The ability to quantify pathogen DNA levels, both in soil and seed tubers, assists potato growers to make informed decisions on implementing management practices to reduce disease risk.

PREDICTA Pt was originally developed to assess the risk of black dot, root knot nematode and powdery scab before planting, because management options are limited once a potato crop has been established.

Disease risk categories have been added for:

- Verticillium wilt caused by *Verticillium dahliae*. This includes quantifying populations of specific root lesion nematodes that increase *V. dahliae* infection when levels in soil are low.
- Warm climate root knot nematodes species (*Meloidogyne javanica*, *M. incognita*, *M. arenaria*) to enhance the value of the service for growers in Queensland, Western Australia and New South Wales. This has led to more agronomists from these regions seeking accreditation to deliver PREDICTA Pt.

Tests for pathogens that cause sclerotinia stem rot and leak rot developed outside this project, have been evaluated in potato crops and are now reported by PREDICTA Pt with population density categories to benchmark results in a specific paddock with the rest of industry.

New tests have been developed for pathogens that cause silver scurf and pink rot. Seed is the primary source of silver scurf inoculum, and disease risk thresholds have been established for the PREDICTA Pt Peel service.

Testing of tuber and plant samples confirmed the test developed for *Phytophthora erythroseptica, P. cryptogea* and *P. drechsleri* DNA, detects the *Phytophthora* spp. that cause pink rot in Tasmania and other states. Developing disease risk categories has been challenging because inoculum levels below the detection limit in soil can cause disease. However, when inoculum is detected crops are three times more likely to develop pink rot with over 10% incidence compared to crops where inoculum was not detected.

Inoculum on seed tubers can contribute to disease risk and in some cases, this is more important than inoculum in the soil. For other pathogens, seed is an important source of inoculum to infect clean paddocks.

The focus in developing PREDICTA Pt Peel has been to provide risk assessments for silver scurf, common scab and rhizoctonia. The service also includes tests for pathogens that cause black dot and pink rot, as seed inoculum contributes to disease risk.

Systems developed to test peel also enable quantitative testing of root and stem samples as a research tool to investigate observations in potato crops and trials.

Existing tests for *Globodera rostochiensis* (golden potato cyst nematode) and *G. pallida* (pale potato cyst nematode) have been calibrated and are now available to assist with surveillance.

The resource manual and training course has been upgraded to support delivery of the new tests and peel testing service to industry. The manual now covers 11 diseases plus protocols for sample collection and interpretation of results.

Use of PREDICTA Pt by growers and agronomists continues to increase and is being used by;

- seed growers to improve the percentage of crops meeting certification standards,
- fresh market growers to adjust management strategies to lower overall risk of black dot,
- Processing growers to justify cost of implementing management options for root knot nematodes,
- agronomists evaluating crop choices in rotations,
- agribusiness to compare and evaluate disease control treatments.

Further information on how PREDICTA testing can assist growers in managing disease risk is available in the guide to Testing for Seedborne and Soilborne Pathogens of Potatoes.

Keywords

Soilborne disease; Seedborne disease; Potato; Diagnostics; DNA; PREDICTA.

Introduction

Soil borne diseases are a major cost for the Australian potato industry and are difficult to manage. The most effective practices need to be implemented before or during planting.

PREDICTA Pt was developed by the Australian Potato Research Program (APRP1 & 2 – PT04016; PT09023) and first delivered to growers in mid-2013 to provide growers with a pre-plant risk assessment for black dot, root knot nematode and powdery scab based on soil DNA concentrations of the respective pathogens. Initial uptake of the service was mostly from South Australia, Victoria and Tasmania, where tests had been calibrated.

DNA concentrations of nine other pathogen tests (*Rhizoctonia solani* AG2.1, *R solani* AG3, *Verticillium dahliae*, *Streptomyces* txtA gene, *Meloidogyne javanica/incognita/arenaria*, *M. fallax*, *Pratylenchus crenatus*, *P. neglectus*, and *P. penetrans*) were reported as "Tests under development" so growers could compare paddocks and monitor changes over time. Additional research and field validation were required to progress their commercial usage.

Industry also identified a need for preplant risk assessment for pink rot caused by *Phytophthora* spp., this required development of a new DNA test.

Inoculum on seed tubers can contribute to risk of silver scurf, common scab, rhizoctonia, pink rot and black dot and in some cases, it can be the primary source of infection. Seed can also an important way to introduce pathogens to uninfected paddocks.

To monitor this risk posed by seed required development of new sampling protocols, kits, and a new test for *Helminthosporium solani*, the cause of silver scurf.

This project was supported by the Australian Potato Industry to build on the existing capability of PREDICTA Pt to develop disease risk thresholds for existing tests under evaluation, expand application to include peel of seed tubers, develop tests for pathogens that cause pink rot and silver scurf, calibrate potato cyst nematode tests and investigate sampling protocols and interpretation of results for growers in Queensland, Western Australia and New South Wales.

Methodology

Development of new tests

Silver scurf and pink rot were identified as requiring DNA tests for their causal agents.

DNA tests targeted gene regions with high copy number, e.g. the Internal Transcribed Spacer (ITS) region of the genome to improve assay sensitivity in soil. Tests were designed as TaqMan Minor Groove Binder (MGB) assay chemistry to improve specificity and quantification.

To design the test for *Helminthosporium solani*, the cause of silver scurf, three Australian isolates were sequenced and used in combination with existing sequence data in public databases for *H. solani* and related non-target species.

Pink rot of potatoes in Australia is mainly caused by *P. erythroseptica*, and occasionally by *P. drechsleri* and *P. cryptogea*. A review of the literature found that qPCR (quantitative Polymerase Chain Reaction) assays had already been published for this group of *Phytophthora* (Atallah and Stevenson, 2006; Cullen et al., 2007), but the test design did not comply with SARDI's requirements. Therefore, a TaqMan MGB assay was designed in the ITS to detect all three species. The new test was evaluated for efficiency and specificity by testing DNA from the target and closely related non-target species.

Controlled environment pot trials were conducted to investigate relationships between added *P. erythroseptica* levels, DNA concentrations detected by the test, and disease expression.

Calibration of potato cyst nematode tests

SARDI had previously developed qPCR assays for golden potato cyst nematode (*Globodera rostochiensis*) and pale potato cyst nematode (*Globodera pallida*). This project conducted experiments in collaboration with Dr Matthew Back (Harper Adams University, United Kingdom) to determine the detection limit for both tests. This involved extracting populations of *G. pallida* and *G. rostochiensis* at Harper Adams University, confirming identification, and assessing the average egg count per cyst for each species. Known numbers of cysts were then added to soil (free of both species) and supplied to SARDI. Five replicate dilution series were produced for each species using uninfested soil to produce individual samples weighing 500 g. These were then tested to determine the detection limit of the assays.

Development of PREDICTA Pt peel testing.

The key to developing the peel testing service was the development of a simple protocol to stabilize the peel samples; existing research methods based on drying samples in ovens before couriering, were not suitable for a grower service.

Four methods were evaluated to preserve samples; drying, preservative powders, preservative solutions and desiccants to absorb moisture during transit. Methods were evaluated using peel samples from tubers in long-term storage with high fungal and bacterial inoculum loads. Samples were prepared and held for 7 days at room temperature (22°C) before assessment. The best option was evaluated on 59 samples submitted by agronomists.

Peel disease risk categories were developed for common scab (*Streptomyces* txtA gene), Rhizoctonia (*R. solani* AG2.1 and AG3) and silver scurf (*H. solani*) using a combination of previous data from commercial potato crops (PT09023), controlled environment studies (PT09019), and new data from commercial fields collected by this project.

Validation of tests

To validate the tests, pathogen DNA concentrations in soil and peel samples from commercial paddocks were compared to disease expression in the sampled areas, as described in Ophel Keller (2015). For this study, paddocks and crops were sampled in South Australia, Tasmania, Western Australia, New South Wales, and Queensland.

The aim was to produce data to develop disease risk categories for new tests for *P. erythroseptica/ drechsleri/ cryptogea* and *H. solani*, and existing tests for *Verticillium dahliae*, *Rhizoctonia solani* AG2.1 and AG3, *Streptomyces* txtA gene, *Meloidogyne hapla, M. javanica/incognita/arenaria*, *Pratylenchus crenatus*, *P. neglectus*, and *P. penetrans*. Results for the latter tests had been reported as tests under evaluation.

Data generated in this project was combined with data from project PT09023 conducted in APRP2. This allowed results from new regions to be compared with previously established relationships for *Colletotrichum coccodes*, *Spongospora subterranea* and *M. fallax*.

Tests for *Rhizoctonia solani* AG4, *Pythium* clade I, *Pythium* clade F, *Macrophomina phaseolina*, *Botrytis cinerea* and *Sclerotinia sclerotiorum/minor* were used to assess pathogen levels from specific regions and growers. The need for these tests was identified to support growers in the broader potato growing regions.

To expand the data set and value add previous work, selected stored DNA samples from PT09023 (soil and peel) were tested using assays not available at the time, including tests for root lesion nematodes, *H. solani* and *P. erythroseptica/ drechsleri/ cryptogea*.

Plot trials to investigate yield loss

To develop yield loss categories for *Verticillium dahliae* and *Pratylenchus* spp, grid plot trials were conducted in four centre pivots in South Australia planted to the potato variety Innovator. All trials were conducted in 2018/19 season in the south-east region of South Australia. In each paddock 16-40 plots (approximately 10m^2) were soil sampled for pathogen DNA testing at planting. Plots were located along the direction of planting and peel pathogen DNA testing conducted on planted seedlots.

Eight plants were sampled from selected plots at 75 to 80 days after planting and examined for disease symptoms prior to DNA testing of root and stem samples. Eight plants from each plot were assessed for yield and disease incidence, and peel samples tested to measure pathogen DNA concentrations. Data was assessed to determine the relationship between preplant pathogen levels and in crop infection levels and yield.

A similar trial was conducted at the long-term rotation trial site at Forthside experimental station in Tasmania (Sparrow 2015), managed by the University of Tasmania. Soil from 48 plots and seed peel samples collected prior to planting were assessed to measure pathogen DNA concentrations, followed by assessment of yield and disease incidence of the Ranger Russet crop.

Pathogen detection in soil

Eight paddocks with known or expected presence of *R. solani* AG2.1 and AG3, *Streptomyces* txtA gene, and *P. erythroseptica, P. drechsleri*, *P. cryptogea* were sampled. Soil samples were collected using a 15cm long by 1cm diameter corer to produce composite samples of 30 cores following a 'W' pattern across the sampling area. Four separate 1 ha areas were sampled 8 times, giving a total of 32 samples from each paddock to determine the sampling intensity required to achieve 80 and 95% confidence levels for detecting the pathogen.

Trials were also undertaken to determine if lupins and broad beans can increase DNA levels of *Phytophthora erythroseptica* in soil samples.

Production of materials and involvement in industry communications, extension, and practice change programs.

The PREDICTA Pt manual on potato soilborne disease risk management was updated. This included updating three existing disease chapters and adding nine new chapters, including one on sampling and interpretation of seed peel samples.

A short grower focused publication was also produced to summarise the benefits of testing soil and peel samples to manage the risk of soil borne diseases of potatoes.

To broaden uptake of the technology and foster practice change using outputs of this project, project staff engaged with industry communication and extension programs. Training courses were conducted to accredit participants in or servicing the Australian potato industry including agronomists and growers (or their employees) to access the PREDICTA Pt service.

Outputs

Enhancements to PREDICTA Pt service

New capability

PREDICTA Pt Peel

- Provides commercial service to growers via accredited agronomists for the testing of 17 pathogens with an indication of disease risk provided for common scab, silver scurf and specific rhizoctonia diseases.

  https://pir.sa.gov.au/research/services/molecular_diagnostics/predicta_pt

(refer Appendix 1, Development of peel testing service page 21)

Potato cyst nematode (PCN)

- Detection limits of existing tests for golden potato cyst nematode (*Globodera rostochiensis*) and pale potato cyst nematode (*Globodera pallida*) was determined.
- The PCN tests are available to industry through SARDI's PREDICTA research testing services.

  https://pir.sa.gov.au/research/services/molecular_diagnostics/predicta_research

(refer Appendix 1, Potato cyst nematode page 36)

New DNA tests

Two DNA tests developed and added to PREDICTA Pt (Soil and Peel services) and PREDICTA research reports.

- DNA test developed for *Phytophthora erythroseptica, P. cryptogea* and *P. drechsleri* (causal agents of pink rot) and developed as soil and peel test in SARDI PREDICTA format, capability to detect pathogens in soil and plant tissue samples confirmed, with assays available for routine assessment of samples.
- DNA test designed for *Helminthosporium solani* (cause of silver scurf) and developed as soil and peel test in SARDI PREDICTA format, capability to detect pathogens in soil and plant tissue samples confirmed, with assays available for routine assessment of samples.

(refer Appendix 1, Development of new pathogen DNA tests page 23)

Addition of disease risk categories

PREDICTA Pt soil

Provision of disease risk categories have been added to reporting for;

- Verticillium wilt caused by *Verticillium dahliae*
- Root knot nematode caused by *Meloidogyne javanica/incognita/arenaria*

PREDICTA Pt peel

Disease risk categories have been developed and are reported for:

- Common scab caused by *Streptomyces* spp. with txtA gene
- Silver scurf caused by *Helminthosporium solani*
- Stem canker and black scurf caused by *Rhizoctonia solani* AG3
- Deformed tubers and black scurf caused by *Rhizoctonia solani* AG2.1

(refer Appendix 1, Validation of new pathogen DNA tests page 24, Calibration of tests page 36)

Other DNA tests added to service

PREDICTA Pt

Three DNA tests added to PREDICTA Pt as tests under evaluation (reported with population density categories generated from pre-plant soil testing prior potato production).

- *Sclerotinia sclerotiorum/minor* (developed in GRDC/SARDI Bilateral project DAS1802-011BLX)
- *Pythium* clade I (developed in MLA project SHP.005)
- *Rhizoctonia solani* AG4 (developed by SARDI)

(refer Appendix 1, Evaluation of tests for other pathogens not developed in this project page 74)

Upgrade of PREDICTA Pt manual

- Chapters on verticillium wilt, root lesion nematodes, rhizoctonia disease, common scab, pink rot, silver scurf, leak rot, sclerotinia stem rot added to support PREDICTA Pt use, interpretation of results and disease risk management.
- Existing chapters on powdery scab, root knot nematode and black dot have been reviewed and updated.
- Module developed on sampling tubers.
- Modules on DNA testing and soil sampling have been updated.

Detecting *Phytophthora*, a challenge

- Low levels of *P. erythroseptica*, *P. cryptogea* and *P. drechsleri* are difficult to detect pre-plant. However, where *Phytophthora* was detected the risk of pink rot occurring was greater compared to areas within the same or adjoining fields where it was not detected.

(refer Appendix 1, Pink rot page 26)

Sampling requirements

- Soil sampling requirement to test for the presence of pathogenic *Streptomyces*, *Rhizoctonia solani* AG3 and *R. solani* AG2.1 in a paddock prior to planting established.

(refer Appendix 1, Evaluation of repeated sampling, page 65, 71)

Tissue sample testing

- Techniques for in-crop DNA testing of root samples, stem sections and developing tubers developed for assessing infection level and causes of loss in productivity of potato crops.

Training, awareness and industry support

PREDICTA Pt training

Five training workshops were conducted, and 51 participants were accredited to deliver PREDICTA Pt. Training covered soil and seed borne pathogens and the diseases they cause, factors driving disease risk, sampling protocols and interpreting test results. A list of accredited agronomists available on PREDICTA Pt webpage

(only includes accredited agronomists that approve to be publicly listed)

https://pir.sa.gov.au/research/services/molecular_diagnostics/predicta_pt

Awareness

Abbreviated guide produced to support grower understanding of PREDICTA Pt service

(Appendix 2: Guide – Testing for Seedborne and Soilborne Pathogens page 80).

Michael Rettke presented at seven potato industry grower and agronomist meetings covering use of the PREDICTA Pt service for pre-plant risk assessment, managing disease risk and understanding effects of treatments and management strategies on inoculum levels and disease outcomes.

Articles produced highlighting what PREDICTA Pt does and what growers are using it to achieve.

Michael Rettke co presented Episode 1 of the InfoVeg TV series| PREDICTA Pt (2017) introducing viewers to the PREDICTA Pt service.

https://ausveg.com.au/infoveg/infoveg-tv/

Industry support

Project staff provided support to accredited agronomists and their clients to facilitate uptake and foster appropriate use of PREDICTA Pt to maximise value to growers.

Support was also provided to research use of the technology including.

- Characterise pathogen levels in trial sites.
- Monitor changes in pathogen levels over time.
- Testing soil and tissue samples from field trials to assess effects of different soil treatments and crop sequences and investigate causes of disease symptoms.

Testing of samples, technical assistance and interpretation was provided to other industry funded potato research and development activities, including:

- PT16001, PT16002 - quantification of pathogen DNA levels in soil samples.
- PT19000 – quantification of *Phytophthora erythroseptica* in growth media, soil and tissue samples.
- PT19001 – assistance with development of sampling and sample handling procedures.

Outcomes

The PREDICTA Pt service started in 2013. Utilisation of the service prior to this project was static around 200-250 samples per year, with samples mostly from potato seed and processing producers in Tasmania, south-east of South Australia and to a lesser extent Victoria.

During this project sample numbers have increased to 400-500 samples per year. Each sample represents between 5 – 25 ha of production. Most of the increased demand has come from the fresh potato sector and seed producers servicing both the fresh and processing sectors.

Accreditation of an extra 51 agronomists has also contributed to increase in demand by expanding the user base in established and new regions.

Most of the larger users of the service and many smaller ones have conducted testing for 3 or more years. There are also agronomists trained in the early years of the service that continue to use PREDICTA Pt. These agronomists are an important resource for industry and the service, as they have refined knowledge to their specific regions, soil types, varieties, and management practices. This knowledge can be used to adjust the risk categories to the situation. These agronomists are also well positioned to extract value out of enhancements to the service.

Many of the PREDICTA Pt samples are collected to assess pre-plant risk for specific diseases and have become a routine part of the growers' program to assess, monitor and manage soilborne disease risks. This has been driven by the positive experiences of agronomists/growers, and increased knowledge and activity generated by the research project.

Also, the new testing options and support offered to agronomists and agribusinesses conducting on-farm trials and investigations has showcased the power of the technology to inform decision making and practice change.

Examples of grower benefits realised through using PREDICTA Pt include:

- To improve the percentage of seed crops meeting certification by avoiding paddocks where PREDICTA Pt had detected a risk of powdery scab.
- To avoid growing highly susceptible fresh market varieties in paddocks with risk of powdery scab. Growers of these varieties had previously lost crops because powdery scab.
- To assess risk of black dot to schedule paddocks, planting time and variety selection in combination with disease management practices, to lower overall risk of black dot.
- To decide if nematicide should be applied to control root knot nematode. High cost of treatment is justified when the risk is substantial.
- To monitor root knot nematode decline in fallow to determine when it is safe to plant.
- To decide when to apply soil treatments to control verticillium wilt on properties where the disease had reduced yields to uneconomic levels in previous crops. Testing identified other paddocks with high levels inoculum, where crops performed well in treated areas compared to untreated strips.
- To assess inoculum levels in paddocks on newly purchased and/or leased land where history of disease is uncertain, on occasions avoiding paddocks with unmanageable disease risk.
- To monitor pathogen levels and inform decisions within seed production operation, assisting to deliver to customer needs.
- To monitor root lesion nematode before and after rotation crops. Testing identified rotation crops that were increasing population of root lesion nematode and these were replaced with better options.

Throughout the project new tests and knowledge have been used as research tools to assess industry trials and problem solving. Between 200 and 500 samples per year are submitted to PREDICTA research for these purposes and sample types include soil, peel, root, and stem samples.

Examples of industry research use of the technology to support growers include:

- Selecting paddocks and seed lots with suitable levels of inoculum to undertake disease management trials – reducing likelihood of not getting result, and to identify presence of inoculum of pathogens that may adversely impact on the objective of the trials.
- Assess treatment effects on pathogen levels in soil, root systems and tubers, for example black dot and powdery scab on fresh market tubers.
- DNA testing peel samples to support observations of disease incidence and the effects of varying treatments, for example black dot and silver scurf.
- Quantifying infection levels of pathogens in root and peel samples from plants with non-specific symptoms, for example root lesion nematodes.
- In-crop testing to monitor changes in pathogen activity, for example *Phytophthora erythroseptica*.
- Pathogens present in poor performing areas of crops confirmed by testing soil and root samples, avoiding misdiagnosis and implementation of costly, but ineffective management strategies.
- Investigating difficult to diagnose causes of tuber symptoms that can be associated with numerous pathogens or non-pathogen related soil conditions. In some cases, testing has identified the probable cause, whereas in other cases it has changed the focus of investigation to other potential causes.

It is too soon to assess industry benefits associated with delivery of the peel testing service, new tests for pathogens that cause silver scurf and pink rot, development of disease risk categories for tests previously reported as tests under evaluation and updated PREDICTA Pt manual. There is considerable scope for industry benefits from PREDICTA Pt to increase over the next five years.

Monitoring and evaluation

This project has achieved its major objectives to enhance the value of PREDICTA Pt to growers to assess preplant risk of soilborne diseases and to develop and deliver a similar service for testing seed tuber peel.

New tests

Two new assays were developed, one for *Phytophthora erythroseptica, P. cryptogea* and *P. drechsleri* which cause pink rot and one for *Helminthosporium solani* which causes silver scurf. These assays have been calibrated to test soil and peel samples. Both are available via PREDICTA Pt, PREDICTA Pt Peel and potato research services.

Silver scurf is primarily caused by inoculum on seed tubers. Disease risk thresholds were developed using new field data obtained by this project and retesting stored DNA from project PT09023. The *Phytophthora* test detected infection in all pink rot samples tested in Tasmania and from other states where pink rot occurs. Unfortunately, preplant inoculum levels in soil below the detection limit of the test can cause disease. However, when inoculum was detected the infected areas were three times more likely to develop over 10% incidence of pink rot. To increase detection rates, sampling intensity and sample enrichment (using lupin seedlings) were investigated. Both were beneficial, but the sampling intensity required may not be economic to implement while enrichment requires further refinement to provide consistent results.

PREDICTA Pt Peel

The key to developing PREDICTA Pt Peel testing service was development of a simple robust method to preserve the sample during transport to SARDI. A range of options were considered, the best and simplest uses desiccants to absorb the moisture.

The peel service was launched with disease risk categories provided for rhizoctonia, common scab and silver scurf.

Enhancing value of PREDICTA Pt

Five new tests have been added to PREDICTA Pt taking the total number of tests reported to 17, and the number of tests reported with disease risk categories has increased from 3 to 5 for soil samples.

Population density categories were developed for other tests, which are used to benchmark soil results to other potato paddocks and seed peel results to other seed lots.

New data and a greater focus on seed inoculum has increased confidence in test interpretation, particularly for pathogens causing rhizoctonia disease and common scab. This knowledge has been incorporated into the PREDICTA Pt manual.

To improve the uptake of PREDICTA Pt nationally, tests were evaluated in at sites in WA, NSW, and QLD. With exception of WA most validation activities were done by local agronomists. In general, the data generated was not as detailed due to the commercial pressures on both growers and agronomists at planting and harvesting. On the positive side agronomists get direct experience with the sampling processes and data generated, making implementation of practice change and/or future testing more likely. They are also able to provide feedback on practicality of protocols, along with views on how testing will be utilised.

An additional positive outcome of evaluating tests in a broader range of regions has been the identification of the need to add extra tests. For example, leak rot caused by *Pythium* spp, was identified as being important by growers in NSW and QLD. As a result, an existing assay for *Pythium* clade I (not developed in this project) was added to the PREDICTA Pt service. This assay has been partially validated by this project, but further work is recommended, especially for the crisping sector.

Training

The PREDICTA Pt service is made available to growers through accredited persons. Training courses were delivered to any person in or servicing the Australian potato industry wishing to access the PREDICTA Pt service. The one-day

training workshops are well received and are meeting client's expectations. Incremental changes have been made to this workshop based on feedback from participants at each workshop and to reflect progression of knowledge about tests, sampling and interpretation.

Knowledge and enhancements to the service have been incorporated into an updated PREDICTA PT soilborne disease manual, including a description of the service, soil and peel sampling requirements and how to use test results. Basic biology, key risk factors, broad management options and use of PREDICTA Pt testing are detailed for 11 diseases (previously 3). These additional chapters address feedback from agronomists indicating a need for more information on pathogens reported in the tests under evaluation section of the PREDICTA Pt report. While the focus is on pathogens for which disease risk is indicated, use of results for the other tests is increasing.

Access to accredited agronomists

Most growers in the main southern production areas have access to local accredited agronomists to undertake PREDICTA Pt testing. In response to grower requests, contact details for accredited agronomists who have agreed to be publicly listed are now maintained on the PREDICTA Pt website. In total 108 people have been accredited to deliver PREDICTA Pt of which 55% have submitted samples.

Growers located in other areas of Australia are not as well serviced. Accreditation of 9 agronomists during this project within WA, NSW and QLD has improved access in some regions. There are still a significant number of producers outside the main production areas that do not have an accredited agronomist in proximity. Post workshop feedback reinforces that participants want face to face training. The interaction with other participants at the workshop is a valuable part of the workshop experience, with group sizes of 10 to 15 participants working well. Convincing and getting agronomists who only service one or a few potato growers as part of their diverse overall activities to travel for PREDICTA Pt training is challenging. Support is provided to facilitate testing if requested by these growers, but more flexible options for accreditation are needed, such as on-line training.

Project staff engaged with the industry communication and extension program where appropriate to assist with uptake of the technology and foster practice change using outputs of this project.

Activities such as field validation and trials have built industry awareness around using PREDICTA Pt. When growers and agronomists start to use or consider using PREDICTA services, guidance is often required to build confidence. Without this support adoption of the technology would be much lower.

Research use of technology

Use of the technology for research has traditionally been driven by industry funded projects. This project has fostered increased testing by agribusinesses through increased awareness and wider recognition of the value of quantitative data that PREDICTA assessments provide.

In crop tissue testing

In previous projects, testing in-crop samples was focused primarily on tuber symptoms. Exception being the testing of rhizoctonia symptoms to determine which *Rhizoctonia solani* anastomosis group(s) were present.

In this project in-crop DNA testing of stem, root and developing tuber samples assisted in validation of DNA assays for pathogens that cause potato early dying. This expanded interpretation of disease risk from being solely based on tuber symptoms to also include potential for loss in total yield.

In-crop testing has proven to be a powerful technique for investigating disease symptoms caused by pathogen complexes and is particularly useful for monitoring pathogens such as root lesion nematodes where visual assessment is often of limited value. Agribusinesses have recognised this and are now using it to underpin observations in trials.

Tissue testing has highlighted misdiagnosis of symptoms on potato plants and tubers. Incorrect diagnosis can lead to the application of unnecessary treatments and failure to control the problem. As an example, peel testing has highlighted inconsistencies in the visual diagnosis of silver scurf (caused by *Helminthosporium solani*) and black dot (caused by *Colletotrichum coccodes*). Visual symptoms can vary between varieties and growing conditions and are not always easily discernible.

Incubation followed by microscopic examination is the traditional method of disease diagnosis. PREDICTA Pt does not replace traditional diagnostic services, however by quantifying 17 pathogens in a sample, it is a useful tool to aid in diagnosis of symptoms.

Results of PREDICTA research testing has provided a valuable learning experience for users of the service.

Conclusion

This project has provided a strong basis for the potato industries to improve management of soilborne diseases. The DNA testing services are now available across Australia and adoption is increasing. Support for agronomists and growers is important to build confidence and underpin continued uptake of the technology.

Recommendations

PREDICTA Pt services

Build agronomist confidence to use the PREDICTA Pt services to assist growers to minimise losses from soilborne and seedborne disease by:

- Measuring inoculum levels to assess disease risk.
- Monitoring to understand pathogen loads in different cropping systems.
- Make better informed decisions to manage the risk of soilborne diseases.

PREDICTA research service

Recommended that researchers use this service to enhance outcomes for the potato industry by:

- Evaluating impact of management practices on pathogen levels.
- Testing plant samples to investigate causes of disease.
- Improving outcomes and knowledge gain from on-farm trials.

Training and awareness

Publicise project outputs through the industry communication program including:

- Enhancements to PREDICTA Pt soil testing service.
- Introduction of PREDICTA Pt seed peel testing service.
- Reasons why growers should use the service and benefits being achieved.
- Importance of adequate sampling when using PREDICTA Pt.
- Productivity losses occurring from verticillium wilt and what to look for.
- Understanding risk of common scab, rhizoctonia disease and silver scurf from seedborne inoculum.
- Importance of correct diagnosis of symptoms.

Build agronomist network for delivery:

- Continue PREDICTA Pt workshops so growers have access to trained and experienced agronomists.
- Develop on-line version of PREDICTA Pt training to increase access for agronomists and growers that find it difficult to attend training courses.
- Ensure existing accredited agronomists are kept up to date, including on outputs from this project.

Future Research, Development and Extension

Areas identified by this project where knowledge or control options are lacking include:

- Lack of cost-effective options to reduce productivity loss from verticillium wilt and black dot.
- Limited information on the impact of seed infection on vigour for *Verticillium dahliae*, *Meloidogyne hapla*, *Pratylenchus* spp.
- Evaluate *Pythium* clade I test for assessing the risk of leak rot, including investigating if specific assays for *Pythium ultimum* or *Pythium aphanidermatum* are required.
- Develop pathogen enrichment techniques to improve detection of *Phytophthora erythroseptica*.
- Evaluate if *Macrophomina phaseolina* is impacting productivity or tuber quality.

Refereed scientific publications

Journal article

None to report.

Whole book

Rettke, M.A., 2021. Potato soilborne diseases risk management. Ag Communicators, SARDI. 409pp.

Rettke, M.A., 2021. Testing for soilborne and seedborne pathogens - Potatoes. Ag Communicators, SARDI. 40pp.

Chapter in a book or Paper in conference proceedings

Chitrangi, R., Tegg, R., Rettke., M., Wilson C., 2019. Pink rot of potato, a re-emerging problem in Tasmania: isolate diversity, fungicide resistance, pathogenicity & population dynamics. *EAPR Pathology & Pests Symposium*, Switzerland, 2 September 2019.

References

Atallah, Z, Stevenson, W.R. (2006) A methodology to detect and quantify five pathogens causing potato tuber decay using real-time quantitative polymerase chain reaction. Phytopathology 96(9): 1037-45.

Cullen, D.W, Toth, I.K., Boonham, N., Walsh, K., Barker, I., and Lees, A.K. (2007) Development and Validation of Conventional and Quantitative Polymerase Chain Reaction Assays for the Detection of Storage Rot Potato Pathogens, *Phytophthora erythroseptica*, *Pythium ultimum* and *Phoma foveata*. Journal of Phytopathology. 155(5): 309-15.

Johnson, D.A., and Cummings, T.F. (2015) Effect of Extended Crop Rotations on Incidence of Black Dot, Silver Scurf, and Verticillium Wilt of Potato. Plant Disease: 99:257-52.

Orlando, V., Grove, I.G., Edwards, S.G., Prior, T., Roberts, D., Neilson, R., and Back, M. (2020) Root-lesion nematodes of potato: Current status of diagnostics, pathogenicity and management. Plant Pathology 69(3):405-17.

Ophel Keller, K. (2015) Diagnostic tests for soilborne pathogens – International collaboration. Horticulture Australia, Final Report PT09023.

Rettke, M.A. (2019) Improved soilborne disease diagnostic capacity for the vegetable industry. Hort Innovation Final Report VG15009.

Rodriguez, D.A., Secor, G.A., Gudmestad, N.C., and Franel,L.J. (1996) Sporulation of *Helminthosporium solani* and infection of potato tubers in seed and commercial storages. Plant Disease. 80:1063-70.

Sparrow L. (2015) Six years of results from a potato rotation and green manure trial in Tasmania, Australia, Acta Horticulturae, 21-24 October 2013, Santiago, Chile, pp. 29-35.

Tegg, R. S., Corkrey, R., Herdina, H., McKay, A. C., Crump, N. S., de Boer, R. F., Wiechel, T. J., and Wilson, C. R. 2015. Modeling pathogen DNA content and visual disease assessment in seed tubers to inform disease in potato progeny root, stolon, and tubers. Plant Dis. 99:50-57.

Intellectual property, commercialisation and confidentiality

Intellectual property was generated in this project relating to the calibration of disease risk thresholds in potato crops for new and pre-existing pathogen DNA tests. This information has been made publicly available for the benefit of the Australian potato industry.

Background intellectual property for the quantification of soil-borne organisms using quantitative PCR is owned by SARDI on behalf of IP partners. The background IP includes the DNA extraction from soil samples and technology related to quality assurance.

DNA testing (Potato test panel) is delivered through SARDI's PREDICTA research testing services to researchers and agronomists accredited to deliver PREDICTA Pt.

Providing commercial services (such as the existing PREDICTA Pt and PREDICTA B) for pre-plant risk assessment of soil-borne disease is an important part of the delivery of research outcomes to industry. SARDI is committed to operating these services via a network of accredited persons, to quality control advice, so growers have access to the technology at critical times.

Even at a high level of adoption within the Australian potato industry the number of tests that would be conducted is relatively low (100's to several 1000 samples per year), however the benefits to the industry from this testing are substantial.

Acknowledgements

We would like to thank the growers across Australia who allowed us access to their properties to undertake the field validation work, as well as for their contribution of time, expertise and resources. The assistance of agronomists and various service providers to collect and assess samples is also acknowledged. Several growers hosted specific trials and we are especially appreciative of the assistance they provided. During the project other growers and industry personnel have generously provided their time and expertise to assist the project.

We thank our collaborators on the project, Dr Robert Tegg and Dr Calum Wilson from the University of Tasmania.

The project leader would like to acknowledge the guidance of Barbara Hall, Dr Alan McKay and Dr Daniele Giblot-Ducray, the research contribution of Dr Mohsen Khani and the technical assistance of Russell Burns, Nigel Percy, Ian Bogisch, Sue Pederick, Matt Rowe, Han Chow, Dr Herdina and Ina Dumitrescu (SARDI).

Thanks to Dr Steve Milroy from Murdoch University, Rachel Lancaster from Environmental and Agricultural Testing Services and John Duff from Queensland Department of Agriculture and Fisheries for assisting with field validations.

We gratefully acknowledge the funding and support from the Fresh Potato and Processing Potato Industries and the Commonwealth Government through Hort Innovation.

Appendix 1: Supporting information

Development of peel testing service

Service Development

Systems, framework and underpinning technology established for the peel testing service were built on experience of operating the PREDICTA Pt soil testing service since 2013.

Peel sample integrity

Previously, sample deterioration in transit to the laboratory was a problem.

To develop a protocol for sampling and handling of potato peels for commercial testing services, methods were desktop studied or evaluated in full. Focus was on the preservation of peel samples to stabilise pathogen DNA levels in the sample during movement from the collection point (anywhere in Australia) to SARDI's laboratories. Options considered included packing the sample with moisture absorbent material to reduce water activity of the sample, adding powders to modify pH of the sample, modification of the atmosphere surrounding the sample (oxygen scavengers and vacuum packaging), immersion in a preserving solution, and simplified drying procedures.

Four methods were evaluated to preserve sample integrity.

- Drying prior to posting; robust but not convenient for most agronomists.
- Preservation with powders; chemicals tested were ineffective.
- Preservation in solution; works but not practical.
- Addition of desiccant bags to absorb moisture during transit; preferred option.

Above systems were tested using peel samples from potatoes from long-term storage with high natural fungal and bacterial inoculum loads. Peel taken from this type of sample are most prone to deterioration. Samples were prepared and held for 7 days at room temperature (22°C) before examination for sample deterioration and DNA testing for comparison against immediate drying at 40°C.

The system developed using desiccants allows clients to prepare and dispatch samples on the same day. It uses 4 calcium chloride based desiccant packs (125g each) attached to the inside of a press seal bag. Peel (approximately 200g) is placed within a porous paper bag wedged in between the desiccant packs and the press seal bag closed for shipment. System is designed to remove sufficient moisture to reduce pathogen growth.

Drying immediately after peel preparation is the most effective technique to stabilise samples. Once dry, samples are stable even under challenging temperature situations. Investigation into low cost (under $200) drying (or partial drying) systems has confirmed off the shelf equipment demonstrated adequate temperature control to dry samples placed in high wet strength sampling bags within 24 hours. The stabilised samples are then placed in secondary bio-secure packaging for shipment via courier or the express post system. Consultation with potential clients highlighted their reservations in using this system. Reasons included that process must be completed over several days (high preference for sample preparation to be single operation), requires equipment that has limited other uses and may not be at location where required. It is considered impractical for use by clients intending to submit occasional low volumes of samples.

Addition of powder or moisture absorbent substances to the peel samples was tested first, due to the ease of use by the sampler. None of the added substances provided adequate protection against sample breakdown under conditions that could be expected to occur during transport, while not compromising the sample for pathogen DNA testing.

A liquid preserving solution was formulated in consultation with a food technologist. This nontoxic food grade solution preserved samples as long as complete coverage of the peel was achieved. This system was not practical, posed additional challenges to process with established validated sample preparation protocols, and with the constraints and requirements that apply to sending liquids through postal services.

Low-cost vacuum sealing equipment is available, and samples can be packaged immediately after peeling with

samples handled individually to minimise chances of cross contamination. These advantages make it a simpler and more efficient system than drying. However preliminary testing of vacuum packaging found it was ineffective, with high levels of bacterial decay occurring in some samples.

The developed system using desiccant bags allows clients to dispatch peel samples immediately after sampling and is the preferred method for submission of PREDICTA Pt peel samples by accredited agronomists.

Some clients who submit high volumes of peel samples will have the capacity to dehydrate the samples, accumulating them before dispatching in batches for testing, which is also an acceptable method.

Prototype service testing

PREDICTA Pt service is delivered through accredited agronomists. Peel sampling and testing is a completely new process for agronomists to undertake. To evaluate the peel testing service, agronomists who had requested peel testing were provided access to a prototype version of the service, reported via PREDICTA research. This included provision of sample submission bags with desiccants, along with written advice on preparation and submission. Three clients successfully submitted 59 peel samples for testing using the prototype service.

PREDICTA Pt Peel sample kits have been prepared and the methods for sample preparation incorporated into the PREDICTA Pt manual supplied as part of training and accreditation of agronomists to access the service. A training module on the peel testing service has been added to the PREDICTA Pt training course.

Other tissue sample types

The desiccant bag system is also suitable for other tissue samples. Sampling and testing protocols have been developed for stem sections, root systems and developing tubers. Ability to test these sample types provides valuable insight into infection by pathogens where visual assessment is of limited value e.g. assessing infestation levels by root lesion nematodes.

Development of new pathogen DNA tests

Silver scurf and pink rot were identified for the development DNA tests of their causal agents.

SARDI Molecular Diagnostics Centre strategy is to design qPCR assays in gene regions with high copy number, e.g. the Internal Transcribed Spacer (ITS) region of the genome to improve assay sensitivity and use TaqMan Minor Groove Binder (MGB) assay chemistry to improve specificity.

Silver Scurf

Silver scurf of potatoes is caused by *Helminthosporium solani*. DNA was extracted from three Australian isolates of *H. solani*, and the ITS sequenced. The new sequence information was used in addition to that available in public database and along with sequences of closely related non-target species, to design the assay. Test design is available to requesting parties under MTA.

The assay was shown to efficiently detect DNA extracted from three Australian isolates of *H. solani*, and did not detect DNA extracted from related species, including *H. velutinum* and *H. chlorophorae* (the two most closely related species) and *Leptosphaeria maculans*.

To confirm the specificity, the assay was used to assess non-infected and heavily infected tuber samples. Results ranged from below detection for non-infected samples to 509,097 kDNA copies / g dry peel, for a heavily infected peel sample. Infected seed tubers are the main source of inoculum causing silver scurf, not inoculum in the soil. Soils were tested, and *H. solani* levels were below detection in most samples. When detected levels were low, with 19 kDNA copies / g soil being the highest level detected in a pre-plant soil.

Calibration standards and PCR reagents were prepared, and the TaqMan MGB assay for *H. solani* incorporated into SARDI's PREDICTA delivery platform to enable routine assessment of samples.

Pink rot

Pink rot of potato is mainly caused by *Phytophthora erythroseptica*. Two other *Phytophthora* species have been shown to cause pink rot, namely *P. cryptogea* and *P. drechsleri*. A review of the literature found that qPCR assays had already been published for the detection of *Phytophthora* causing pink rot (Atallah and Stevenson, 2006; Cullen et al., 2007), but they did not comply with all of SARDI's criteria. Analysis of information available in public databases showed that *P. erythroseptica*, *P. cryptogea* and *P. drechsleri* are genetically closely related. Therefore, a new TaqMan MGB qPCR assay targeting the ITS was designed to detect all three species. Test design is available to requesting parties under MTA.

The assay was shown to efficiently detect DNA extracted from two Australian isolates of each *P. erythroseptica*, *P. cryptogea* and *P. drechsleri*, and did not detect pure DNA extracted from other *Phytophthora* species, including *P. cactorum*, *P. cinnamomi*, *P. medicaginis*, *P. megasperma*, *P. trifolii* and *P. vignae*.

To confirm the specificity observed with DNA from pure cultures, the assay was used to assess field samples from diverse origins (SA, TAS, VIC and Qld). Results ranged from below detection to 9,000 copies / g sample with most positive samples showing low levels (100-500 copies / g sample).

Calibration standards and PCR reagents were prepared, and the TaqMan MGB assay for *P. erythroseptica*, *P. cryptogea* and *P. drechsleri* incorporated into SARDI's PREDICTA delivery platform to enable routine assessment of samples.

Validation of new pathogen DNA tests

Silver Scurf

Assessment of disease risk

Incidence of silver scurf was assessed from regions where paddocks were mainly grown for fresh market production. Black dot can cause very similar symptoms to silver scurf and microscopic examination or DNA testing is often required for positive diagnosis. DNA levels of *H. solani* were measured on peel from harvested tubers, which indicated there was misdiagnosis between silver scurf and black dot and that it likely occurs in commercial production.

Peel testing of seed tubers indicates that the *H. solani* test provides a useful assessment of the risk of infection of daughter tubers, Figure 1. Data collected over three seasons and across multiple states shows that seed tubers are the main source of inoculum causing infection of daughter tubers. Seed with below detection infection levels of *H. solani* in peel were planted in 15 paddocks. In 11 of these paddocks, infection level of *H. solani* on peel of harvested tubers was very low (< 1 log DNA). The other four paddocks had low to moderate infection levels. Peel samples from tubers with 5% incidence of silver scurf had levels of approximately 3 log DNA *H. solani*. In paddocks where seed with detectable levels of *H. solani* was planted, harvested tubers from 27% of paddocks had infection levels of above this threshold value of 3 log DNA *H. solani*. This compares with no paddocks exceeding this threshold where seed with undetectable levels were planted.

Silver scurf can increase in severity and incidence in storage after harvest, so any level of infection needs to be managed on potatoes placed in long term storage. (Rodriguez et al. 1996). *H. solani* was detected in the peel of daughter tubers from 94 and 73% of paddocks (processing crops) where seed with and without detectable levels *H. solani* respectively were planted. This data highlights the need to assess infection levels of tubers of crops that are being selected for long term storage.

Inoculum of *H. solani* in the soil is known to decline to low levels within 4 years after a potato crop (Johnson and Cummings 2015). Of 363 soil samples tested prior to planting, DNA of *H. solani* was detected in 5% of samples (18 samples). Infection of daughter tubers was tested at 10 of these locations, with DNA of *H. solani* being detected on nine samples, Figure 2. Only one of the infected samples was from a site where *H. solani* on the seed planted was known to be below detection, so it is not possible to draw conclusion on the importance of soil inoculum from this data.

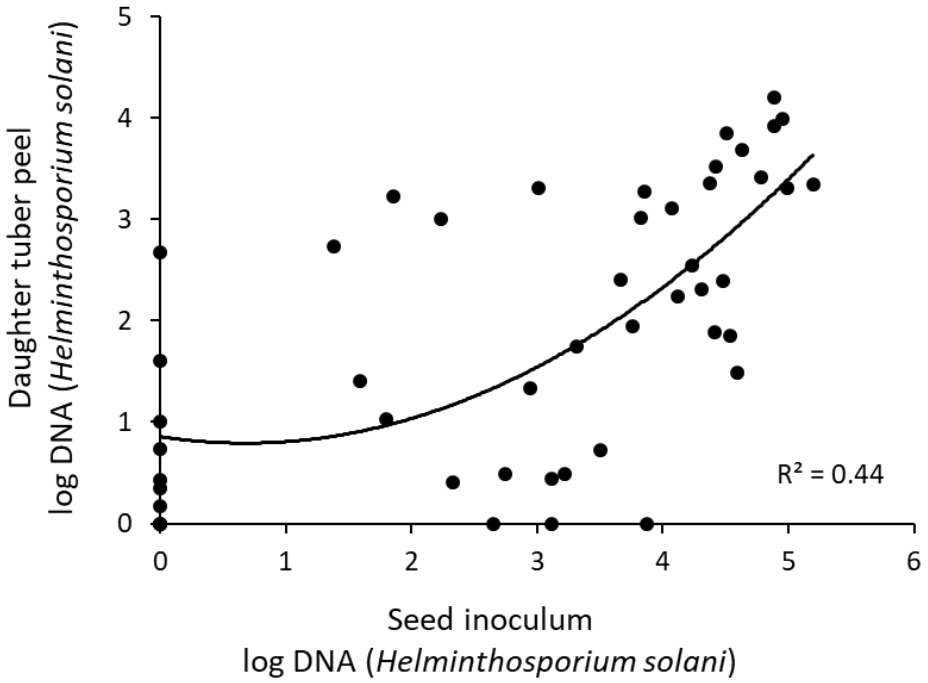

Figure 1: Relationship between inoculum level of *Helminthosporium solani* on seed peel and infection levels of harvested daughter tubers. (Averaged data for paddocks where the same seed was planted in at least two 1 ha areas).

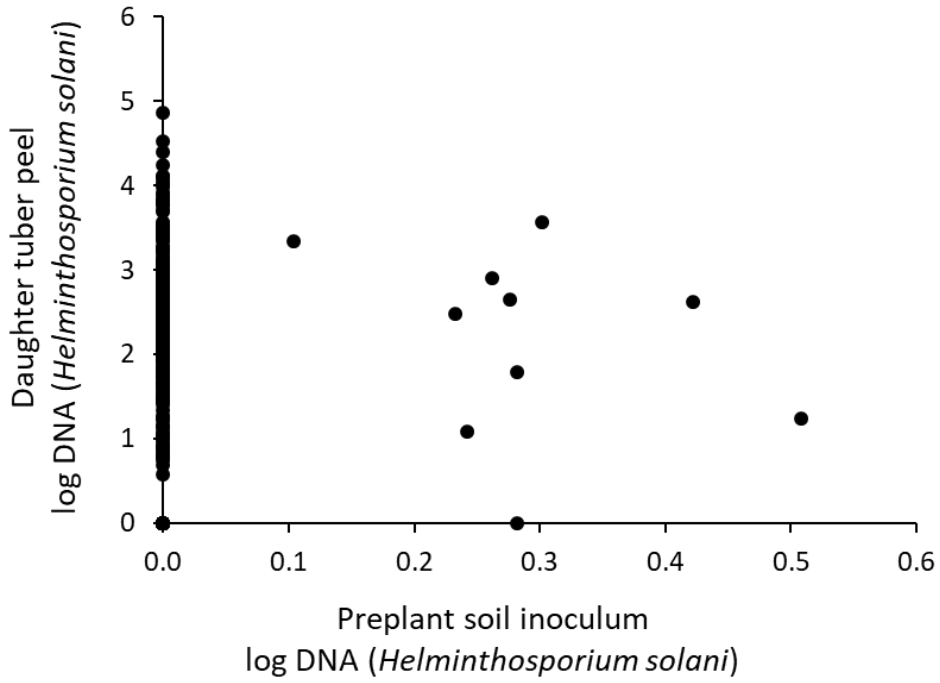

Figure 2: Relationship between inoculum level of *Helminthosporium solani* in soil prior to planting and infection levels of harvested daughter tubers. (Data from 1 ha areas).

Pink rot

Assessment of disease risk

Soil testing was undertaken for *P. erythroseptica*, *P. drechsleri* and *P. cryptogea* (*Phytophthora* EDC) in 1 ha sampling areas over 3 seasons and the incidence of pink rot assessed at harvest. Limited incidence of pink rot occurred at validation sites located outside Tasmania. Visual assessments were supported by DNA testing of tissue and peel samples of harvested tubers. Testing of rotted tubers from Tasmania detected high levels of *Phytophthora* EDC DNA supporting visual observation that symptoms were pink rot. Pink rot was confirmed as the cause of rots in two sampling locations in South Australia. Rots observed on tubers from other regions around Australia were not consistent with pink rot and in some cases confirmed as being caused by other pathogens such as *Pythium* spp. and *Fusarium* spp..

Due to the low incidence of pink rot in other regions, only data from Tasmania are included in the analysis.

Detection of *Phytophthora* EDC DNA in soil samples taken from 1 ha areas prior to planting was associated with an increased risk of pink rot occurring, Table 1. When detected, there was almost double the risk of > 2% incidence and almost triple the risk of > 10% incidence of pink rot.

The data also indicates that inoculum levels below the level of detection still pose a risk of pink rot occurring. One third of sampling areas where *Phytophthora* EDC DNA was below detection in the pre-plant soil sample developed pink rot on more than 2% of tubers assessed at harvest.

While detection of *Phytophthora* EDC indicates an increased risk of pink rot, there is a poor relationship between the level of *Phytophthora* EDC DNA detected in soil prior to planting and the incidence of pink rot on harvested tubers, Figure 3. Using average test results of four soil samples from different 1 ha areas of a paddock to assess risk of pink rot occurring in the paddock was not substantially better than using 1 sample to assess the risk in a 1 ha sampling area, Figure 3 and Figure 4.

Phytophthora EDC DNA was detected on 29% of seed samples planted at validation sites in Tasmania including at 31% of sites where *Phytophthora* EDC DNA was below the level of detection in the soil prior to planting. Infected seed can contribute to disease risk but did not explain the incidence of pink rot at sites where the pathogen was not detected in the soil prior to planting, Figure 5. Infection of seed tubers may have contributed to disease risk at some sites.

Table 1: Incidence of pink rot in 1 ha sampling areas where test for *Phytophthora erythroseptica, P. drechsleri, P. cryptogea* detected DNA in preplant soil samples compared with when not detected. (Data from 104 sampling locations in Tasmania).

Log DNA (Phytophthora EDC)	Percentage of paddocks with pink rot incidence exceeding:		
	2%	5%	10%
Below detection	33	19	12
Detected	59	45	32

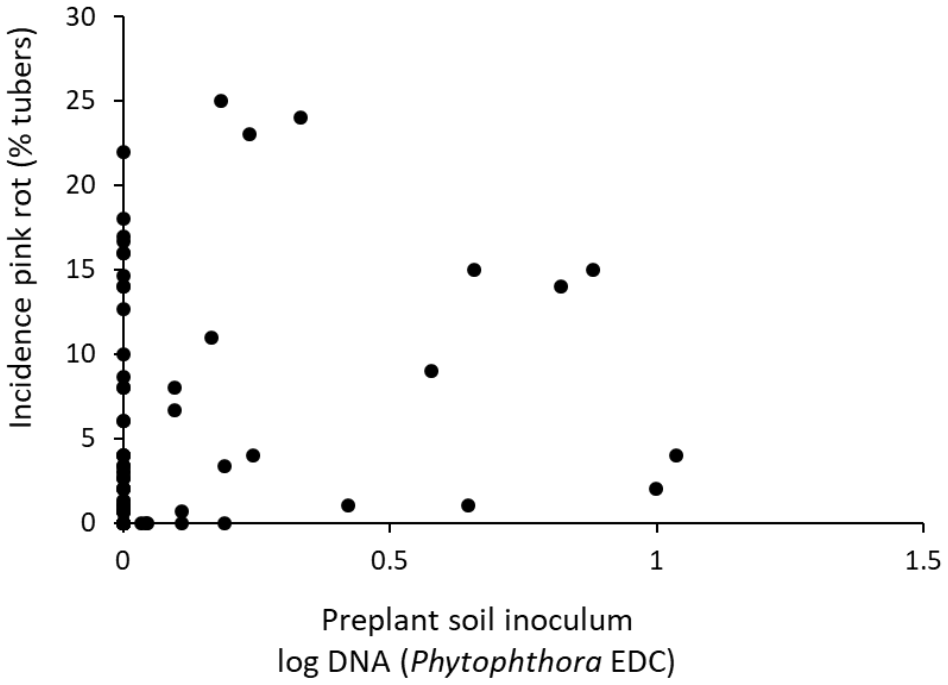

Figure 3: Relationship between inoculum level of *Phytophthora erythroseptica*, *P. drechsleri*, *P. cryptogea* in the soil at time of planting and incidence of pink rot on harvested daughter tubers. (Data: 104 sampling points, Tasmania).

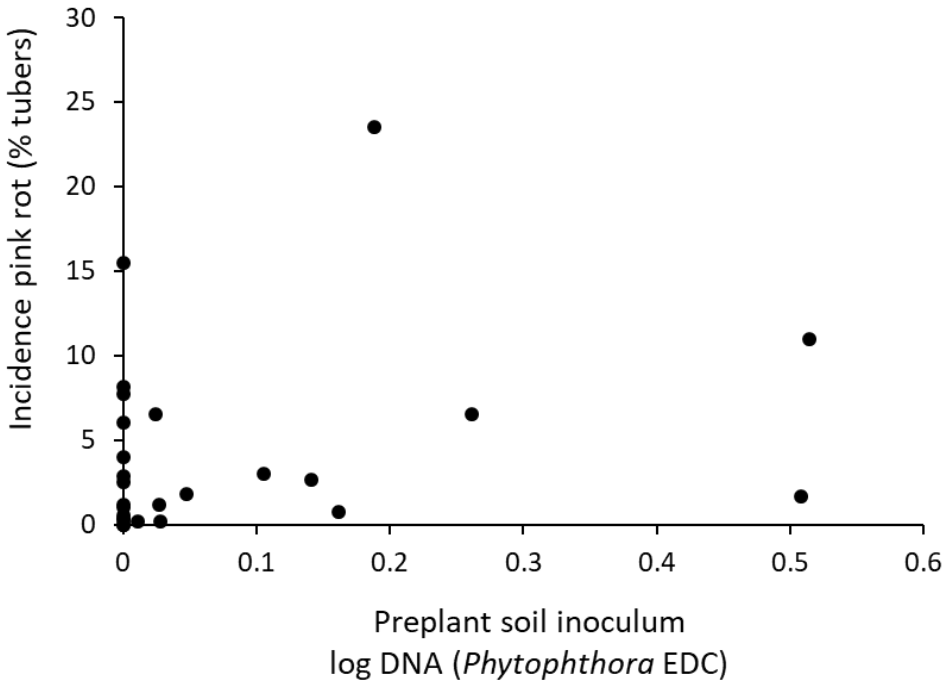

Figure 4: Relationship between inoculum level of *Phytophthora erythroseptica*, *drechsleri*, *P. cryptogea* in the soil at time of planting and incidence of pink rot on harvested daughter tubers. (Data: Average of 4 samples from each 26 paddocks assessed in Tasmania).

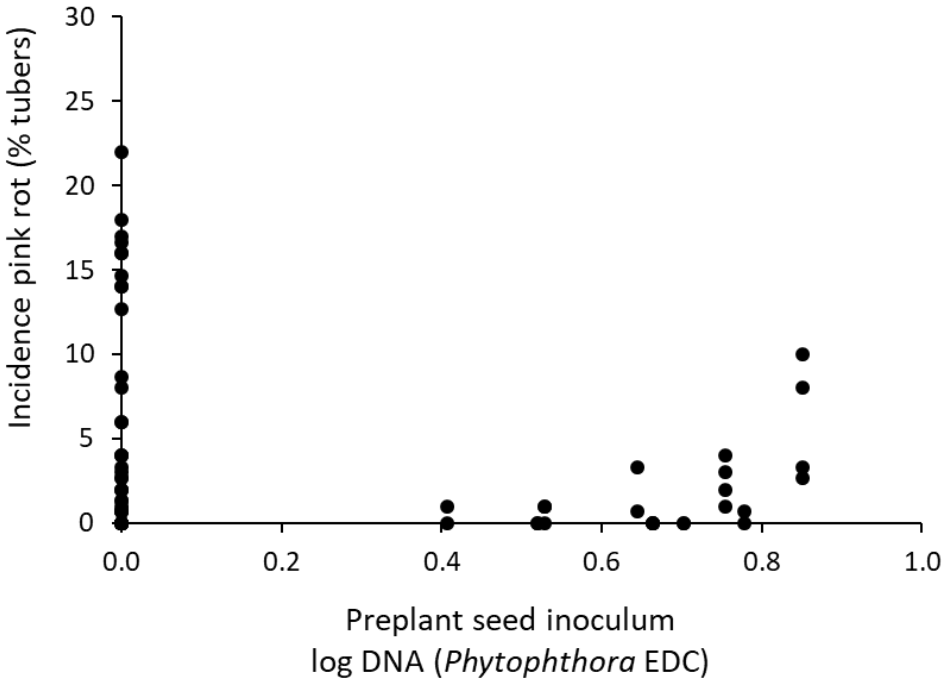

Figure 5: Relationship between inoculum level of *Phytophthora erythroseptica*, *P. drechsleri*, *P. cryptogea* on seed peel and incidence of pink rot on harvested daughter tubers. (Data: 29 seedlots planted at 100 sampling points, Tasmania)

Grid trials

Grid trials were conducted at four centre pivots in South Australia planted with Innovator potatoes. These trials were not targeting pink rot, but growers identified that pink rot may be a risk in these paddocks. *Phytophthora* EDC DNA levels were below detection in all soil samples taken at planting and on peel of seed planted in assessment plots.

Two paddocks had challenging topography that resulted in wet and dry areas developing. In one paddock plots were not located in wet areas; however, additional testing of roots and tubers from wet areas did not find any symptoms of pink rot. In the other paddock, pink rot developed in 9 of 39 plots tested, of which 8 were in confirmed wet areas. No pink rot was observed on tubers or detected in the peel of tubers from the other 30 plots grown under favourable to slightly dry moisture conditions. Productivity was compromised and rots were observed in the plots located in wet spots, irrespective of whether *Phytophthora* spp. were present. Other pathogens such as *Pythium* spp. also cause rots in these conditions. Impact of waterlogged soils resulting in unfavorable growing conditions and development of pink rot on yield are shown in Figure 6.

In the paddock with the most uniform topography and soil moisture conditions, no pink rot was observed, and DNA was not detected in any of the root or tuber samples tested. At the remaining site, where variation in soil moisture levels was evident, no tubers with pink rot were observed, but *Phytophthora* EDC DNA was detected in harvested tubers at 4 of 20 plots.

A further grid trial was conducted in a sprinkler irrigated paddock in Tasmania planted with Ranger Russet potatoes. *Phytophthora* EDC DNA levels were below detection in all soil samples taken at planting and on peel of seed planted in assessment plots. Testing of underground stems and root systems at harvest detected low levels (< 1 log DNA *Phytophthora* EDC) at 4 of 48 plots and no tubers with pink rot were observed.

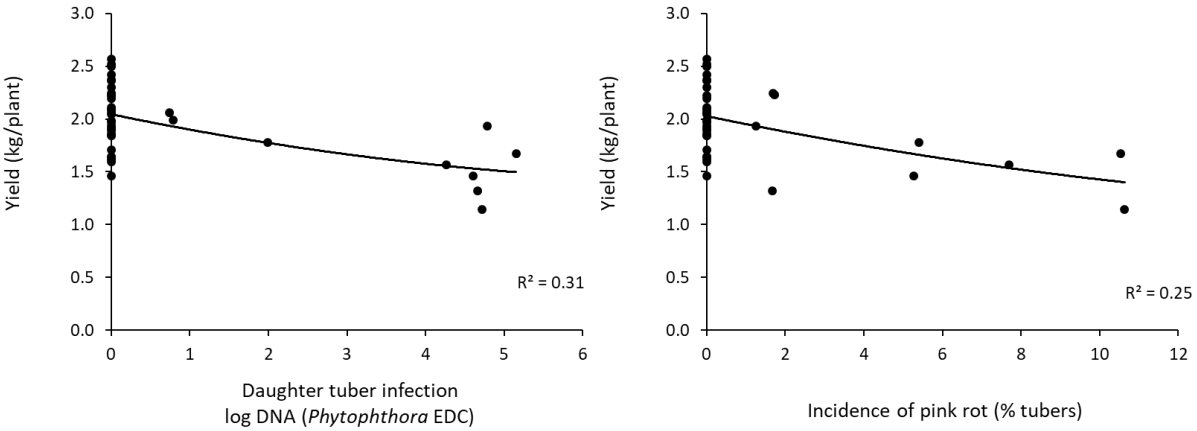

Figure 6: Relationship between infection level by *Phytophthora erythroseptica*, *P. drechsleri*, *P. cryptogea* and yield (left) and incidence of pink rot and yield (right) of potatoes from plots at grid trial site A in South Australia. Plots that developed pink rot were in low wet areas of the paddock.

Variety susceptibility

Three main varieties were planted at validation sites assessed in Tasmania. Russet Burbank, Ranger Russet and Innovator were all susceptible, with multiple 1 ha assessment areas having a pink rot incidence of >10%. Top Cat was planted in one paddock included in assessments, with pink rot incidence of 1-4% recorded in 3 of the 4 1 ha sampling areas, suggesting it is also susceptible.

Decline in soil levels

Soil inoculum levels of *P. erythroseptica*, *P. drechsleri*, *P. cryptogea* were monitored in three paddocks where pink rot had occurred. Inoculum was only detected in pre-plant testing at one site. At each site soil samples were collected using PREDICTA Pt sampling protocols and the *Phytophthora* EDC test was used to monitor the change in pathogen levels, Table 2. The test detected the pathogen in soil at all sampling locations following harvest. With the exception of site I, the levels in the soil were low considering an infected crop had recently been harvested. At site I where high levels were recorded, there were many carryover tubers resulting in high density of volunteer potatoes emerging after harvest and during the following carrot crop. By 3 months, levels at this site had dramatically declined to levels similar to what was detected at the other two sites. The first non-detection at a site occurred at 5 months, with non detection at a second site occurring when sampled at 8 months. The pathogen was still present at these sites, as the *Phytophthora* EDC test detected pathogen at a subsequent sampling time at both locations. At the other four sampling areas the *Phytophthora* EDC test detected pathogen in the soil at all timings up to 12 months, but not at two locations at 16 months after potato harvest.

Table 2: Change in *Phytophthora erythroseptica, P. drechsleri*, *P. cryptogea* levels in the soil monitored at 2 sampling locations in each of 3 sites where an infected potato crop had been grown in Tasmania.

Time after harvest (months)	Soil Inoculum (kDNA copies / g soil (*Phytophthora* EDC))					
	Site G		Site H		Site I	
	Area 1	Area 3	Area 1	Area 3	Area 1	Area 2
Preplant	0	0	0	0	10	9
After Harvest	7.1	1.0	2.1	2.0	106	378
3	1.0	2.1	1.4	2.9	1.5	2.4
4	8.1	2.5	0.3	1.2	0.8	2.8
5	1.9	1.8	0.2	1.4	0	5.6
8	0.5	0.6	0.9	0	0	1.3
12	1.6	0.8	0.3	0	0.1	0.5
16	0.7	0	0	1.8		
Incidence pink rot at harvest (% tubers)	17	13	10	8	4	2

Evaluation of repeated sampling

Seven paddocks with known presence of *P. erythroseptica, P. drechsleri*, *P. cryptogea* inoculum from previous testing or potato crop histories were identified in Tasmania. Sites G and H where the decline in soil levels had been monitored were included. Soil samples were collected using the standard protocol for PREDICTA Pt. Four separate 1 ha areas were each sampled 8 times, giving a total of 32 samples from each paddock. The detection rate, range in level and average level of *Phytophthora* EDC DNA detected in the 8 samples in each 1 ha area are reported in Table 3. This data highlights the challenge of detecting *P. erythroseptica, P. drechsleri*, *P. cryptogea* in paddocks at levels known to pose disease risk. In sampling areas where *Phytophthora* EDC tests detected the pathogen, it was only detected in between 1 and 6 of the 8 samples collected, never in all 8 samples. Based on statistical analysis of the data using a non-parametric approach, 7 samples would be required to have an 80% confidence level of detecting the pathogen if present. To achieve a 95% confidence level of detection would require 12 samples to be tested. Taking less samples per paddock is not adequate, as there is an unacceptable risk of failure to warn of the presence of inoculum in a paddock.

On one occasion a very high detection was recorded (505 kDNA copies / g soil (*Phytophthora* EDC)) suggesting that plant material with an active infection was present in at least one core of this sample. This could be a carryover tuber in the soil or an infected host plant.

Table 3: Detection rate, range and average level of *Phytophthora erythroseptica, P. drechsleri, P. cryptogea* detected by repeated soil sampling (8 times) in the same 1 ha area at 4 locations in each of 7 paddocks.

Paddock	Sampling area	Soil Inoculum (kDNA copies / g soil (*Phytophthora* EDC))		
		Positive detections out 8	Range of levels detected	Average
A	1	2	0.0 – 2.9	0.5
	2	2	0.0 – 0.8	0.2
	3	0	0 – 0	0.0
	4	0	0 – 0	0.0
B	1	6	0.0 – 2.2	0.8
	2	6	0.0 – 1.9	0.8
	3	1	0.0 – 0.7	0.1
	4	3	0.0 – 0.8	0.3
C	1	2	0.0 – 0.7	0.1
	2	1	0.0 – 0.5	0.1
	3	0	0 – 0	0.0
	4	2	0.0 – 1.2	0.2
D	1	0	0 – 0	0.0
	2	4	0.0 –0.8	0.3
	3	1	0.0 – 505	63
	4	0	0 – 0	0.0
E	1	0	0 – 0	0.0
	2	1	0 – 3.5	0.4
	3	0	0 – 0	0.0
	4	2	0.0 – 1.1	0.2
G	1	4	0.0 – 1.7	0.5
	2	2	0.0 – 1.3	0.3
	3	4	0.0 – 1.2	0.3
	4	0	0 – 0	0.0
H	1	3	0.0 – 2.4	0.6
	2	3	0.0 – 1.4	0.4
	3	4	0.0 – 1.8	0.5
	4	4	0.0 – 0.7	0.3

Controlled environment experiments

Controlled environment experiments were conducted in Tasmania. A ferrosol soil was sourced from a non horticultural site for the pot trials. DNA testing using the *P. erythroseptica*, *P. drechsleri*, *P. cryptogea* assay did not detect the pathogens that cause pink rot at this site. Soils were inoculated with *P. erythroseptica* infected millet seed at rates of 0, 10, 31, 62, 125 and 250 g per soil batch (8 pots). After 2 weeks settling-incubation period the range of levels achieved were higher (136 to 32307 kDNA copies *Phytophthora* EDC / g soil) than desired, and well above those typically detected in soil samples from commercial potato paddocks prior to planting (0.1 to 5 kDNA copies / g soil). Emergence of Russet Burbank potatoes was reduced and slowed when sown into the inoculated pots. The two highest levels of inoculation resulted in non-emergence of the potatoes. When dug up the flesh of seed tubers had disintegrated just leaving the skin tissue.

A second experiment was conducted to monitor change in levels in soil inoculated with *Phytophthora erythroseptica* infected millet seed with rates as low as 0.02 g per pot. After 4 weeks incubation average levels in pots were 18 kDNA copies / g soil, above those typically detected in soil samples from commercial potato paddocks prior to planting.

These trials demonstrated the strong relationship between level of spiking and quantification of *P. erythroseptica* by the developed DNA test, Figure 7. However, as pink rot was found to occur in fields where inoculum in soil samples was below the level of detection of the *Phytophthora* EDC test, no further pot trials were conducted.

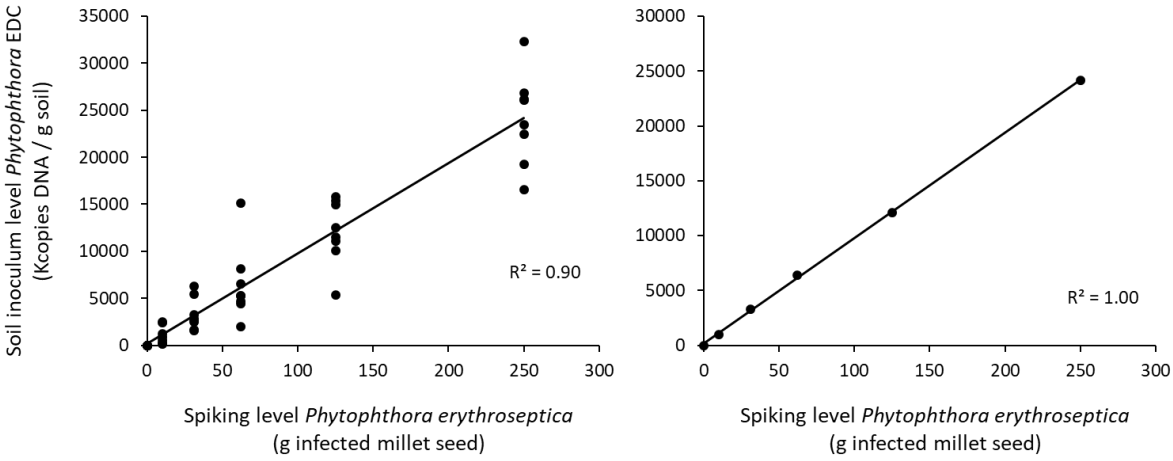

Figure 7: Relationship between amount of millet seed infected with *Phytophthora erythroseptica* added to pots and the level of *Phytophthora* EDC in the soil of individual pots (left) and average of 8 replicate pots (right).

Enrichment of samples

Preliminary trials were undertaken to determine if lupins and broad beans have potential to increase DNA levels of *P. erythroseptica* in soil samples. Bulked soil collected at the time of harvest from the grid trial plots at the site where pink rot occurred in South Australia was used for initial enrichment trials. At the time of harvest the level in soil from infected plots was 14 kDNA copies g / soil *Phytophthora* EDC. Level was below detection at the time of trial setup, after approximately 1 year of storage of moist soil at 7°C. Six samples of 500g dried soil were moistened to saturation and lupins (25g surface sterilised seed) planted into the surface and grown in lidded containers for six days at 25°C. The technique lifted the levels of *Macrophomina phaseolina*, *Pythium* clade I and *Pythium* clade F by 10 to 100 fold, but *Phytophthora* EDC levels were below detection in all samples after the incubation period.

A second series of trials was conducted in Tasmania using freshly collected soil samples from pink rot monitoring sites and soil from an area where pink rot infected potatoes had recently grown. All samples were dried prior to use in trials. In six of these soil samples, *Phytophthora* EDC levels ranged from 3-25 kDNA copies / g soil, Table 4. Following similar technique to that described above with longer incubation times of 10-15 days the *Phytophthora* EDC levels in the six samples ranged from 2 -2477 kDNA copies / g soil and 3-348 kDNA copies / g soil after lupins and broad beans respectively. Broad beans are slower to germinate and grow than lupins, requiring a longer incubation time. When soil was moistened only, average detected levels of *Phytophthora* EDC dropped (range 1-8

kDNA copies / g soil). In soil samples collected from sites where more than 1 year had elapsed since the harvest of an infected crop, results were inconsistent, Table 4. Enrichment of samples allowed detection of *Phytophthora* EDC at two sites where it was not detected prior to enrichment. However, enrichment of samples with detectable levels also resulted in levels below detection.

Moistening soil alone demonstrated limited beneficial effect for improving detection of *P. erythroseptica* with the *Phytophthora* EDC test.

Results using soil samples from where infected potatoes had recently grown demonstrate potential to develop an enrichment technique to improve *Phytophthora* EDC test detection, however, results with samples where potatoes had not been grown for 1-2 years were inconsistent. It is possible the technique is raising the DNA levels in samples where active *Phytophthora* infection is present, but not where resting oospores are present. Further work is required to understand the usefulness and limitations of this technique to improve the capability of the developed *Phytophthora* EDC test to detect inoculum and provide indication of the risk of pink rot occurring.

Table 4: Change in soil inoculum level measured with *Phytophthora* EDC test resulting from incubating soil samples collected from sites where pink rot had occurred in a previous potato crop.

Sample source	Soil Inoculum (kDNA copies / g soil (*Phytophthora* EDC))			
	Initial level Dry soil	Incubated Water	Incubated Lupins	Incubated Broad beans
Infected crop - Recent				
Site 1	5.3	1.2	16.0	46.8
	4.1	1.1	10.1	347.9
	3.2	2.2	2.5	2.6
	2.7	2.2	8.8	113.7
	25.2	8.1	102.6	0.0
	3.5	1.5	2477.2	4.8
Infected crop - 1 year ago				
Site 1	0.0		0.0	
			0.0	
Site 2	0.0	0.0	0.7	
			12.5	
Site 3	0.2	1.0	0.0	
			0.6	
Site 4	0.0	2.5	1.8	
			3.6	
Site 5	4.5	0.4	1.2	
			5.2	
Site 6	2.3	0.6	2.4	
			2.5	
Site 7	1.2	0.6	1.8	
Infected crop - 2 years ago				
Site 1	0.0	0.0	0.0	
		0.9	0.0	
			0.0	
Site 2	0.4	0.0	0.0	
		0.0	0.0	
			1.3	

Other pathogens as indicators

Paddocks with a long history of potato production where drainage and soil conditions make conditions conducive to pink rot are expected to be at highest risk. In Tasmania, high levels of *Spongospora subterranea* inoculum is also likely to build up in these paddocks as both pathogens are favoured by high moisture conditions and repeated potato cropping. In Tasmania high levels of *S. subterranea* inoculum in the soil prior to planting were associated with increased incidence of pink rot, Figure 8. Presence of *S. subterranea* is not required for pink rot to occur. While a high incidence of pink rot may occur in paddocks with low levels of *S. subterranea*, detection of high levels should alert growers that a paddock has an increased likelihood of severe pink rot occurring.

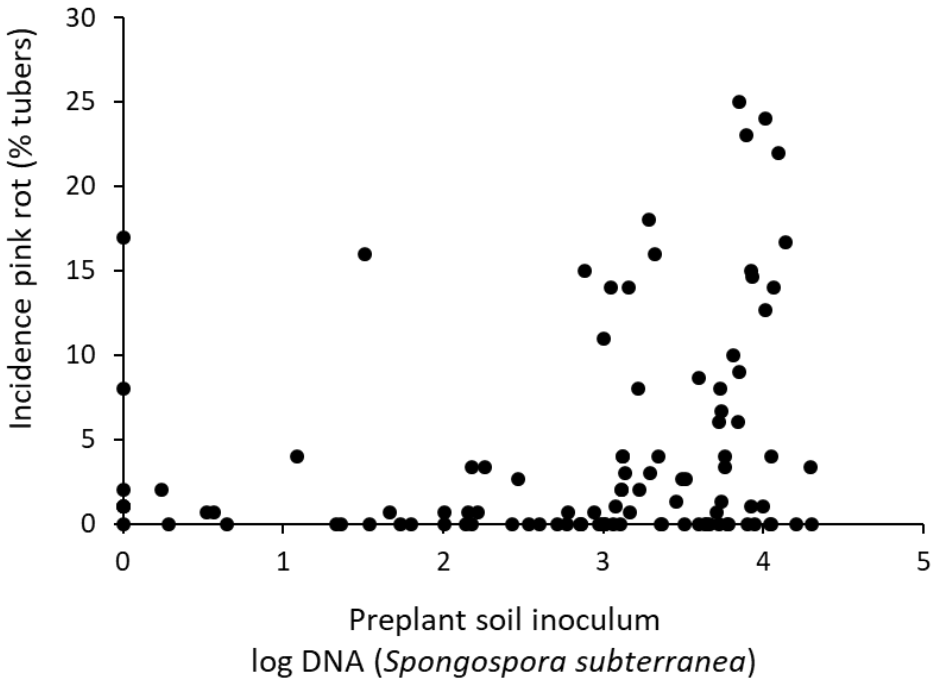

Figure 8: Relationship between inoculum level *Spongospora subterranea* in the soil at time of planting and incidence of pink rot on harvested daughter tubers. (Data: 104 sampling points, Tasmania).

Calibration of tests

Potato cyst nematode

SARDI had pathogen DNA assays available for the detection of golden potato cyst nematode (*Globodera rostochiensis*) which is present in Australia and pale potato cyst nematode (*Globodera pallida*) not known to be present in Australia, prior to commencement of this project. Both tests had been checked for specificity, however only limited evaluation of test sensitivity had been conducted. Spiking experiments were conducted in collaboration with Dr Matthew Back from Harper Adams University, Newport (United Kingdom) to ascertain the detection limit for cysts of both species.

Populations of *G. pallida* and *G. rostochiensis* were isolated at Harper Adams University, identification confirmed by DNA testing at their laboratory. Bulk soil free of both species and samples spiked with known number of cysts were supplied to SARDI. Average egg count per cyst was 218 for *G. pallida* and 214 for *G. rostochiensis*. For each species, five replicate dilution series were produced from the bulked and spiked soil and 500 g samples tested to determine the detection limit of the assays developed by SARDI.

The sensitivity of the test for 50% detection rate was determined as 1.49 ± 0.33 cysts/kg for *G. rostochiensis* and 1.41 ± 0.27 cysts/kg for *G. pallida*. Based on analysis data it is recommended that 2 PCR be conducted on the DNA extracted from each sample, to optimise the chances of detection at low levels.

Powdery scab, black dot, root knot nematode

PREDICTA Pt results for the pathogens that cause powdery scab, black dot and root knot nematode have been reported with an indication of disease risk since 2013. As a part of calibration of other tests, and in regions where validation had not previously been conducted, testing and assessment of disease incidence for powdery scab, black dot and root knot nematode was conducted. Data generated in this project supports previous findings for these pathogens and the risk thresholds established for pre-plant soil testing using PREDICTA Pt.

Environment, variety and management impact the level of root knot nematode, powdery scab and black dot incidence. Growing conditions vary within a region depending on topography, planting time and soil type, and difference can be as great as between regions. As a guide the thresholds for disease risk previously developed are applicable. The level of disease expression (incidence and severity) increases or reduces depending on site specific conditions. Susceptibility of the variety and sensitivity of the market to symptoms on tubers are also important factors in making decisions on what actions should be implemented to mitigate risk.

An indication of disease risk has been added to reporting for the combined test for *Meloidogyne javanica/incognita/arenaria*. This test had already been calibrated against traditional tray extraction tests, documented in Hort Innovation Final Report VG15009 (Rettke, 2019). Analysis of available thresholds from published literature, previous studies in Australia and commercial data indicates that the thresholds established for *Meloidogyne fallax* are applicable to use with the *M. javanica/incognita/arenaria* test. All three species can cause tuber symptoms on potatoes. Symptoms are mostly caused by *M. javanica*, followed by *M. incognita*. Damage from these warm climate species is most common in production areas of Queensland and some areas of Western Australia and New South Wales. *M. javanica* was detected in tubers with galling symptoms from semiarid inland production regions, particularly the Riverland region of South Australia where it caused economic loss. *M. fallax* also causes galling of tubers in main potato production areas of Western Australia and cooler production areas of New South Wales. *M. hapla* is widely distributed but has not been associated with economic levels of tuber galling. It does infest potato root systems and tubers.

Verticillium dahliae and other soilborne pathogens effecting yield

Impact on yield at field validation sites

At validation sites in South Australia where yield was assessed, *V. dahliae* was the pathogen that most consistently produced a downward trend in yield with increasing pre-plant soil inoculum levels. Depending on the variety, average yield reductions of 8-21% were associated with preplant soil inoculum levels of 1.3 log DNA *V. dahliae* when compared to yield from sites where *V. dahliae* was not detected.

There is a high proportion of variation in yield that is unaccounted for when comparing results from validation sites located in commercial crops. Factors such as seed vigour, virus load, nutrition, irrigation and weather events impact on yield at each site.

Detection of high levels *V. dahliae* in the soil prior to planting indicated that a site is likely to have reduced yield potential when growing Innovator potatoes, Figure 9. For this variety no other pathogens were associated with a downward trend in yield with increasing pre-plant inoculum levels. Other pathogens are known to reduce yield and it is likely that they had an impact on productivity at some sites, but not to the extent or as consistently as *V. dahliae*.

For the innovator variety there was a strong relationship between levels of *V. dahliae* in the soil and infection of the daughter tubers, Figure 9. Infection of tubers may not reflect infection of the plant or impact of productivity by *V. dahliae*.

For other varieties where yield was assessed at enough sites to analyse (Ranger Russet, Russet Burbank, Nooksac) downward yield trends were found with more than one pathogen, including with *V. dahliae*. For these varieties the relationship between levels of *V. dahliae* in the soil and infection of the daughter tubers was not as strong as found with Innovator, Figure 9. Ranger Russet and Nooksac are considered to have moderate resistance to verticillium wilt.

Yield decline of the Ranger Russet variety was associated with increased levels of *V. dahliae* (Figure 9), *C. coccodes* (Figure 10) and *R. solani* AG3 (Figure 10) in the soil prior to planting. All sites planted to Ranger Russet with high levels of *V. dahliae* also had high levels of *C. coccodes*, though not all sites with high levels of *C. coccodes* had high levels of *V. dahliae* (Figure 11). In 5 of 6 sites where *V. dahliae* was below detection and high levels of *C. coccodes* were detected in the soil prior to planting, yield was lower than the average yield when neither pathogen was detected, suggesting that *C. coccodes* contributing to reduced yield. *R. solani* AG3 was more frequently detected in sites with high levels of *V. dahliae* (Figure 11) and *C. coccodes* (not shown) that were grown in a short potato rotation (<4 years*). R. solani* AG3 may have been adding to yield losses at these sites.

In the combined data across years for Russet Burbank, there appeared to be no association between reduced yields and high preplant inoculum levels of *V. dahliae,* Figure 9. There were several paddocks with high preplant levels of *V. dahliae* that appeared to escape disease, producing higher than expected yields. These paddocks were in a temperate coastal region and crops were planted early in the season. At remaining sites yield decline of the Russet Burbank variety was associated with increased levels of *V. dahliae* in the soil prior to planting. Yield of Russet Burbank potatoes trended down with increasing levels of *C. coccodes* and *S. subterranea* inoculum in the soil prior to planting, Figure 12. At sites where Russet Burbank was planted the inoculum level of these two pathogens was related to each other ($r^2 = 0.68$), but levels of neither pathogen were strongly related to inoculum levels of *V. dahliae*, Figure 13. Infection levels of plants were not monitored during crop growth to determine which pathogen was responsible for yield loss at validation sites. High levels of *C. coccodes* on harvested tubers were related to high pre-plant inoculum levels and associated with low yielding crops, suggesting that high levels of infection of these crops may have contributed to early plant decline and yield loss, Figure 14. While it is more likely the yield loss is associated with *C. coccodes*, Russet Burbank is susceptible to severe root infection and root galling from *S. subterranea* and this may also have contributed to yield loss. High levels of *S. subterranea* on harvested tubers were related to high pre-plant inoculum levels, but not strongly associated with low yielding crops, Figure 15. As Russet Burbank is moderately resistant to powdery scab development on tubers, level of tuber infection is not a good indication of root infection.

Less sites were assessed for the variety Nooksac. Several sites with high inoculum levels of multiple pathogens were planted. These paddocks not only had poor yields, but also had high levels of root knot nematode, common scab, and deformed tubers, highlighting the risk of planting such paddocks. Low yield was associated with high inoculum levels of *V. dahliae* (Figure 9), *M. fallax*, *P. neglectus*, and *R. solani* AG2.1 (Figure 16) in the soil prior to planting. All these pathogens have the potential to cause and may have contributed to yield loss of Nooksac potato crops.

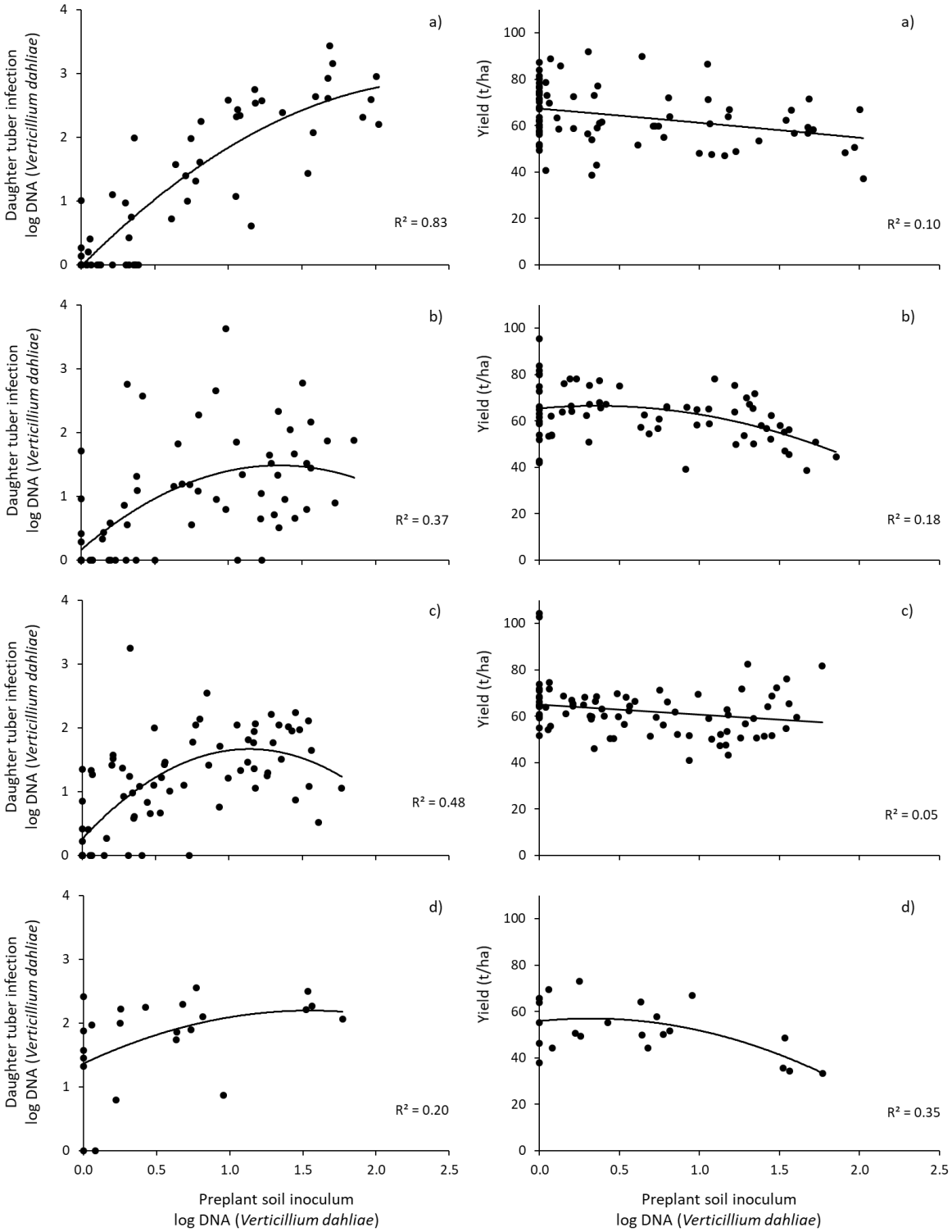

Figure 9: Relationship between inoculum level of *Verticillium dahliae* in the soil prior to planting and (left) infection level of daughter tubers and (right) yield of four varieties a) Innovator, b) Ranger Russet, c) Russet Burbank and d) Nooksac grown in South Australia. (Data from 1 ha areas measured in up to 5 seasons).

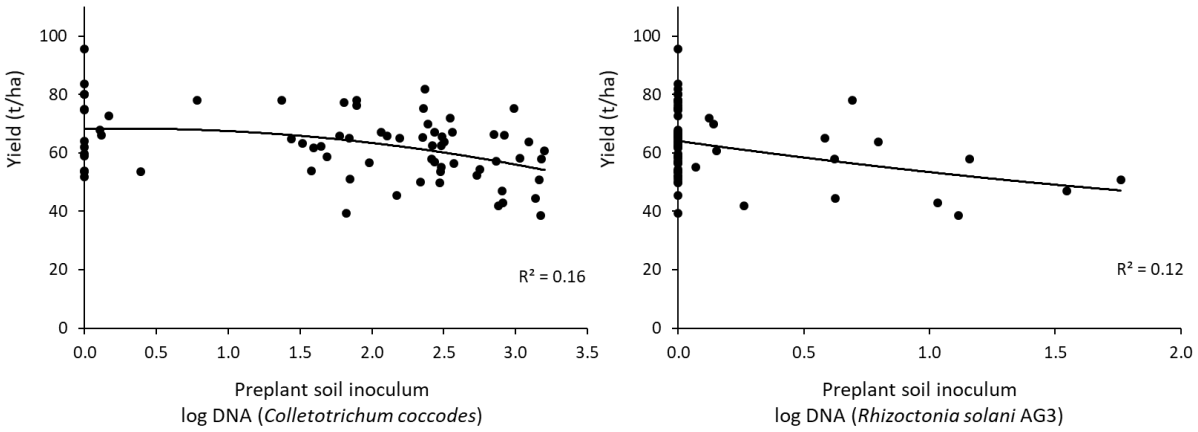

Figure 10 Relationship between inoculum level of *Colletotrichum coccodes* (left) and *Rhizoctonia solani* AG3 (right) in the soil prior to planting and yield of Ranger Russet potatoes grown in South Australia. (Data from 1 ha areas measured in 4 seasons).

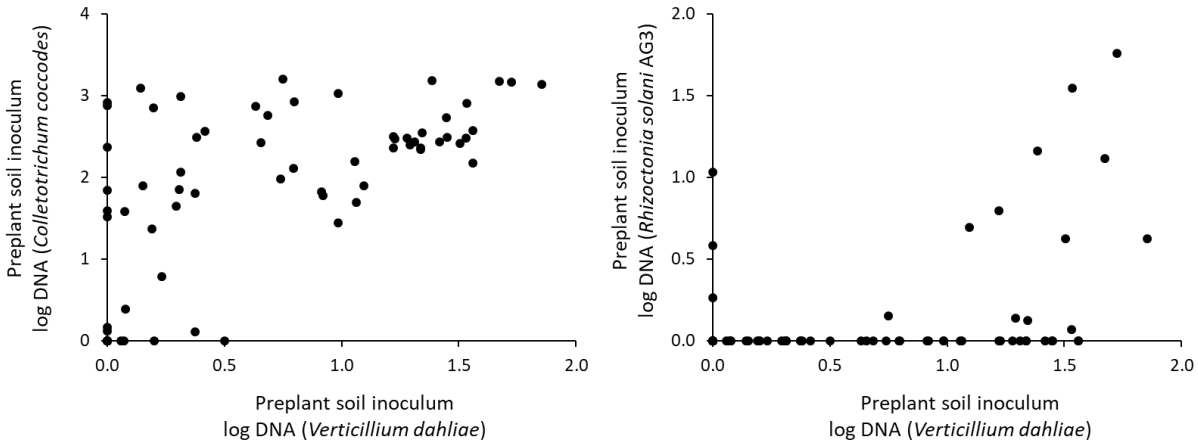

Figure 11: Relationship between inoculum level of *Verticillium dahliae* in the soil prior to planting and the levels of *Colletotrichum coccodes* (left) and *Rhizoctonia solani* AG3 (right) in the soil prior to planting where Ranger Russet potatoes were grown. (Data from 1 ha areas measured in 4 seasons).

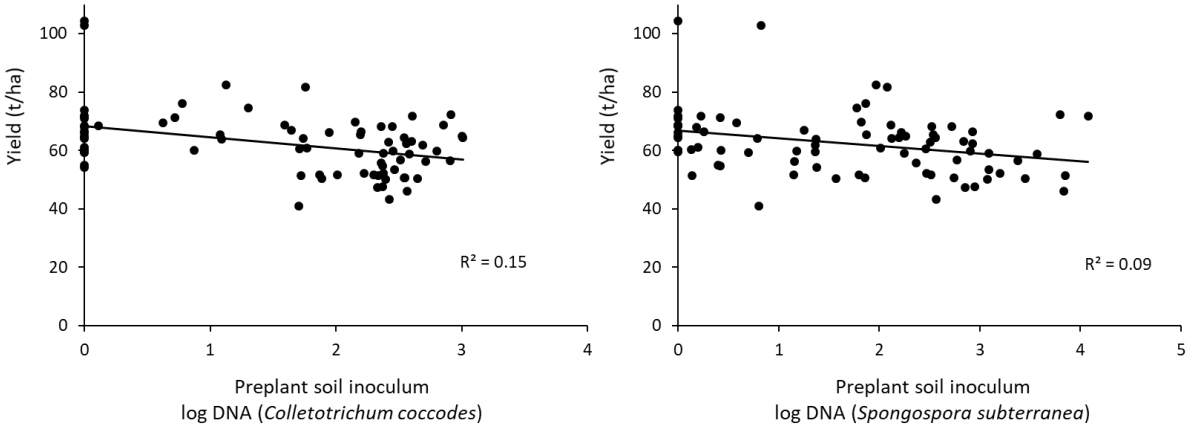

Figure 12: Relationship between (left) inoculum level *Colletotrichum coccodes* in the soil prior to planting and yield and (right) inoculum level of *Spongospora subterranea* in the soil prior to planting and yield, of Russet Burbank potatoes grown in South Australia. (Data from 1 ha areas measured in 2 seasons).

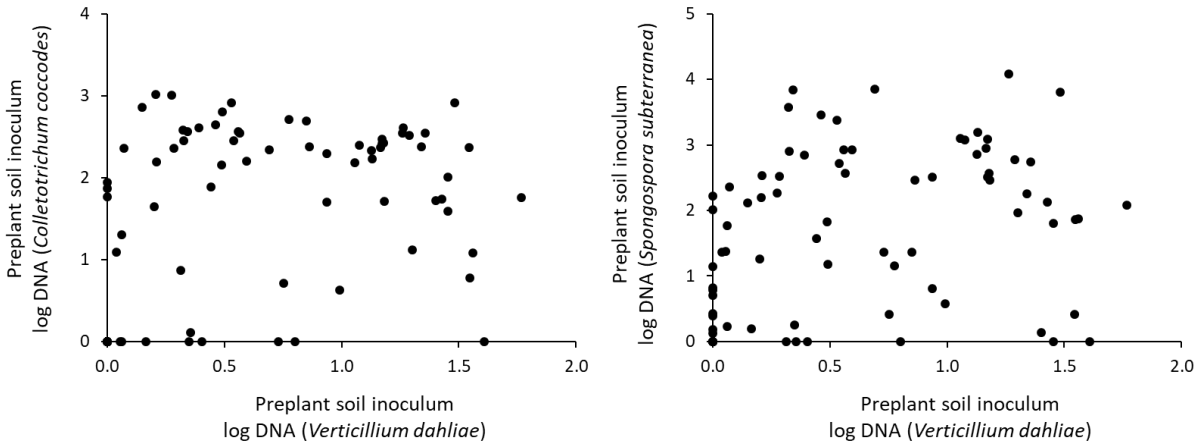

Figure 13: Relationship between inoculum level of *Verticillium dahliae* in the soil prior to planting and the levels of *Colletotrichum coccodes* (left) and *Spongospora subterranea* (right) in the soil prior to planting where Russet Burbank potatoes were grown. (Data from 1 ha areas measured in 2 seasons).

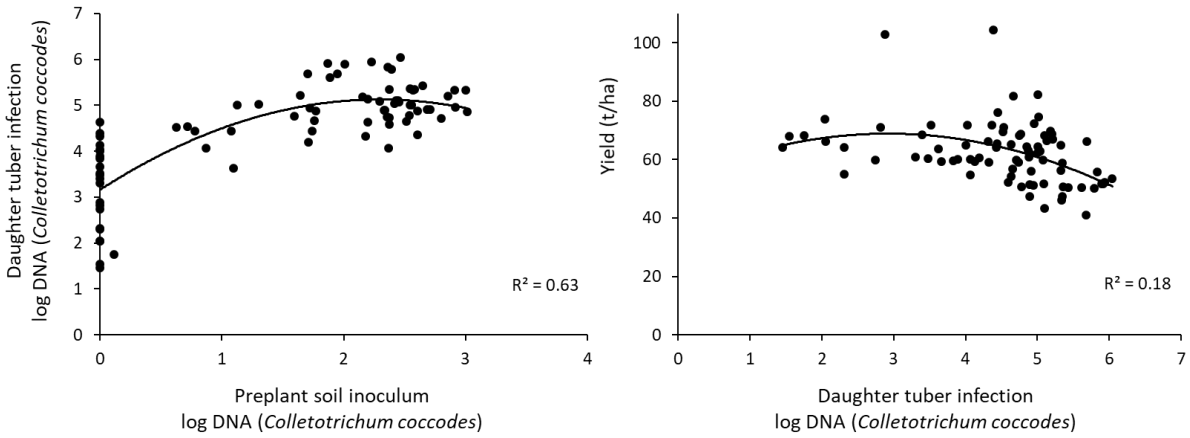

Figure 14: Relationship between (left) inoculum level *Colletotrichum coccodes* in the soil prior to planting and infection of daughter tubers of Russet Burbank potatoes and (right) infection level of daughter tubers by *Colletotrichum coccodes* and yield of Russet Burbank potatoes. (Data from 1 ha areas measured in 2 seasons).

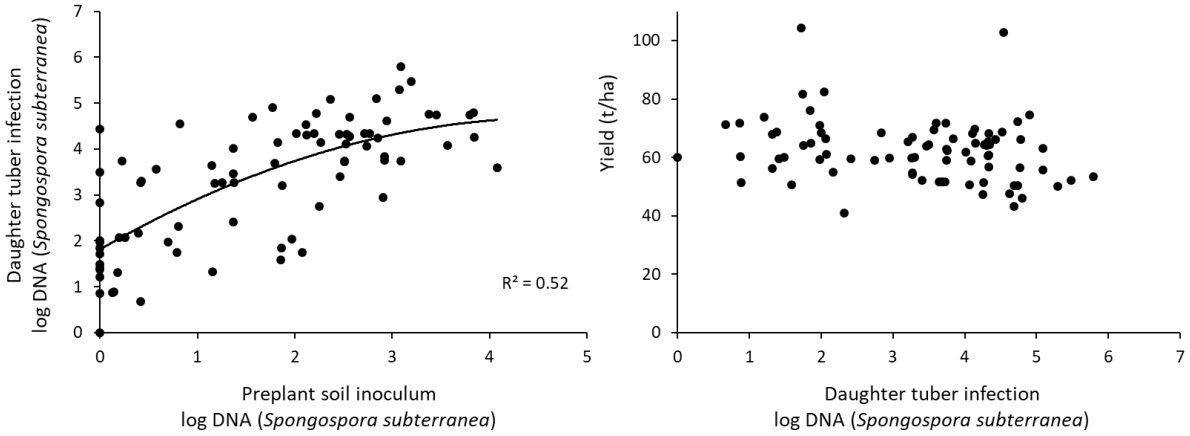

Figure 15: Relationship between (left) inoculum level *Spongospora subterranea* in the soil prior to planting and infection of daughter tubers of Russet Burbank potatoes and (right) infection level of daughter tubers by *Spongospora subterranea* and yield of Russet Burbank potatoes (right side). (Data from 1 ha areas measured in 2 seasons).

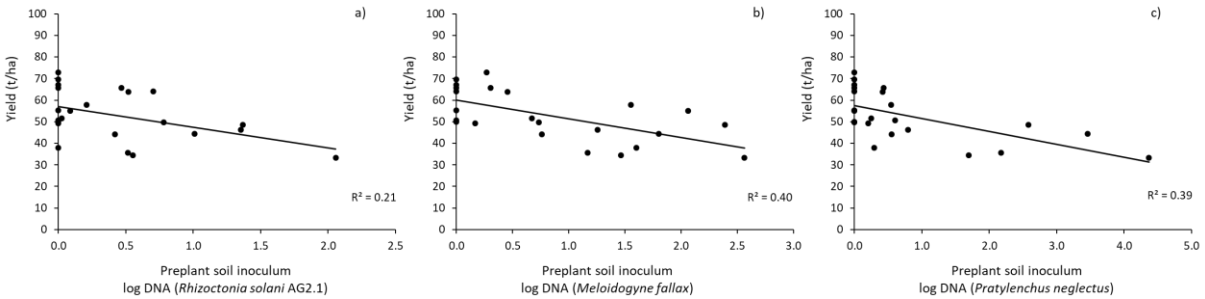

Figure 16: Relationship between inoculum level in the soil prior to planting of a) *Rhizoctonia solani* AG2.1, b) *Meloidogyne fallax* and c) *Pratylenchus neglectus* and the yield of Nooksac potatoes grown in South Australia. (Data from 1 ha areas measured in 4 seasons).

Impact on yield in grid trials (South Australia)

Grid trials were conducted at four centre pivots in South Australia targeting the effect of *Verticillium dahliae* and *Pratylenchus* spp. on the yield of potato variety Innovator. In each paddock 16-40 plots (approximately 10m^2) were soil sampled for pathogen DNA testing just after planting. The range in pathogen levels detected at each paddock are shown in Table 5. Seed samples were collected from along planting lines for pathogen DNA testing and are shown in Table 6.

Based on analysis of combined data from the 4 sites, *V. dahliae* was the pathogen for which the strongest relationship was found between inoculum levels in the soil at the time of planting and yield, Figure 17.

The impact of wet areas within the paddock was obvious at site A, with wet areas associated with tuber rots caused by *Phytopthora* spp. Plots affected by pink rot were omitted from this analysis, with the impact of these wet areas on yield presented in Figure 6 of this report.

Table 5: Range of pathogen DNA levels in soil sampled from grid trial plots at the time of planting at four sites (A-D) in South Australia.

Pathogen DNA test	Site A		Site B		Site C		Site D	
	Min. level	Max. level	Min. level	Max. level	Min. level	Max. level	Min. level	Max. level
Spongospora subterranea pgDNA/g soil	99	1015	26	231	BD	29	BD	15
Colletotrichum coccodes pgDNA/g soil	6	543	12	387	47	356	18	359
Rhizoctonia solani AG2.1 pgDNA/g soil	BD	128	BD	524	BD	538	7	826
Rhizoctonia solani AG3 pgDNA/g soil	BD		BD	3	BD	1	BD	
Rhizoctonia solani AG4 pgDNA/g soil	BD		BD		BD	302	BD	
Verticillium dahliae pgDNA/g soil	BD	30	3	113	6	18	4	82
Streptomyces txtA gene pgDNA/g soil	BD	153	15	316	49	380	30	95
Pratylenchus crenatus nematodes/g soil	<0.1	0.9	BD	<0.1	BD		<0.1	0.3
Pratylenchus neglectus nematodes /g soil	BD	0.3	BD	2.4	BD		BD	0.5
Pratylenchus penetrans nematodes /g soil	BD		BD	5.7	0.6	3.3	BD	<0.1
Meloidogyne fallax pgDNA/g soil	BD	3	BD	11	BD	4	BD	80
Meloidogyne hapla pgDNA/g soil	BD	7	4	359	15	225	BD	
Meloidogyne javanica/incognita/arenaria pgDNA/g soil	BD		BD		BD		BD	
Phytophthora erythroseptica/ drechsleri/ cryptogea kDNA copies/g soil	BD		BD		BD		BD	
Helminthosporium solani kDNA copies/g soil	BD		BD		BD		BD	
Sclerotinia sclerotiorum/minor kDNA copies/g soil	BD	1	BD	2168	BD	4	BD	

BD = Below level of detection

Table 6: Peel pathogen DNA levels of Innovator variety seed tubers planted in grid trial plots at four sites (A-D) in South Australia.

Pathogen DNA test	Site A	Site B		Site C	Site D
	Plot 1-39	Plot 1 -20	Plot 21 -40	Plot 1 -20	Plot 1 -8
Spongospora subterranea pgDNA/g soil	8646	69	31	BD	3546
Colletotrichum coccodes pgDNA/g soil	67054	41377	90996	140773	48440
Rhizoctonia solani AG2.1 pgDNA/g soil	2280	820	1233	737	91
Rhizoctonia solani AG3 pgDNA/g soil	18667	3490	2498	14498	11395
Rhizoctonia solani AG4 pgDNA/g soil	BD	BD	BD	BD	BD
Verticillium dahliae pgDNA/g soil	BD	9	35	39	BD
Streptomyces txtA gene pgDNA/g soil	931	6636	2649	652	200
Pratylenchus crenatus nematodes/g soil	1.6	0.1	<0.1	0.1	0.9
Pratylenchus neglectus nematodes /g soil	BD	BD	BD	BD	BD
Pratylenchus penetrans nematodes /g soil	BD	BD	BD	BD	BD
Meloidogyne fallax pgDNA/g soil	3453	731	344	BD	2609
Meloidogyne hapla pgDNA/g soil	BD	BD	BD	BD	BD
Meloidogyne javanica/incognita/arenaria pgDNA/g soil	BD	BD	BD	BD	BD
Phytophthora erythroseptica/ drechsleri/ cryptogea kDNA copies/g soil	BD	BD	BD	BD	BD
Helminthosporium solani kDNA copies/g soil	20519	38943	34498	7876	11816
Sclerotinia sclerotiorum/minor kDNA copies/g soil	BD	BD	BD	BD	BD
Macrophomina phaseolina kDNA copies/g soil	23	21	BD	BD	9
Pythium clade I pgDNA copies/g soil	BD	BD	BD	BD	0.5

BD = Below level of detection

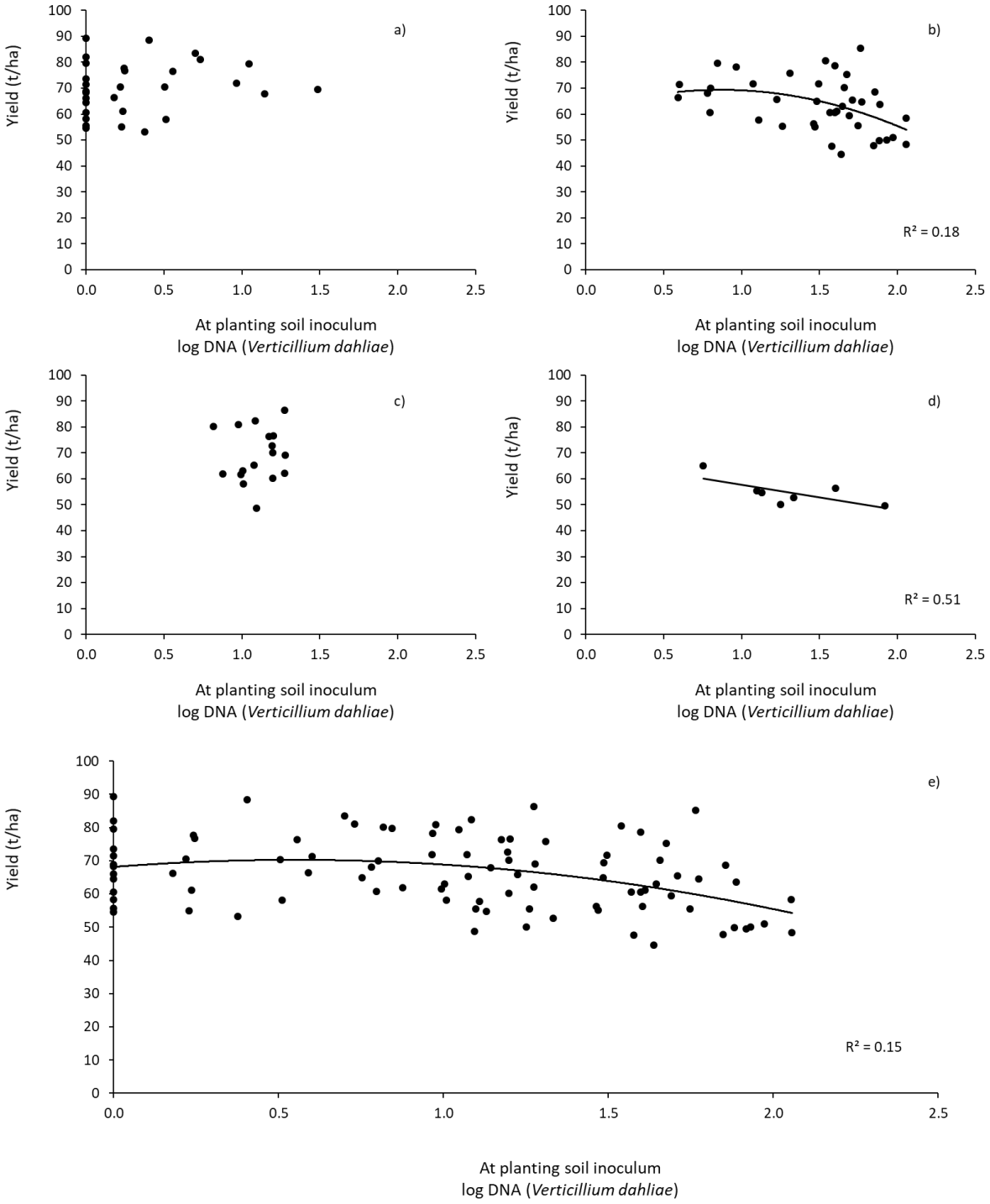

Figure 17: Relationship between inoculum level of *Verticillium dahliae* in the soil prior to planting and yield of Innovator potatoes at four sites (A-D) in South Australia (a,b,c,d respectively) and combined (e). (Data from plots spaced along row lines, plots impacted by pink rot omitted).

Impact on yield in grid trials (Tasmania)

The Forthside rotation crop trial site in Tasmania was planted with a processing potato crop (Ranger Russet). The variations in cropping history across the trial site provide an opportunity to study the impacts of a range of inoculum levels of pathogens within the one paddock (Sparrow 2015). Soil pathogen DNA testing prior to planting was conducted in each of 48 rotation trial plots and the ranges in pathogen level detected are shown in Table 7.

No relationships were found between pre-plant pathogen levels and the total yield or productivity of tubers at this site. With exception of negligible levels of common scab in 2 of 48 plots, tubers were free of soilborne disease symptoms.

In-crop DNA testing of composite root systems from each plot detected high levels (log 4.8-6.5) of *V. dahliae* and high levels (log 5.6-7.2) of *C. coccodes* which is consistent with moderate to high *V. dahliae* and high *C. coccodes* inoculum levels detected by pre-plant soil testing.

In-crop DNA testing of root systems indicated that *S. subterranea* may have impacted crop productivity. Plots with high levels of *S. subterranea* infection of root systems tended to have reduced yields, Figure 18. Infection of root system was related to pre-plant soil inoculum levels, Figure 18.

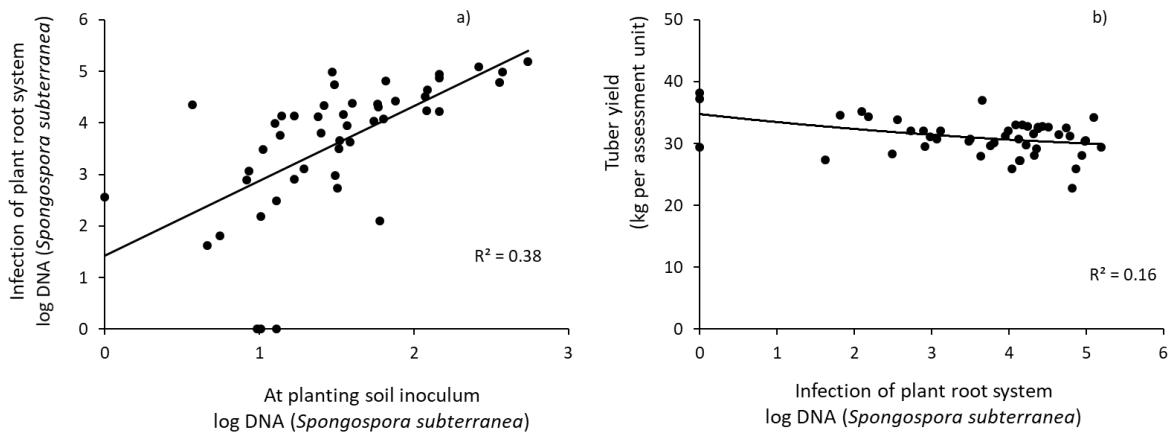

Figure 18: Relationship between a) inoculum level of *Spongospora subterranea* in the soil prior to planting and infection of root system and b) infection of root system by *Spongospora subterranea* and yield of Ranger Russet potatoes at Forthside rotation trial site in Tasmania.

Table 7: Range in pathogen DNA levels of soil sampled from 48 trial plots at the time of planting and peel of Ranger Russet seed tubers (4 samples) planted at Forthside rotation trial site in Tasmania.

Pathogen DNA test	Soil		Seed	
	Min. level	Max. level	Min. level	Max. level
Spongospora subterranea pgDNA/g soil	BD	546	16	93
Colletotrichum coccodes pgDNA/g soil	126	916	BD	
Rhizoctonia solani AG2.1 pgDNA/g soil	7	973	BD	1122
Rhizoctonia solani AG3 pgDNA/g soil	BD	213	BD	194
Rhizoctonia solani AG4 pgDNA/g soil	BD	11	BD	
Verticillium dahliae pgDNA/g soil	9	47	BD	2
Streptomyces txtA gene pgDNA/g soil	BD	41	BD	498
Pratylenchus crenatus nematodes/g soil	BD	0.2	BD	0.2
Pratylenchus neglectus nematodes /g soil	BD	3.3	BD	
Pratylenchus penetrans nematodes /g soil	BD		BD	0.9
Meloidogyne fallax pgDNA/g soil	BD	1	33	134
Meloidogyne hapla pgDNA/g soil	BD		BD	16
Meloidogyne javanica/incognita/arenaria pgDNA/g soil	BD		BD	
Phytophthora erythroseptica/ drechsleri/ cryptogea kDNA copies/g soil	BD		BD	
Helminthosporium solani kDNA copies/g soil	NT		BD	2729

BD = Below level of detection, NT = Not tested

Root lesion nematode *Pratylenchus penetrans*

Impact on yield at validation sites

At validation sites in South Australia, levels of *P. penetrans* ranged from below detection to approximately 10 nematodes per gram soil at sites planted with Innovator and Ranger Russet and from below detection to 4 nematodes per gram soil for potato variety Russet Burbank and Nooksac, Figure 19. For Russet Burbank and Ranger Russet varieties, the average yield was 5% lower when *P. penetrans* was detected in soil prior to planting compared with when not detected. Average yield of sites was similar whether *P. penetrans* was detected or not for variety Innovator. Average yield of variety Nooksac was 15% lower at the 5 sites where *P. penetrans* was detected, compared with average yield from sites where below detection. These sites also had high levels of other yield reducing pathogens that caused disease and may have contributed to yield loss of Nooksac variety potato crops.

When present, increasing population of *P. penetrans* within the range detected in soil prior to planting did not appear to be associated with greater yield loss of the four processing potato varieties at the validation sites assessed.

There is a high proportion of variation in yield that is unaccounted for when comparing results from validation sites located in commercial crops. Factors including seed vigour, virus load, nutrition, irrigation and weather events impact on yield at each site.

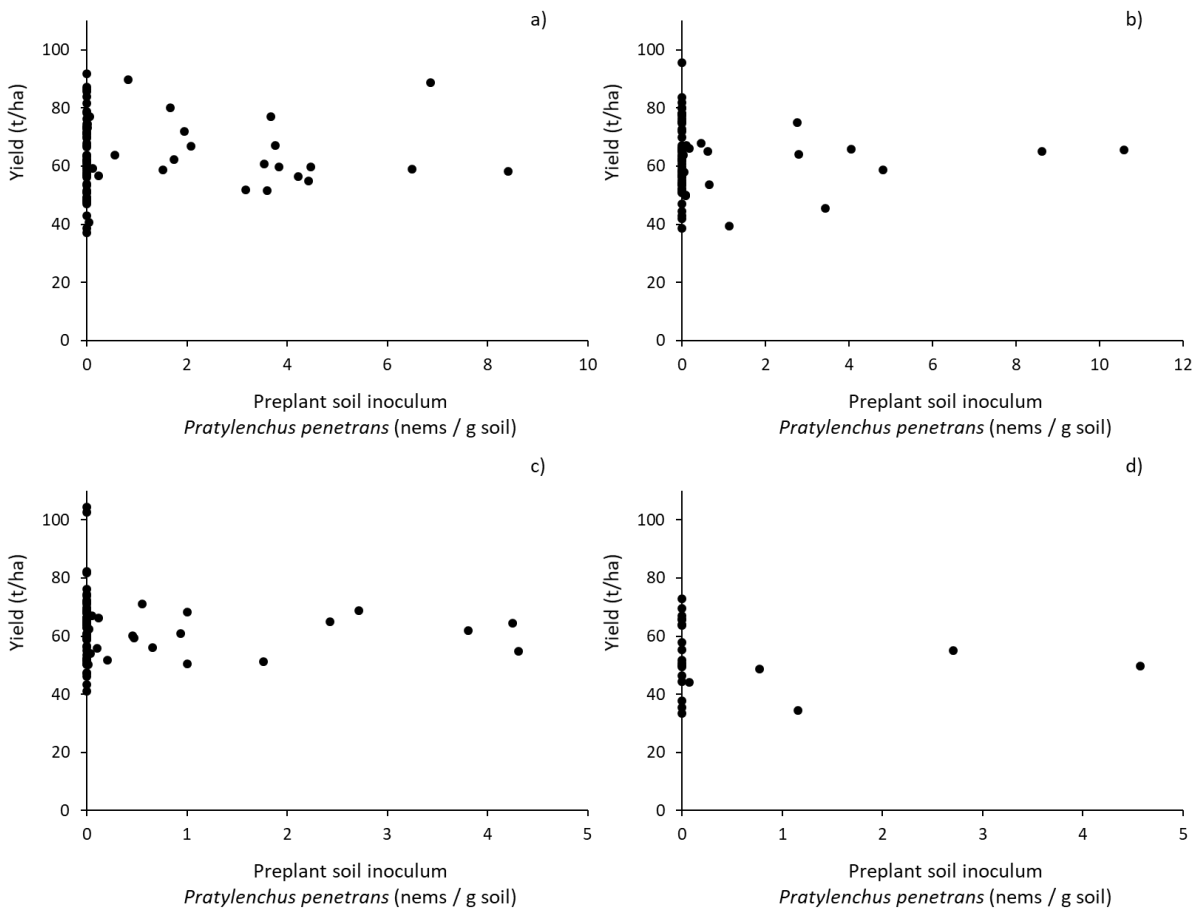

Figure 19: Relationship between inoculum level of *Pratylenchus penetrans* in the soil prior to planting and yield of a) Innovator, b) Ranger Russet, c) Russet Burbank and d) Nooksac potatoes grown in South Australia. (Data from 1 ha areas measured in up to 5 seasons).

Impact on infection and yield in grid trials

Site B in South Australia had the highest levels of *P. penetrans* at planting, with levels ranging from below detection

to 5.7 nematodes per gram soil. *P. penetrans* was also detected in all plots sampled at Site C. *P. penetrans* was below detection in all plots at site A and detected in isolated plots at very low levels at site D.

Data from site B is presented by itself as this site had the wider range in levels, including plots below the level of detection. In-crop testing showed a strong relationship between the level of *P. penetrans* in the soil at planting and the infection level of root systems and harvested tubers, Figure 20. However, despite increasing level of infection no relationship was found between population of *P. penetrans* in the soil at planting and the yield of Innovator potatoes at this site, Figure 20. *P. neglectus* was also detected prior to planting at Site B, along with very low levels of *P. crenatus* in 2 of 40 plots. Highest populations of *P. neglectus* were found in area of the paddock where *P. penetrans* levels were low or below detection. Neither excluding these plots from the analysis or combining population levels of the nematode species detected revealed a relationship between nematode population and yield at site B. Data suggests that under the management applied at Site B, differing infection levels of the root system by *P. penetrans* did not have a significant effect on productivity of the Innovator variety crop grown.

Overlaying data from the 4 sites shows the relationship found between population *P. penetrans* in the soil at planting and infection of root system at site C was consistent with site B, Figure 21. However at site C the same level of inoculum in the soil resulted in higher levels of infection of daughter tubers than at site B. Consistent with pre-plant levels that were very low or below detection at sites A and D, levels of infection of root systems and harvested tubers by *P. penetrans* were very low or below detection from all plots at these sites.

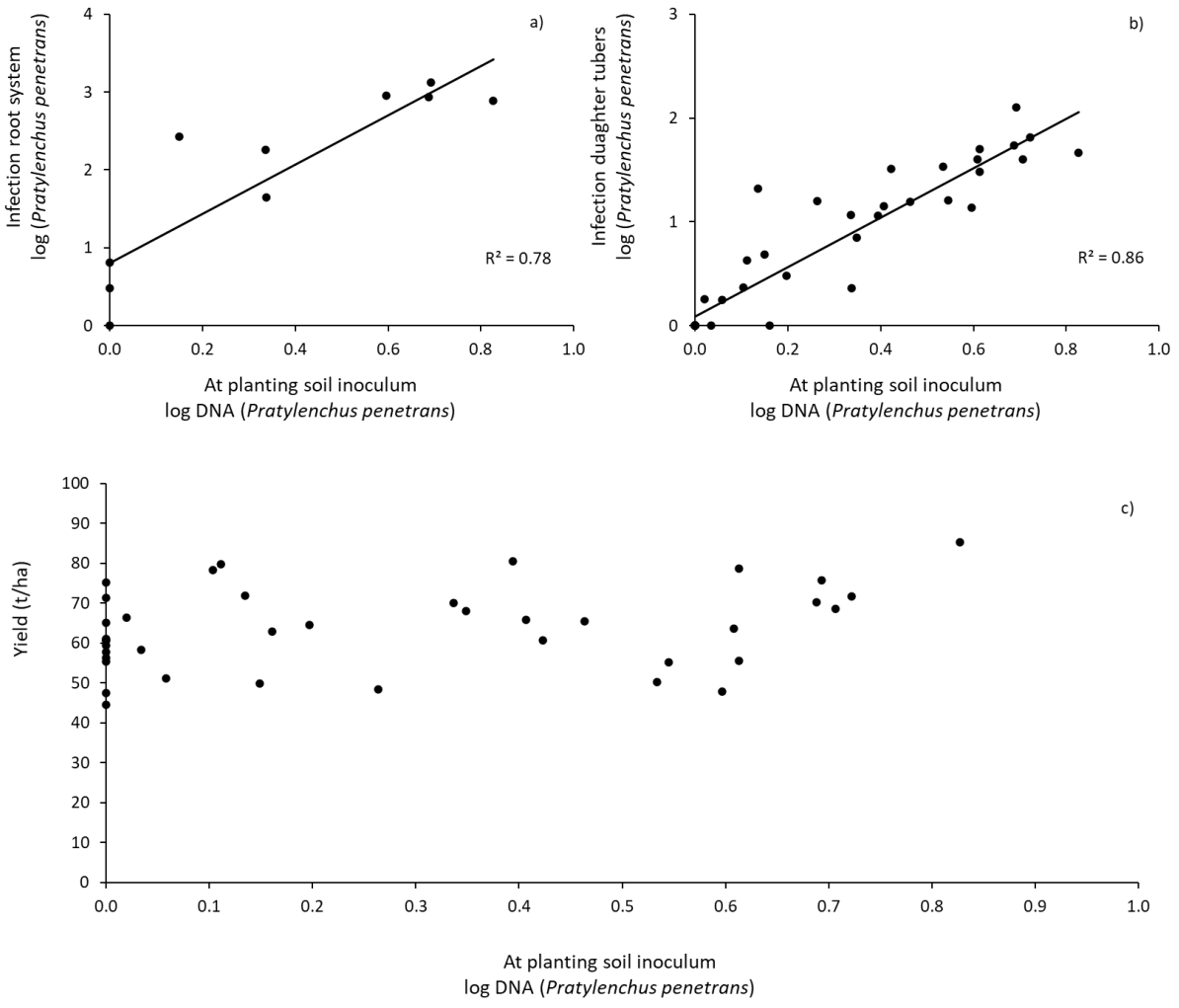

Figure 20: Relationship between inoculum level of *Pratylenchus penetrans* in the soil at planting and a) infection of root system, b) infection of daughter tubers and c) yield at grid trial site B in South Australia. (Data from plots spaced out along row lines).

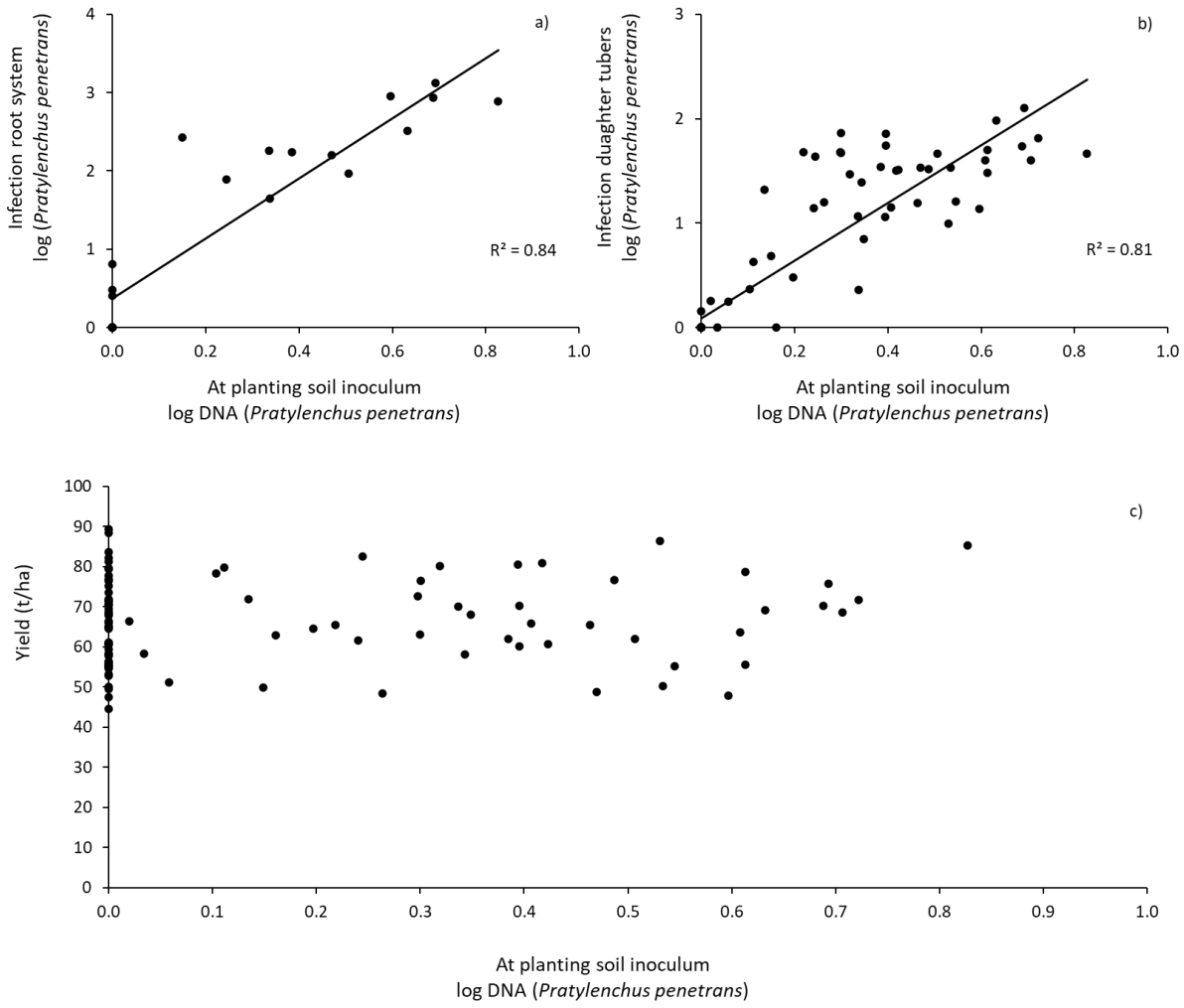

Figure 21: Relationship between inoculum level of *Pratylenchus penetrans* in the soil at planting and a) infection of root system, b) infection of daughter tubers and c) yield at grid trial site a-d combined in South Australia. (Data from plots spaced out along row lines).

Interaction with *Verticillium dahliae*

At grid trial site B in South Australia 40 plots with varying levels of *V. dahliae* and *P. penetrans* were assessed. Ten of these plots were selected and assessed for root infection at 75 days after planting. Levels of *V.dahliae* DNA in peel of daughter tubers (including the stolon end section) were assessed at all plots at harvest. Detection of *P. penetrans* in the soil at the time of planting was associated with higher levels of *V.dahliae* infection of root systems 75 days after planting and the peel of daughter tubers at harvest when compared to plots where *P. penetrans* was below detection, relative to the level of inoculum of *V. dahliae* present in the soil, Figure 22. This interaction between *P. penetrans* and *V. dahliae* is often referred to as the disease potato early dying (Orlando *et al*., 2020).

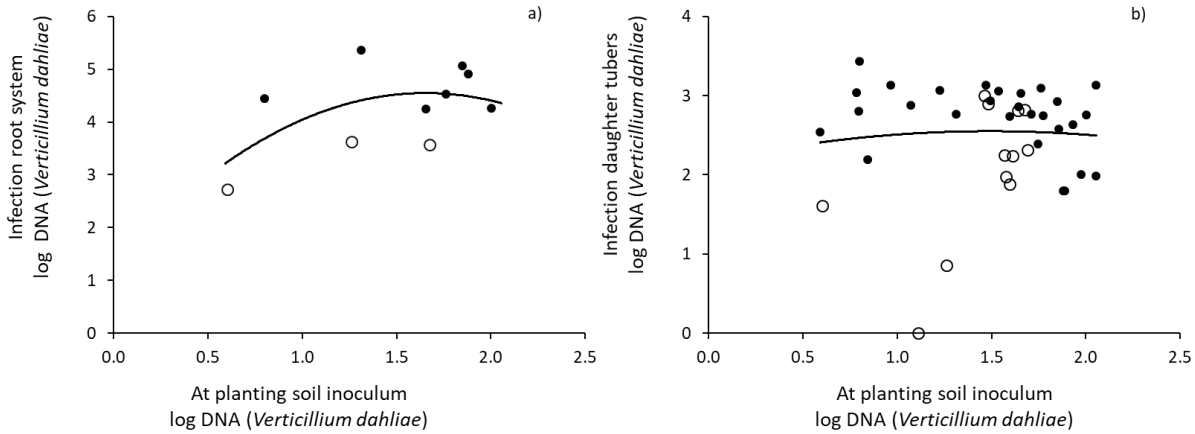

Figure 22: Relationship between inoculum level in the soil at planting of *Verticillium dahliae* and a) infection of root system by *Verticillium dahliae* 75 days after planting, b) infection of daughter tubers by *Verticillium dahliae* at harvest at grid trial site B in South Australia. Solid markers represent plots where *Pratylenchus penetrans* detected in soil at time of planting and circles plots where *Pratylenchus penetrans* below detection in soil at time of planting.

Root lesion nematode *Pratylenchus neglectus*

Impact on yield at validation sites

At validation sites in South Australia, levels of *P. neglectus* ranged from below detection to approximately 12 nematodes per gram soil at sites planted with Ranger Russet and Russet Burbank variety potatoes and from below detection to approximately 5 nematodes per gram soil at sites planted with Innovator and Nooksac, Figure 23. For Russet Burbank the average yield of sites was 6% lower when *P. neglectus* was detected in soil prior to planting compared with when not detected. Average yield of sites was similar whether *P. neglectus* was detected or not for varieties Ranger Russet and Innovator. Average yield of Nooksac was 22% lower at the 14 sites where *P. neglectus* was detected, compared with the 9 sites where it was below detection. Other yield reducing pathogens that also caused disease and may have contributed to yield loss of Nooksac potato crops were present at these sites.

When present, increasing population of *P. neglectus* within the range detected in soil prior to planting did not appear to be associated with greater yield loss of Innovator and Ranger Russet varieties, but may have contributed along with other pathogens to increasing yield reduction of Russet Burbank and Nooksac potatoes.

There is a high proportion of variation in yield that is unaccounted for when comparing results from validation sites located in commercial crops. Factors including seed vigour, virus load, nutrition, irrigation and weather events impact on yield at each site.

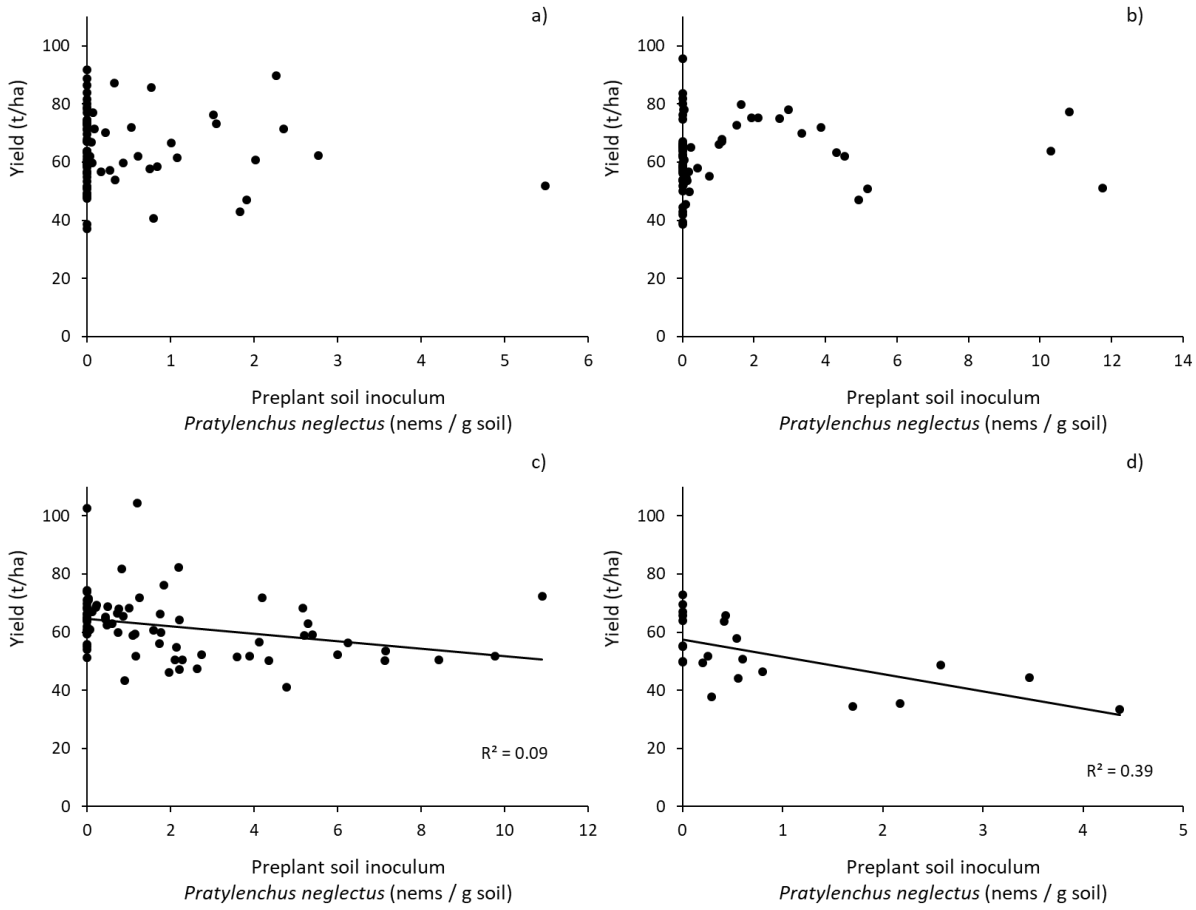

Figure 23: Relationship between inoculum level of *Pratylenchus neglectus* in the soil prior to planting and yield of a) Innovator, b) Ranger Russet, c) Russet Burbank and d) Nooksac potatoes grown in South Australia. (Data from 1 ha areas measured in up to 5 seasons).

Impact on infection and yield in grid trials

Pratylenchus neglectus was present at three of four grid trial sites in South Australia. Only data from site B is presented as detections at sites A and D were limited to a small number of plots. *P. neglectus* populations at the time of planting ranged from below detection to 2.4 nematodes per gram soil at site B. High populations of *P. penetrans* were also present at site B as reported in Table 5. At site B average yield of potato variety Innovator was 4% lower from plots where *P. neglectus* was detected in soil at planting compared with plots where not detected. When present, increasing population of *P. neglectus* in soil within the range detected did not appear to be associated with greater yield loss.

In-crop testing of root systems from plots where *P. neglectus* was detected at low levels confirmed infection, Figure 24. Infection levels of roots was low or below detection from plants tested from plots where *P. neglectus* was not detected in soil at planting. As *P. neglectus* population in the soil at planting increased so did the probability of tubers being infected at harvest. Figure 24. *P. neglectus* does not appear to cause as high infection level of tubers as *P. penetrans* from similar inoculum levels in the soil.

P. neglectus was present at the grid trial site in Tasmania that was planted with Ranger Russet potatoes. *P. neglectus* populations prior to planting ranged from below detection to 3.3 nematodes per gram soil. In-crop testing found a relationship between the level of *P. neglectus* in the soil prior to planting and the infection level of root systems, Figure 25. Infection of harvested tubers was not assessed. Despite increasing level of infection of root systems, no relationship was found between the pre-plant population of *P. neglectus* in the soil and the yield of Ranger Russet potatoes at this site, Figure 25.

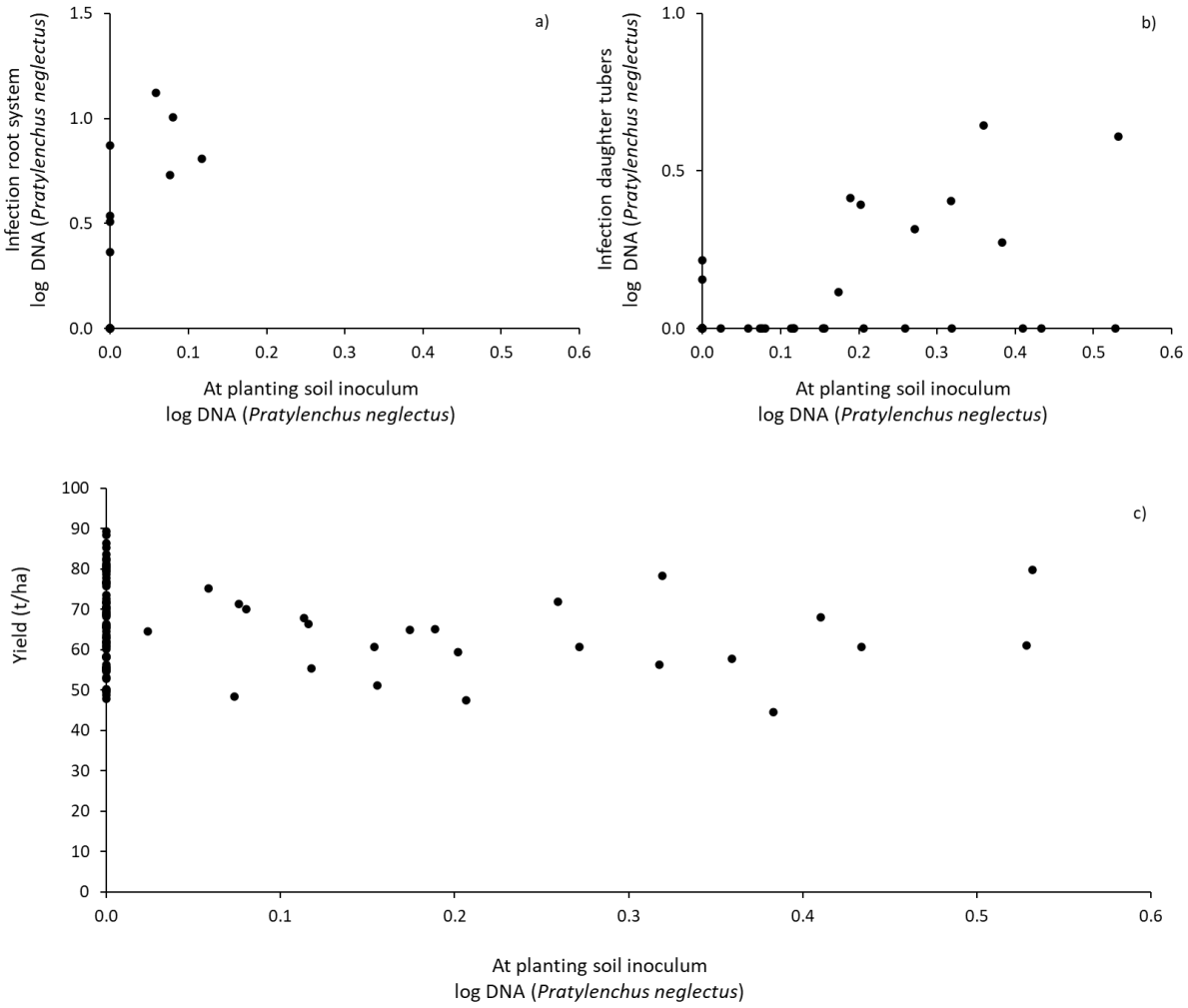

Figure 24: Relationship between inoculum level of *Pratylenchus neglectus* in the soil at planting and a) infection of root system, b) infection of daughter tubers and c) yield at grid trial site b in South Australia. (Data from plots spaced out along row lines).

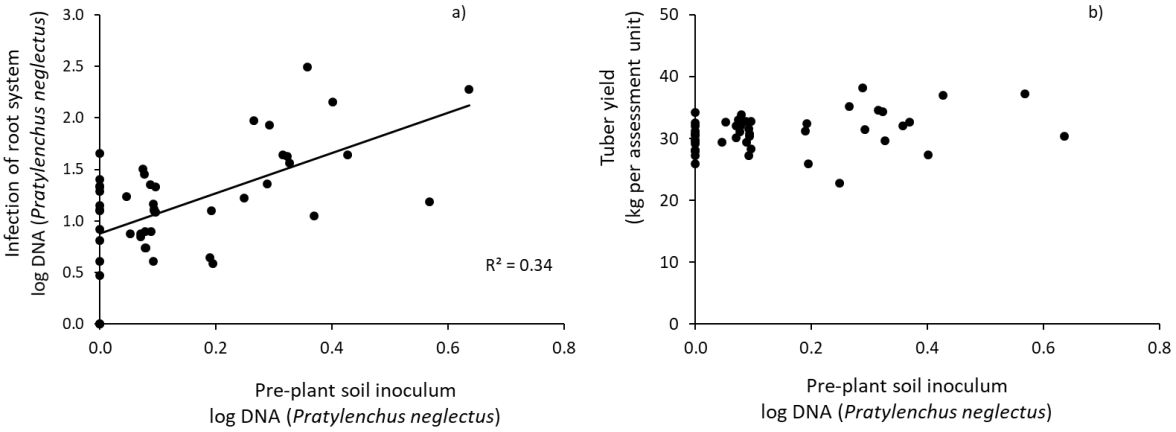

Figure 25: Relationship between inoculum level of *Pratylenchus neglectus* in the soil prior to planting and a) infection of root system and b) yield of Ranger Russet potatoes at Forthside rotation trial site in Tasmania. (Data from 48 trial plots).

Root lesion nematode *Pratylenchus crenatus*

Impact on yield at validation sites

At validation sites in South Australia, levels of *Pratylenchus crenatus* ranged from below detection up to approximately 1.6 nematodes per gram soil, Figure 26. These populations are lower, when compared with *P. penetrans* and *P. neglectus*, but are reflective of levels found in potato cropping systems. In general, levels of *P. crenatus* detected in soil prior to planting are below 2 nematodes per gram soil. For the Ranger Russet variety, the average yield was 12% lower when *P. crenatus* was detected in soil prior to planting compared with when not detected. Average yield of sites was similar whether *P. crenatus* was detected or not for varieties Innovator, Russet Burbank and Nooksac.

When present, increasing population of *P. crenatus* within the range detected in soil prior to planting did not appear to be associated with greater yield loss of any of the four varieties, Figure 26.

There is a high proportion of variation in yield that is unaccounted for when comparing results from validation sites located in commercial crops. Factors including seed vigour, virus load, nutrition, irrigation and weather events impact on yield at each site.

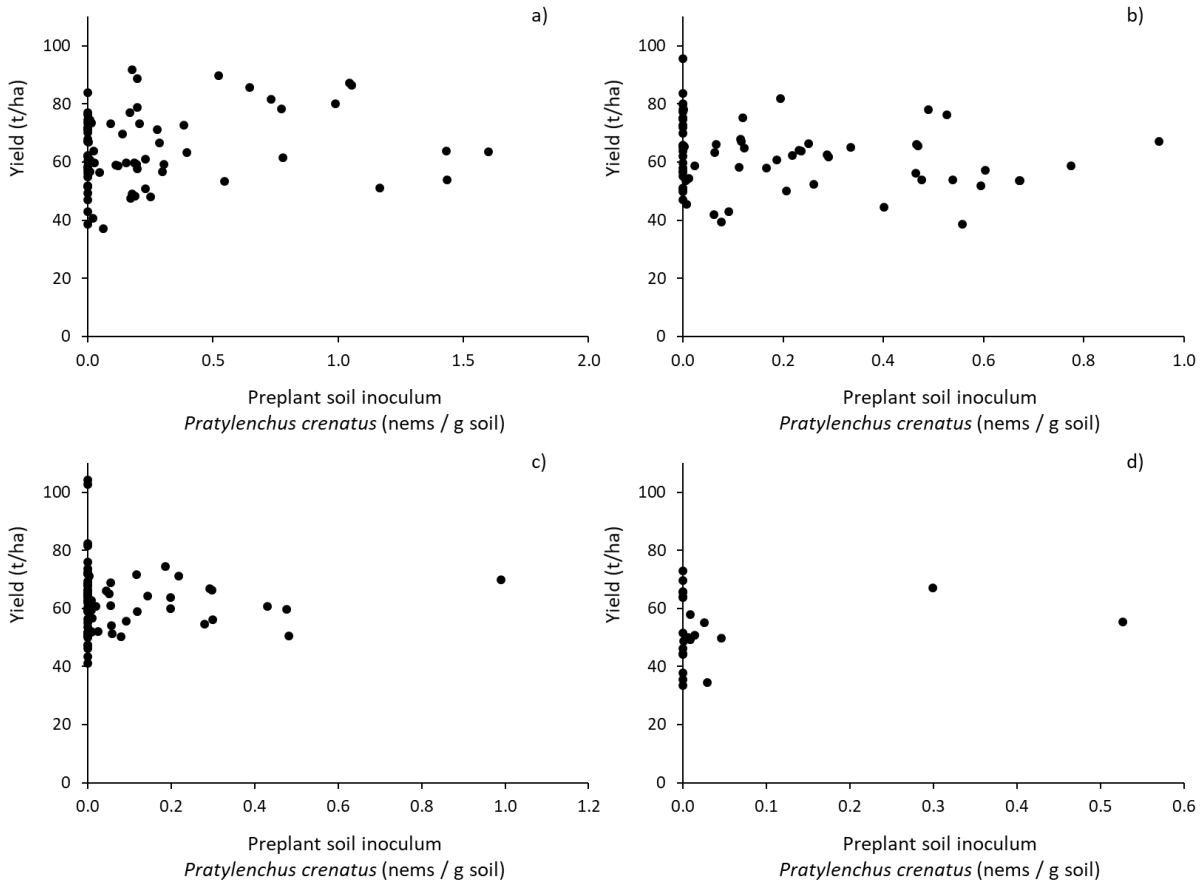

Figure 26: Relationship between inoculum level of *Pratylenchus crenatus* in the soil prior to planting and yield of a) Innovator, b) Ranger Russet, c) Russet Burbank and d) Nooksac potatoes grown in South Australia. (Data from 1 ha areas measured in up to 5 seasons).

Impact on infection and yield in grid trials

Pratylenchus crenatus was detected in all plots at sites A and D in South Australia, with levels ranging from very low (<0.1) to 0.9 nematodes per gram soil. *P. crenatus* was below detection at all plots at site C and detected in isolated plots at low levels at site B. In-crop testing showed relationships between the level of P. crenatus in the soil at planting and the infection level of root systems and harvested tubers, Figure 27. However, despite increasing level of infection no relationship was found between the pre-plant population of P. crenatus in the soil and the yield of Innovator potatoes at either site A or D or when data was combined, Figure 27. *P. crenatus* was present at the grid trial site in Tasmania that was planted with Ranger Russet potatoes. *P. crenatus* populations prior to planting ranged from below detection to 0.2 nematodes per gram soil. Even at these low population levels a relationship was found between the level of *P. neglectus* in the soil prior to planting and the infection level of root systems, Figure 28. Infection of harvested tubers was not assessed. At the levels of infection present at this site no relationship was found between the pre-plant population of *P. crenatus* in the soil and the yield of Ranger Russet potatoes at this site, Figure 28.

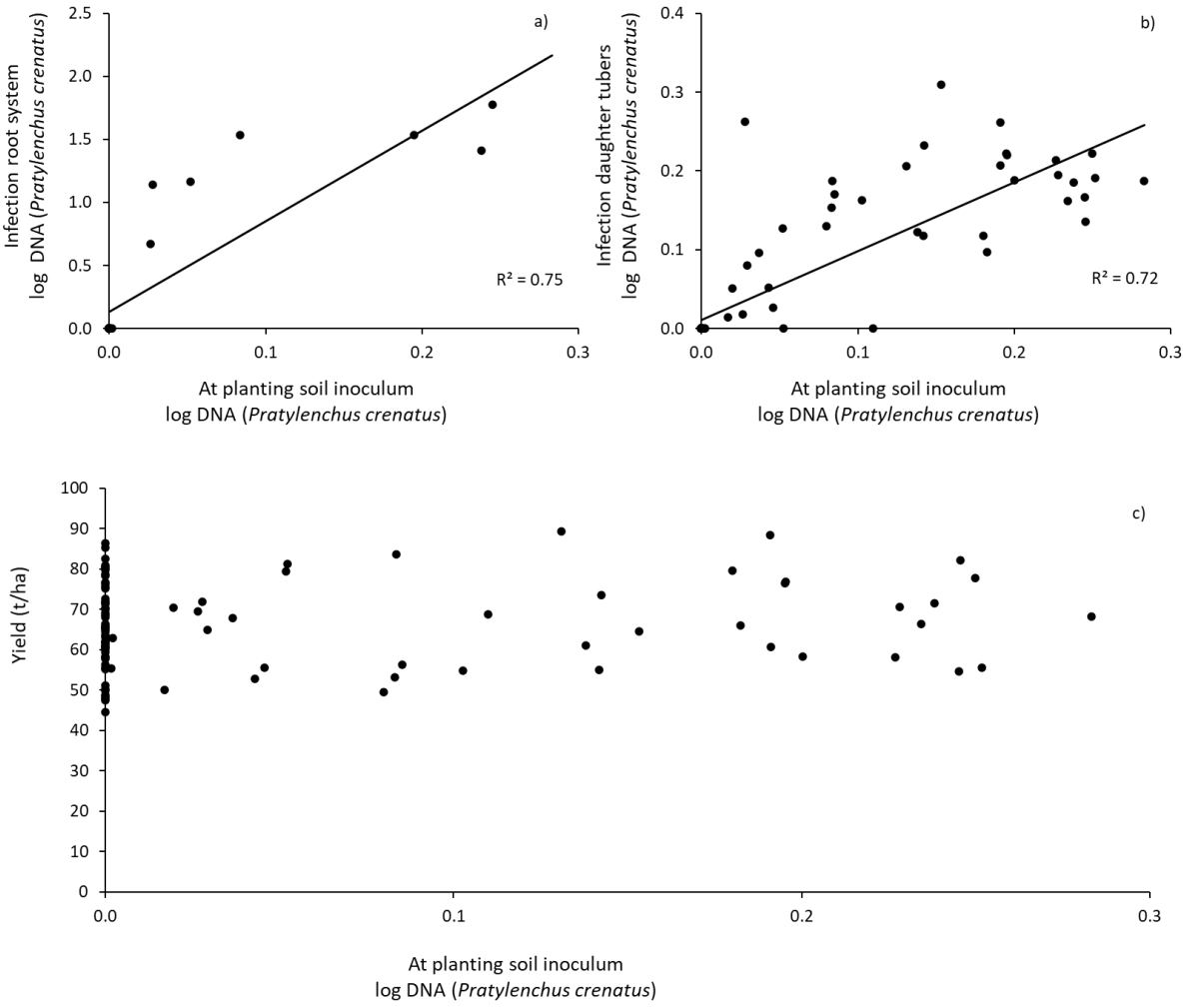

Figure 27: Relationship between inoculum level of *Pratylenchus crenatus* in the soil prior to planting and a) infection of root system, b) infection of daughter tubers and c) yield at grid trial sites A-D combined in South Australia. (Data from plots spaced out along row lines).

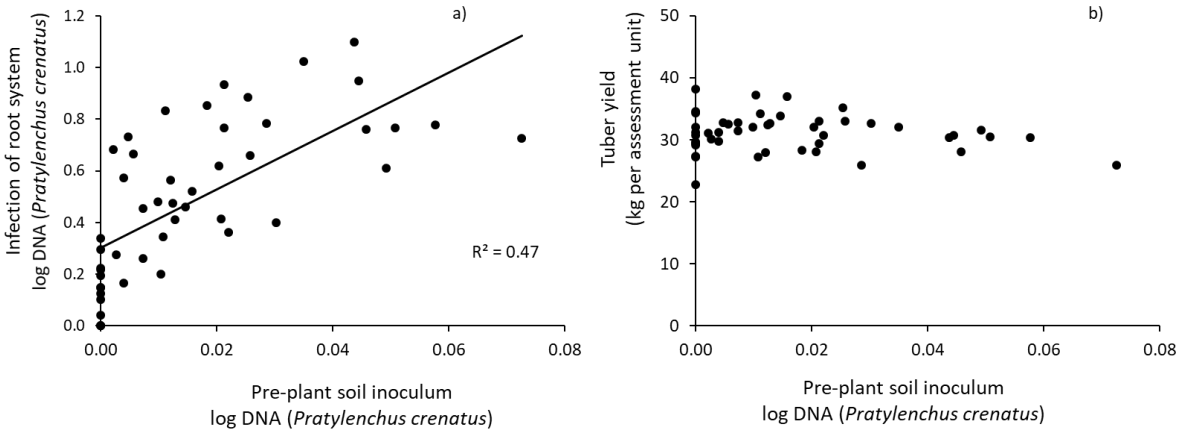

Figure 28: Relationship between inoculum level of *Pratylenchus crenatus* in the soil prior to planting and a) infection of root system and b) yield of Ranger Russet potatoes at Forthside rotation trial site in Tasmania. (Data from 48 trial plots).

Rhizoctonia

Rhizoctonia disease of potatoes in Australia is mainly caused by *Rhizoctonia solani* AG3 and AG2.1. Tests for both these anastomosis groups are reported on PREDICTA Pt. Other *R. solani* AG's may cause symptoms on potatoes, including AG2.2, AG4 and AG8. While they have not been considered of major importance on potatoes in Australia, *R. solani* AG4 was identified for further investigation and has been added to PREDICTA Pt reporting.

Data presented in this section focuses on *Rhizoctonia solani* AG3 and AG2.1, as they are the main anastomosis groups associated with stem canker and black scurf of potatoes in Australia. Reporting includes new data generated in this project and pre-existing data from Project PT02093 which has been reanalysed.

Stem canker

Assessed at validation sites

Inoculum on seed of both *R. solani* AG3 and AG2.1 were a source of disease risk for rhizoctonia stem canker in South Australia, explaining a high proportion of field incidence at the 25 sites assessed, Figure 29. This was especially the case when inoculum level of both AG3 and AG2.1 were added together. Depending on site, stem canker was associated with infection by *R. solani* AG3 or AG2.1 or both. Soil inoculum of one or both *R. solani* AG3 and AG2.1 was present at many sites and contributed to incidence of rhizoctonia stem canker at some sites, Figure 30. Incidence of stem canker was more strongly associated with inoculum on seed tubers than levels in the soil prior to planting.

Previous results relating seed inoculum to risk of rhizoctonia stem canker have not shown as strong a relationship. This is highlighted by reanalysis of data from PT02023 in Figure 31. As both soil and seed inoculum can contribute to risk of rhizoctonia stem canker and several anastomosis groups can cause stem canker, accounting for all sources of inoculum that pose a risk of disease is complex. With this dataset, when seed and soil inoculum of both AG2.1 and AG3 are added together, plantings at all sites have inoculum that poses some level of disease risk for stem canker, Figure 32. There are several sites where disease risk was underestimated by the level of inoculum detected in soil and seed samples. Conditions may have been more conducive for stem canker at these sites, resulting in high incidence of disease from low or below detectable levels of soil inoculum. Alternatively, the sampling intensity was insufficient to detect inoculum on seed.

Assessed at grid trials

At grid trial sites A, B, and C conducted in South Australia underground stems and root system of potato plants were sampled for DNA testing from selected plots. Seed planted at all plots carried inoculum of *R. solani* AG2.1 (Figure 33) and AG3 (Figure 34). Infection levels of underground stems and root systems by *R. solani* AG2.1 tended to be higher in plots where soil inoculum was detected, Figure 33. Inoculum of *R. solani* AG3 was only detected in the soil at time of planting at one plot, and no DNA of *R. solani* AG3 was detected in underground stems and root system samples collected from this plot. Underground plant parts were infected by *R. solani* AG3 at a high proportion of other plots, most likely from inoculum on the seed tubers.

Other anastomosis groups

R. solani AG2.2 was associated with stem canker on samples from a validation site in Tasmania where neither *R. solani* AG2.1, AG3 or AG4 were detected by DNA testing of infected tissue. *R. solani* AG2.2 prefers warmer conditions than AG3 and is more likely to be the cause of stem canker in warm production areas. This data also indicates it can cause stem canker in potato crops in Tasmania. *R. solani* AG2.2 is far less prevalent in main potato growing areas than *R. solani* AG2.1.

Testing of underground stems frequently detected *R. solani* AG4. In several cases there were no symptoms of rhizoctonia stem canker. When stem canker symptoms were observed, either AG2.1 or AG3 were also detected.

R. solani AG8 was detected in pre-plant soil samples at grid trial site B in South Australia. Follow up testing of lesions on roots with *Rhizoctonia* symptoms confirmed they were associated with *R. solani* AG8. Symptoms were confined to the roots. In this trial yield was not reduced at the plots where root symptoms were observed, when compared with plots where no symptoms were observed.

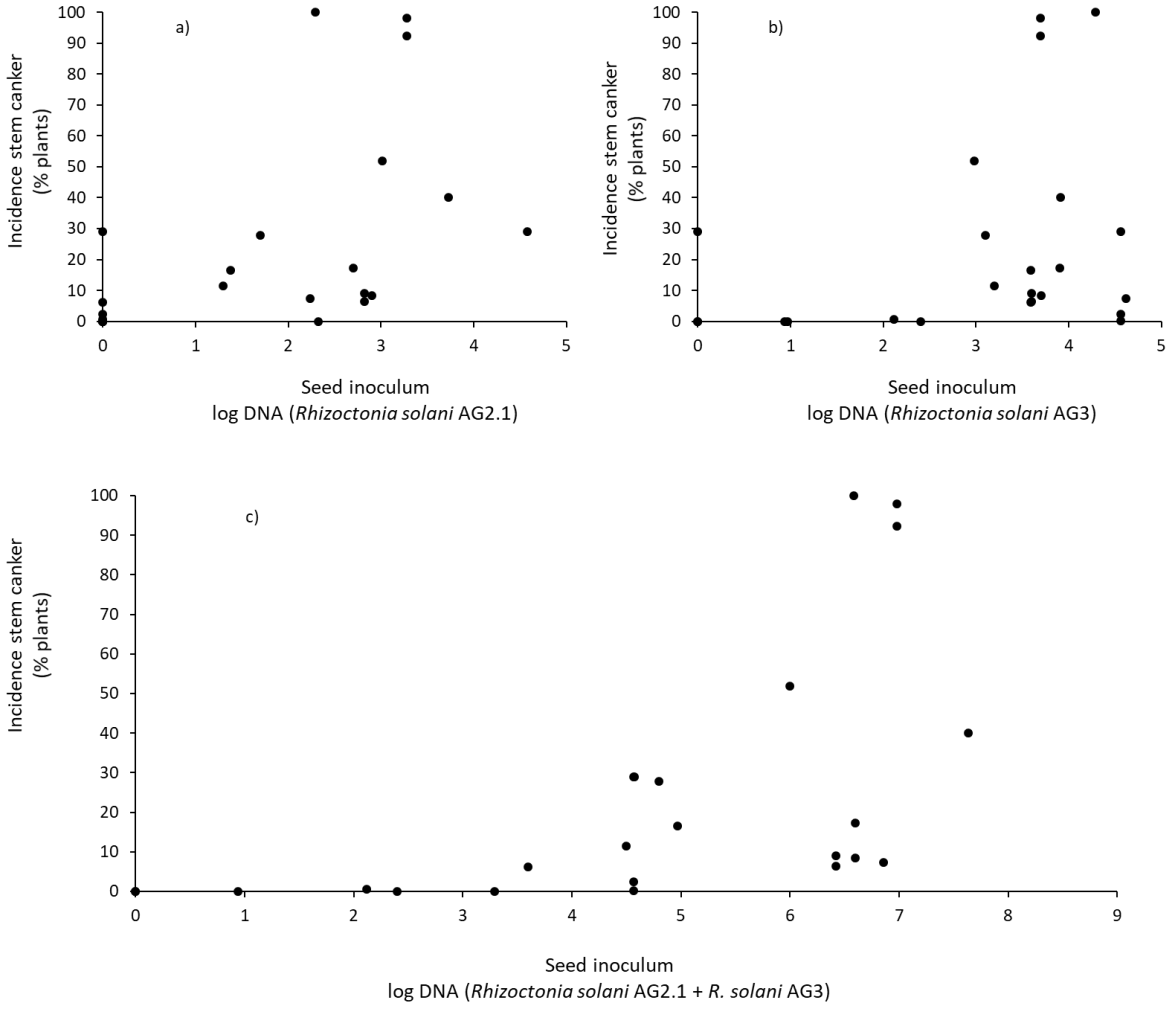

Figure 29: Relationship between inoculum level of *Rhizoctonia solani* on seed peel and incidence of stem canker for a) *R. solani* AG2.1, b) *R. solani* AG3 and c) *R. solani* AG2.1 plus AG3. (Data from 25 sites over 3 seasons in South Australia).

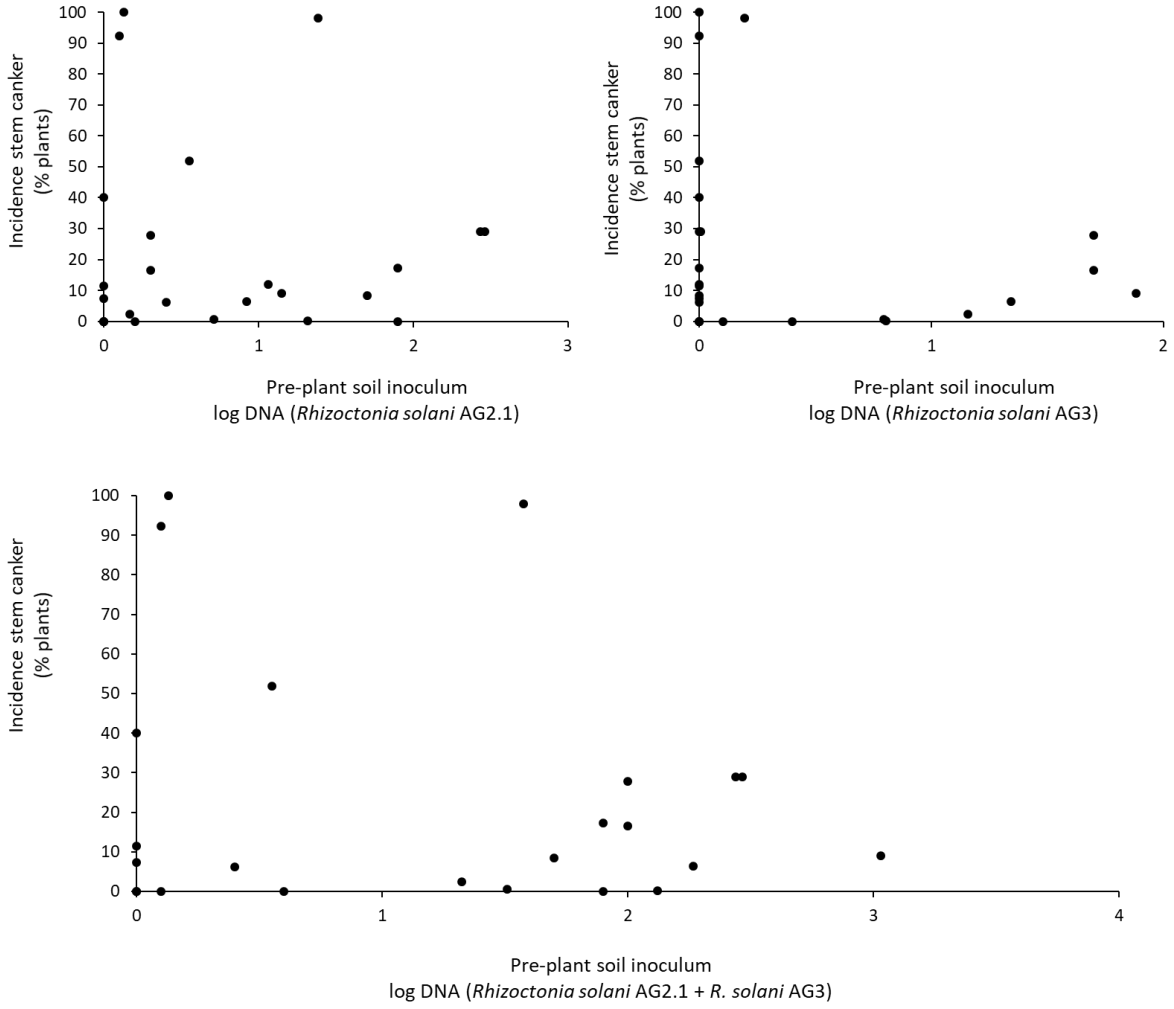

Figure 30: Relationship between preplant soil inoculum level of *Rhizoctonia solani* and incidence of stem canker for a) *R. solani* AG2.1, b) *R. solani* AG3 and c) *R. solani* AG2.1 plus AG3. (Data from 25 sites over 3 seasons in South Australia).

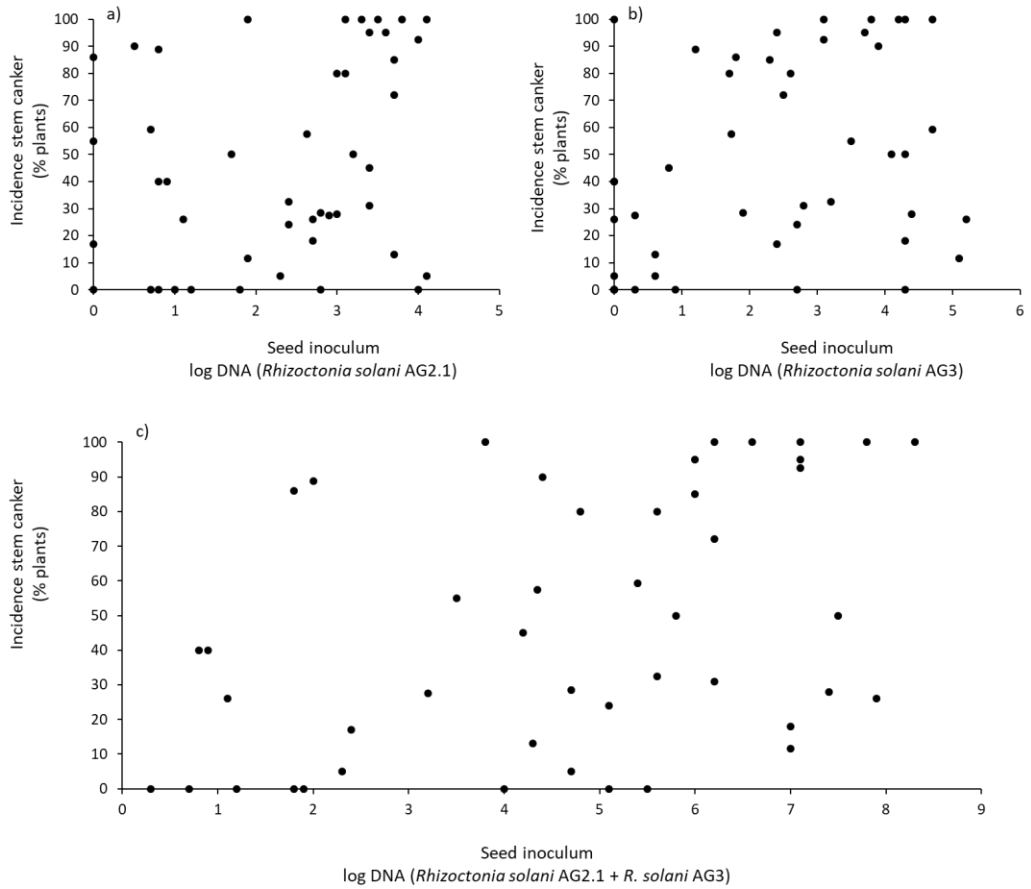

Figure 31: Relationship between inoculum level of *Rhizoctonia solani* on seed peel and incidence of stem canker for a) *R. solani* AG2.1, b) R. *solani* AG3 and c) *R. solani* AG2.1 plus AG3. (Data reanalysed from PT09023 - 46 seedlots - averaged where planted at multiple sites, over 2 seasons).

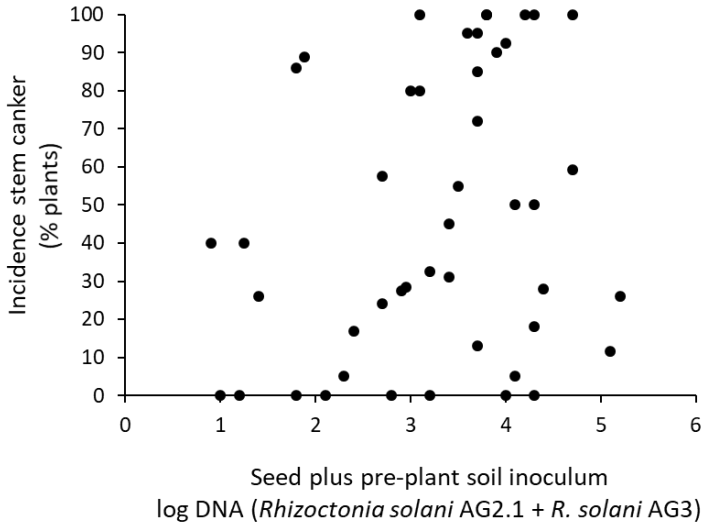

Figure 32: Relationship between inoculum level of *Rhizoctonia solani* (AG2.1 plus AG3) in seed peel plus pre-plant soil and incidence of stem canker (Data reanalyzed from PT09023 - 46 seedlots - averaged where planted at multiple sites, over 2 seasons).

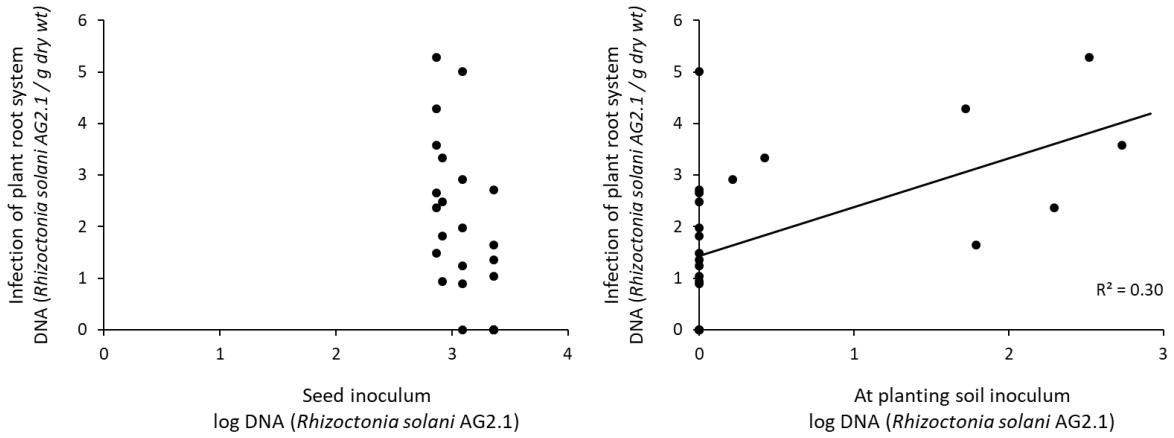

Figure 33: Relationship between inoculum level of *Rhizoctonia solani* AG2.1 on seed peel (left) and in the soil at planting (right) and infection of underground stems and root systems at three grid trial sites in South Australia. (Data from plots spaced out along row lines).

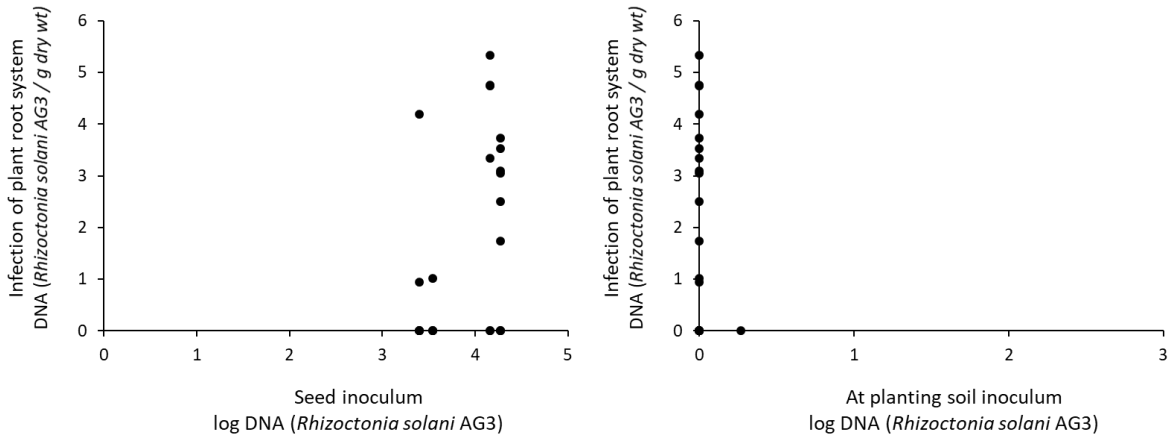

Figure 34: Relationship between inoculum level of *Rhizoctonia solani* AG3 on seed peel (left) and in the soil at planting (right) and infection of underground stems and root systems at three grid trial sites in South Australia. (Data from plots spaced out along row lines).

Black scurf

Rhizoctonia solani AG3 was the main anastomosis group associated with the incidence of black scurf. *R. solani* AG2.1 was also associated with incidence of black scurf. At sites where a high incidence of severe black scurf occurred, it was caused by *R. solani* AG3.

Black scurf caused by *R. solani* AG2.1 is flatter in appearance than the lumpy sclerotia on tubers caused by *R. solani* AG3. *R. solani* AG2.1 is regularly detected by pre-plant soil testing in potato paddocks. Detection of *R. solani* AG3 by pre-plant soil testing is less common, mostly occurring at sites with short rotations. Both *R. solani* AG2.1 and AG3 are commonly detected on seed tubers.

R. solani AG4 has been detected in soil prior to planting potatoes, particularly where brassicas are grown. In this project, AG4 has only rarely been detected on seed tubers. At a site in Queensland and a site in Western Australia where harvested tubers had black scurf caused by *R. solani* AG3, high levels DNA of *R. solani* AG4 were also detected in the composite peel sample.

***R. solani* AG3**

Inoculum on seed of *R. solani* AG3 was found to be important source of disease risk for black scurf, Figure 35. In paddocks where inoculum was not detected in the soil prior to planting, 41% of assessed areas had > 10% incidence of black scurf when infected seed (> 2.0 log DNA (*Rhizoctonia solani* AG3)) was planted. This compares with 5% and 28% of assessed areas when seed with levels below detection and low levels inoculum (< 2.0 log DNA (*Rhizoctonia solani* AG3)), respectively were planted.

To investigate the contribution of soil inoculum to disease risk, sampling areas where either the seed was known to be infected by *R. solani* AG3 (> 2.0 log DNA seed peel) or of unknown status were removed from the validation data set. Presence of soil inoculum in a paddock was associated with a higher probability of black scurf on daughter tubers, Figure 36. Greater than 10% incidence of black scurf occurred at 28% sites where *R. solani* AG3 was detected in the soil prior to planting, compared with 15% of sites when inoculum was not detected in soil prior to planting.

***R. solani* AG2.1**

Inoculum on seed of *R. solani* AG2.1 was found to be important source of disease risk for black scurf, Figure 37. In paddocks where inoculum in the soil was below detection or low (< 0.5 log DNA), 16% of assessed areas had > 5% incidence of black scurf when infected seed (> 1.5 log DNA (*Rhizoctonia solani* AG2.1)) was planted. This compares with none of assessed areas when seed with levels below detection were planted.

To investigate the contribution of soil inoculum to disease risk, sampling areas where either the seed was known to be infected by *R. solani* AG2.1 (> 1.5 log DNA seed peel) or of unknown status were removed from the validation data set. Presence of soil inoculum in a paddock was associated with a higher probability of black scurf on daughter tubers, Figure 38. Greater than 5% incidence of black scurf occurred at 24% sites where *R. solani* AG2.1 was detected in the soil prior to planting, compared with no sites when inoculum was not detected in soil prior to planting.

At 4 of 35 sites where *R. solani* AG2.1 infected seed (> 1.5 log DNA) was planted into infected soil (> 0.5 log DNA), a higher incidence of black scurf (10-28%) was observed than at any site associated with soil or seed inoculum alone.

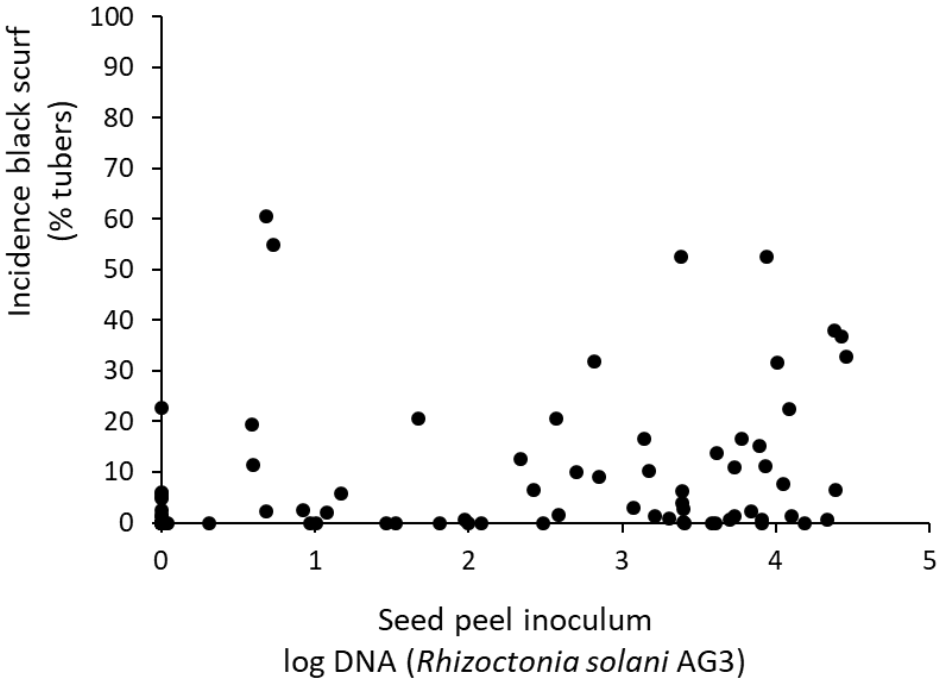

Figure 35: Relationship between inoculum level of *Rhizoctonia solani* AG3 on seed tubers and incidence black scurf at sites where not detected in pre-plant soil samples. (Sites where black scurf caused by *R. solani* AG2.1 were excluded; data from 83 sites over 6 seasons).

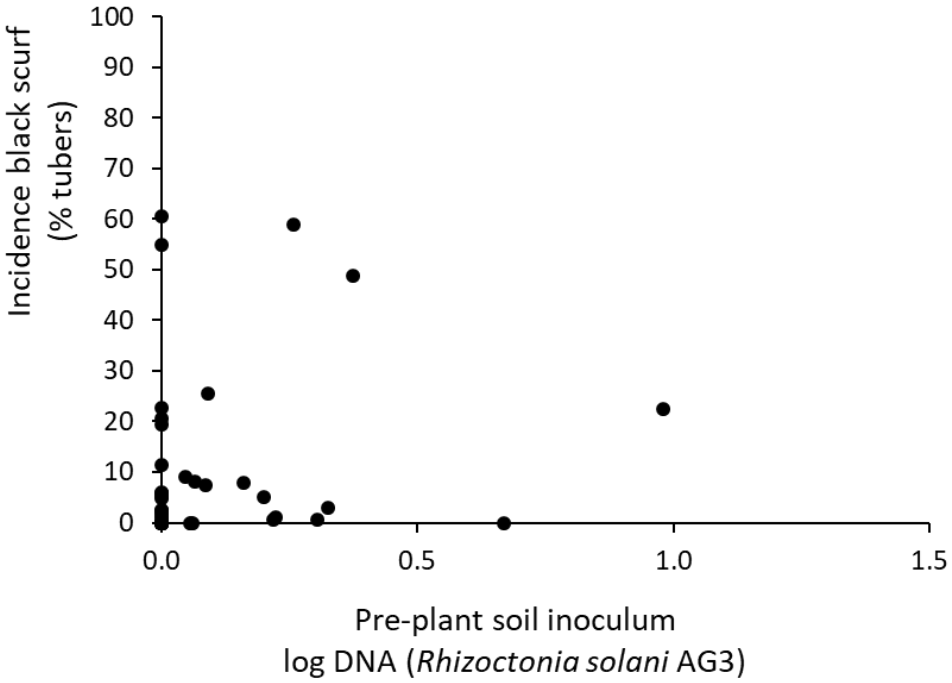

Figure 36: Relationship between inoculum level of *Rhizoctonia solani* AG3 in soil prior to planting and incidence black scurf at sites where seed planted had inoculum levels below 2 log DNA (*Rhizoctonia solani* AG3). (Sites where black scurf caused by *R. solani* AG2.1 were excluded; data from 57 sites over 6 seasons).

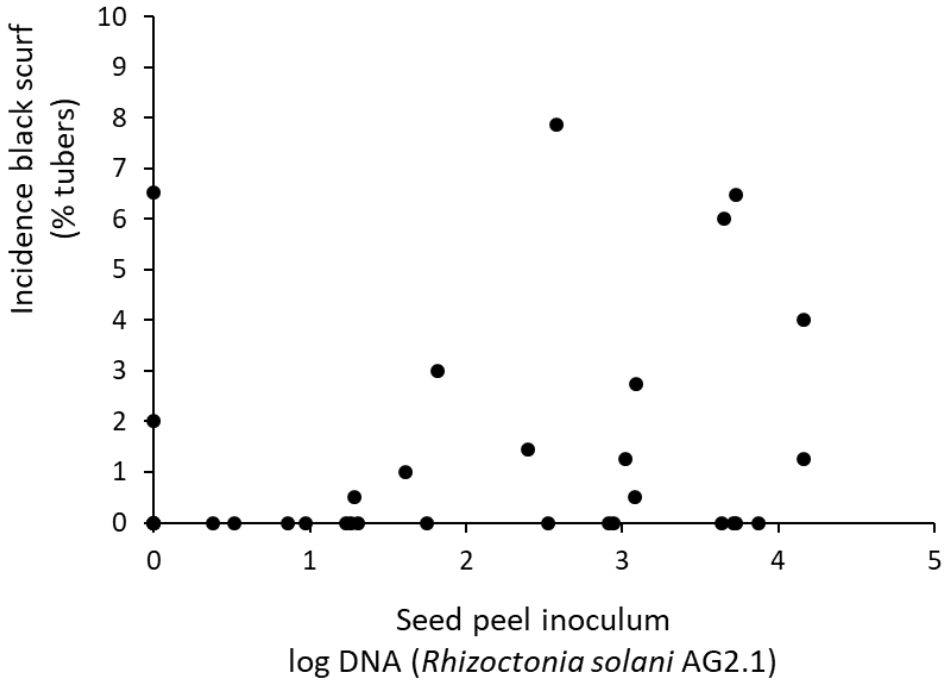

Figure 37: Relationship between inoculum level of *Rhizoctonia solani* AG2.1 on seed tubers and incidence black scurf at sites where inoculum in the soil prior to planting was below 0.5 log DNA (*R. solani* AG2.1). (Sites where black scurf caused by *R. solani* AG3 were excluded; data from 33 sites over 6 seasons).

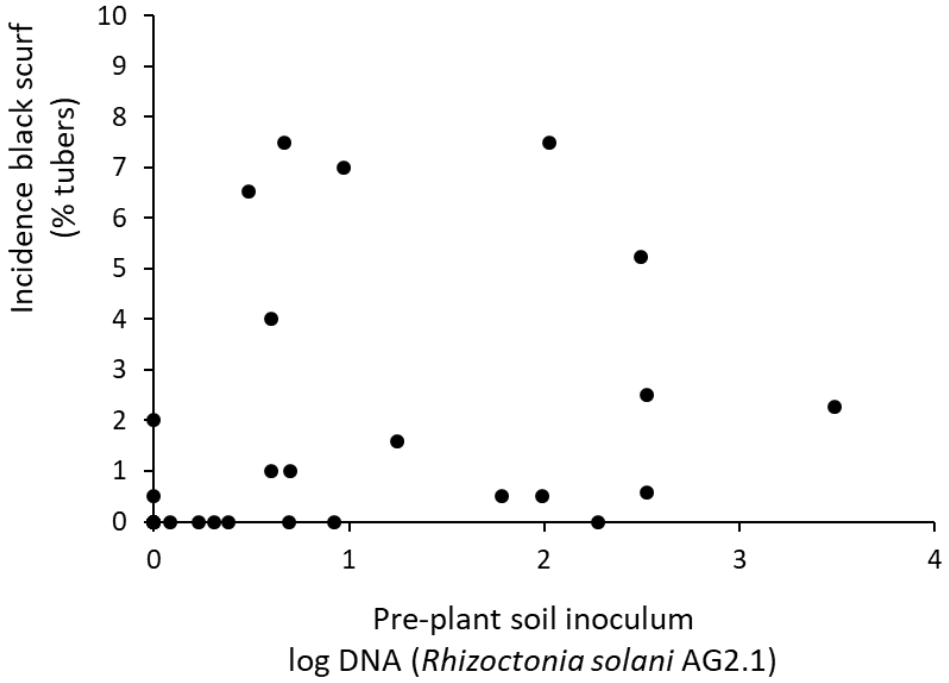

Figure 38: Relationship between inoculum level of *Rhizoctonia solani* AG2.1 in soil prior to planting and incidence black scurf at sites where seed with inoculum level was below 1.5 log DNA (*R. solani* AG2.1) planted. (Sites where black scurf caused by *R. solani* AG3 were excluded; data from 30 sites over 6 seasons).

Evaluation of repeated sampling

Eight paddocks with history of potato production were selected and tested to investigate the number of samples in a 1 ha area and in four 1 ha areas within a paddock required to be confident of detecting pathogens that cause rhizoctonia disease. Testing for *R. solani* AG2.1 and AG3 was conducted on the same samples collected to investigate detection of the pathogen that causes pink rot (refer page 30), plus 1 additional site.

The detection rate, range in level and average level of *R. solani* AG2.1 and AG3 DNA detected by the 8 samples in each 1 ha area are reported in Table 8.

***R. solani* AG3**

Any detection in pre-plant soil samples of *R. solani* AG3 in a paddock is considered to indicate an elevated risk of disease. Rhizoctonia disease has occurred at sites where inoculum has not been detected in either soil or seed testing. For this reason, it is important to determine the intensity of sampling required at a given confidence level to detect the pathogen, if present in a paddock.

Of the 8 paddocks where testing was conducted, there was only one paddock where *R. solani* AG3 was not detected in any of the 32 samples. In other paddocks at least one sample of the 8 tested from any 1 ha area was above detection. There was no paddock where all samples tested were above the level of detection. This confirms that even in paddocks with high levels of inoculum it is still possible to miss the pathogen, if only 1 sample is collected and tested. The range of levels detected in individual samples taken from the same 1 ha area was large, indicating that a single sample does not provide a useful indication of the inoculum level in a 1 ha area. Even when the highest detection exceeded 100 pg DNA/g soil, the lowest was close to or below the level of detection.

Based on statistical analysis of the data using a non-parametric approach, 2 samples would be required to have an 80% confidence level of detecting the pathogen if present. To achieve a 95% confidence level of detection would require 4 samples to be tested. Taking less samples per paddock is not adequate, as there is an unacceptable risk of failure to warn of the presence of inoculum in a paddock. This is also the case, even if the total area being sampled is 1 ha. This analysis assumes that testing of 32 samples in a paddock will detect the pathogen if present at a level that poses a risk.

***R. solani* AG2.1**

Detection in a sample as low as 2 pg /g soil DNA of *R. solani* AG2.1 in pre-plant soil samples can pose a risk of disease. This guidance based on combined field validation data from this and previous project PT09023.

All 8 paddocks sampled were found to have *R. solani* AG2.1 inoculum. There were only 3 sampling areas (all in the one paddock) where average *R. solani* AG2.1 DNA levels were below 2 pg /g soil DNA. In this paddock, a level of 163 pg /g soil DNA was detected in one sample, indicating that this paddock may still have some small areas (hot spots) of high inoculum where disease may occur if conditions are conducive.

For analysis, it would be preferable to have more than 1 paddock with low inoculum levels. Based on statistical analysis of the data using a non-parametric approach, 1 sample would be required to have an 80% confidence level of detecting the pathogen and 2 samples to achieve a 95% confidence level of detection. Considering variation observed in tests results in paddocks E and F, the standard protocol for PREDICTA Pt for a paddock over 10 ha of at least four tests, each sampled from 1 ha areas, is recommended. The range of levels detected in individual samples taken from the same 1 ha area was often large, indicating that a single sample does not consistently provide a useful indication of the average inoculum level in a 1 ha area. Using four samples will increase the level of confidence in the average inoculum level in the paddock.

This data provides greater confidence than previously found in the ability of the standard PREDICTA Pt sampling protocol for paddocks over 10 ha to detect presence of *R. solani* AG3 and AG2.1. The protocol recommends taking at least four samples, each collected from 1 ha areas within a paddock. Level of confidence in the inoculum level, as opposed to presence or absence, would depend on the consistency of multiple (4 or more) test results from a paddock or sampling area. More than 4 samples should be considered for *R. solani* AG3.

Table 8: Detection rate, range and average level of *Rhizoctonia solani* AG2.1 and AG3 detected by repeated soil sampling (8 times) in the same 1 ha area at 4 locations in each of 8 paddocks (A to H).

Paddock	Sampling area	Soil Inoculum (pg DNA / g soil (*Rhizoctonia solani*))					
		AG2.1			AG3		
		Positive detections out 8	Range of levels detected	Average	Positive detections out 8	Range of levels detected	Average
A	1	8	38 – 557	155	6	0 – 94	29
	2	8	35 – 402	132	8	1 – 468	133
	3	8	5 – 185	79	8	3 – 298	66
	4	8	21 – 228	99	8	1 – 418	105
B	1	8	86 – 538	336	4	6 – 71	31
	2	8	85 – 343	273	4	1 – 164	39
	3	8	16 – 429	124	2	0 – 67	9
	4	8	70 – 1571	519	7	0 – 83	24
C	1	8	8 – 153	46	5	0 – 78	13
	2	8	3 – 100	50	6	0 – 264	40
	3	8	9 – 50	29	7	0 – 94	24
	4	8	2 – 250	74	5	0 – 52	9
D	1	8	142 – 1495	432	5	0 – 11	4
	2	8	168 – 585	364	6	0 – 26	8
	3	8	56 – 570	230	8	2 – 192	55
	4	8	95 – 1300	417	8	4 – 282	73
E	1	6	0 – 67	15	6	0 – 25	7
	2	6	0 – 45	16	6	0 – 17	4
	3	6	0 – 151	56	6	0 – 90	13
	4	6	0 – 141	51	2	0 – 1	0.3
F	1	8	202 – 1585	656	0	0 – 0	0
	2	8	58 – 786	263	0	0 – 0	0
	3	8	49 – 1352	480	0	0 – 0	0
	4	8	8 – 190	71	0	0 – 0	0
G	1	1	0 – 5	0.6	6	0 – 181	26
	2	0	0 – 0	0	6	0 – 254	38
	3	1	0 – 163	20	4	0 – 43	6
	4	4	0 – 6	1	1	0 – 10	1
H	1	8	116 – 1626	530	1	0 – 1	0.2
	2	8	157 – 599	347	2	0 – 10	2
	3	8	282 – 645	414	5	0 – 10	3
	4	8	39 – 597	261	3	0 – 31	5

Common scab

Seed and soil inoculum are both sources of disease risk for common scab.

Seed inoculum

Streptomyces seed inoculum contributed to common scab risk at field validation sites. Inoculum level on seed has been related to incidence of common scab in pot trials (Tegg, 2015). Data collected in this project has increased confidence about the role of seed inoculum in field production. When infected seed (> 1.5 log DNA *Streptomyces* txtA gene) was planted in paddocks where inoculum was not detected in the soil prior to planting, 24% of assessed areas had > 10% incidence of common scab, Figure 39. This compares with 4% of assessed areas when seed with levels below detection were planted.

Planting infected seed (> 1.5 log DNA (*Streptomyces* txtA gene)) into infected paddocks (*Streptomyces* txtA gene detected in soil prior to planting) resulted in a higher proportion of assessed areas (42%) having > 10% incidence of common scab, Figure 40, this compares with 24% when infected seed were planted into paddocks where *Streptomyces* txtA gene was below level of detection in the soil prior to planting.

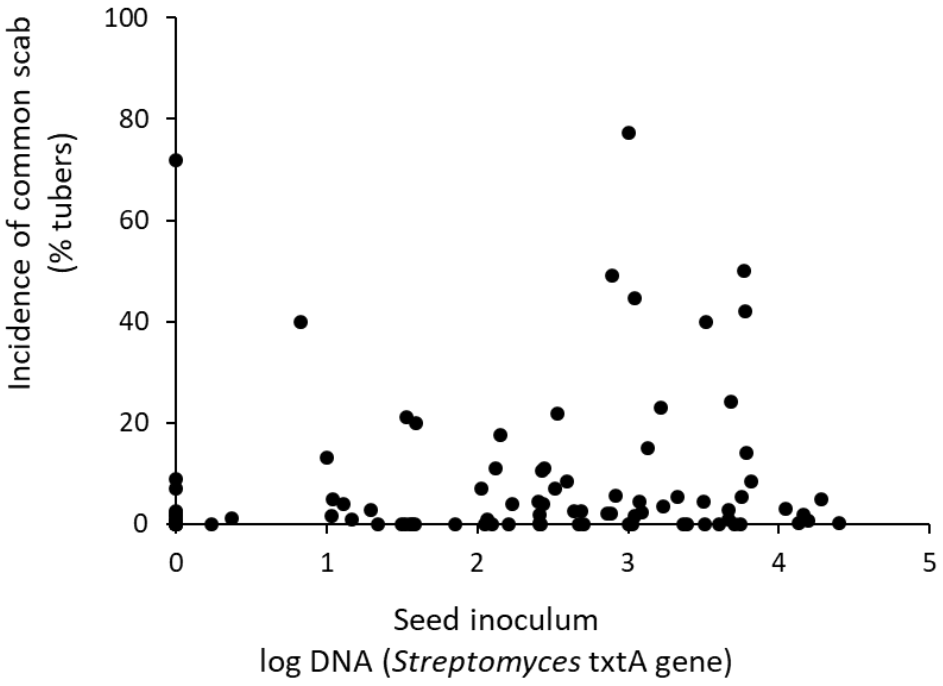

Figure 39: Relationship between inoculum level of *Streptomyces* txtA gene on seed peel and incidence of common scab on harvested daughter tubers when planted at sites where *Streptomyces* txtA gene was not detected in the soil prior to planting. (Averaged data for paddocks where at least two 1 ha areas were assessed for the same seed source).

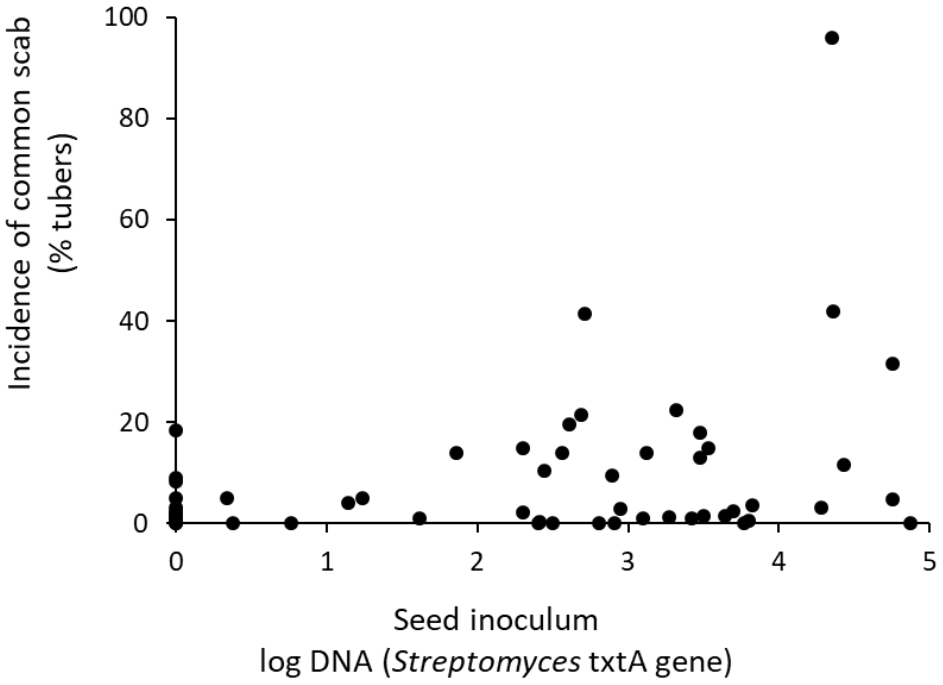

Figure 40: Relationship between inoculum level of *Streptomyces* txtA gene on seed peel and incidence of common scab on harvested daughter tubers when planted at sites where *Streptomyces* txtA gene was detected in the soil prior to planting. (Averaged data for paddocks where at least two 1 ha areas were assessed for the same seed source).

Soil Inoculum

To investigate the contribution of soil inoculum to disease risk, sampling areas where seed known to be infected by pathogenic *Streptomyces* (seed peel DNA testing) or of unknown status were removed from the validation data set. Testing of daughter tubers from these sites indicates that soil inoculum contributes to the level of infection of harvested tubers, with the level of infection rising with increasing level of inoculum in the soil, Figure 41.

Presence of soil inoculum in a paddock was associated with a higher probability of common scab occurring. In paddocks where inoculum of pathogenic *Streptomyces* was detected in the soil prior to planting but not on the seed planted, 27% of paddocks had > 5% incidence of common scab, Figure 42. This compares with 15% of assessed paddocks when inoculum was not detected in soil prior to planting. Incidence of common scab was higher when infected seed was planted, Figure 43.

Pathogenic *Streptomyces* was detected in peel of harvested tubers from 75% of locations where inoculum was not detected on seed or in the soil prior to planting. This indicates that inoculum was present in the soil on seed tubers at these locations. At one site this translated to a very high incidence of common scab, Figure 42.

Four grid trials were conducted in South Australia. Inoculum of pathogenic *Streptomyces* in soil and peel of seed tubers were measured prior to planting. Pathogenic *Streptomyces* was detected in peel of the innovator variety seed planted at all sites, with levels varying from 2.3 to 3.8 log DNA *Streptomyces* txtA gene, Table 6 on page 44. Inoculum was detected in the soil in all plots at sites B, C and D and ranged from below detection to 2.2 log DNA *Streptomyces* txtA gene at site A. Common scab occurred at all sites, Figure 44. As seed and soil inoculum were confirmed to be present at all sites, both sources of inoculum could have contributed to disease incidence at each site. The incidence of common scab was lower at site A, which had the lowest average preplant soil inoculum levels of pathogenic *Streptomyces*. Additionally, this site had variations in drainage and soil moisture which effected which plots developed common scab, with the highest incidence in the plots located in driest areas of paddock.

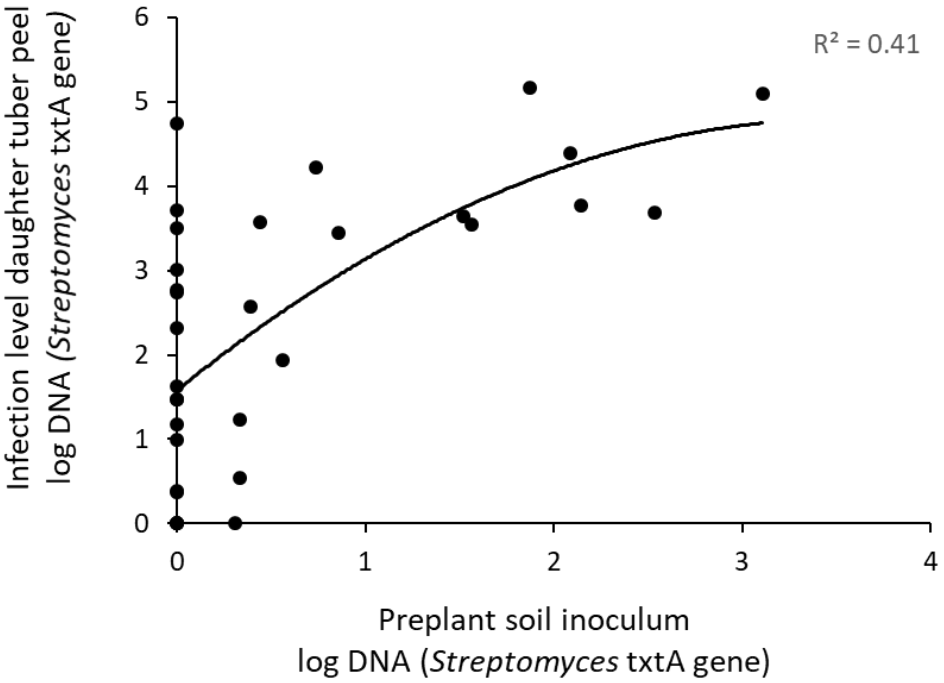

Figure 41: Relationship between inoculum level of *Streptomyces* txtA gene in the soil prior to planting and infection level of daughter tubers when seed planted had below detectable levels of *Streptomyces* txtA gene. (Averaged data for paddocks where at least two 1 ha areas were assessed).

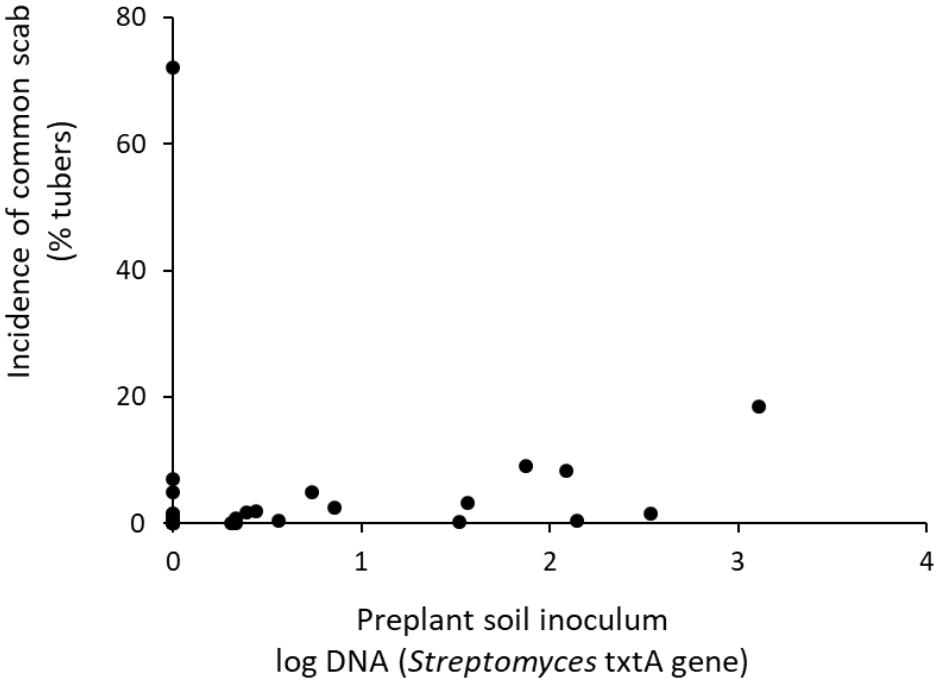

Figure 42: Relationship between inoculum level of *Streptomyces* txtA gene in the soil prior to planting and incidence of common scab on daughter tubers when seed planted had below detectable levels of *Streptomyces* txtA gene. (Averaged data for paddocks where at least two 1 ha areas were assessed).

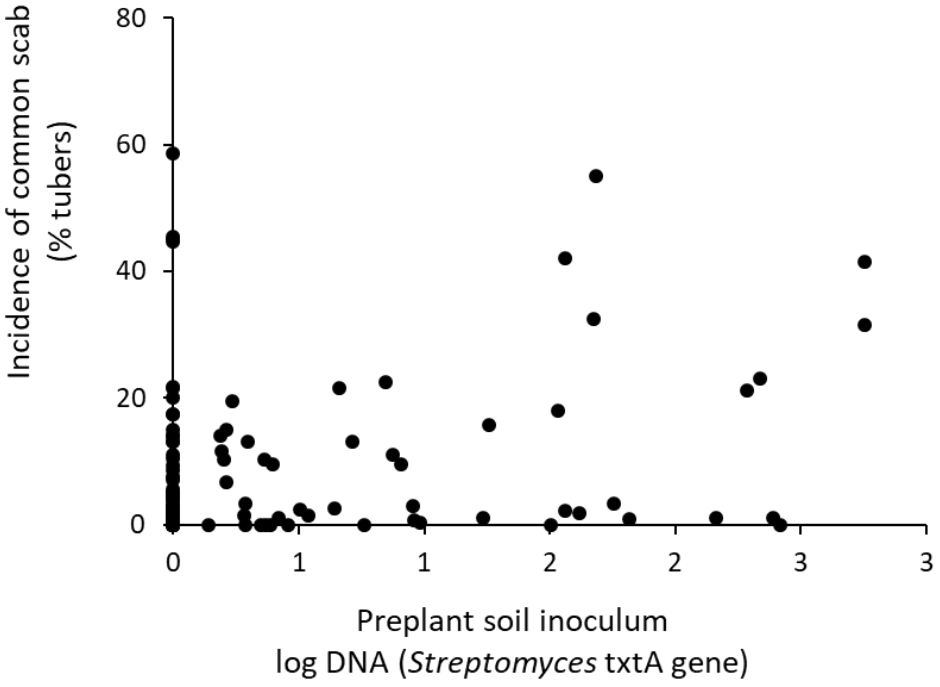

Figure 43: Relationship between inoculum level of *Streptomyces* txtA gene in the soil prior to planting and incidence of common scab on daughter tubers when seed planted had detectable levels of *Streptomyces* txtA gene. (Averaged data for paddocks where at least two 1 ha areas were assessed).

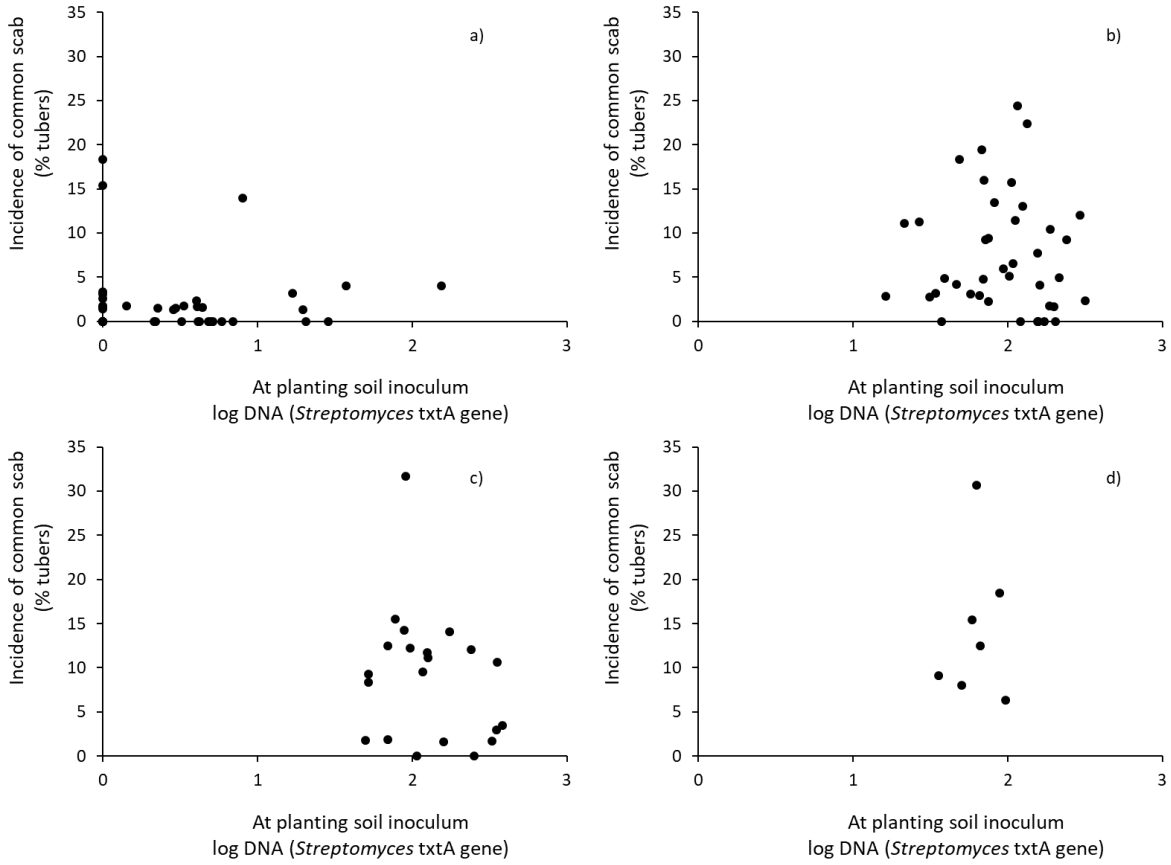

Figure 44: Relationship between inoculum level of *Streptomyces* txtA gene in the soil of grid plots at time of planting and incidence of common scab on daughter tubers at four sites (A-D) in South Australia (a,b,c,d respectively) planted with Innovator variety potatoes.

Evaluation of repeated sampling

Eight paddocks with history of potato production were selected and tested to investigate the number of samples in a 1 ha area and in four 1 ha areas within a paddock required to be confident of detecting the pathogen that causes common scab. Testing for *Streptomyces* txtA gene was conducted on the same samples collected to investigate detection of the pathogen that causes pink rot (refer page 30), plus 1 additional site.

The detection rate, range in level and average level of *Streptomyces* txtA gene DNA detected by the 8 samples in each 1 ha area are reported in Table 9.

Any detection in pre-plant soil samples of *Streptomyces* txtA gene DNA in a paddock is considered to indicate an elevated risk of disease. Common scab has occurred at sites where inoculum has not been detected in either soil or seed testing. For this reason, it is important to determine the intensity of sampling required at a given confidence level to detect the pathogen if present in a paddock.

Detection in the 8 paddocks selected was infrequent. There were two paddocks where *Streptomyces* txtA gene DNA was not detected in any of the 32 samples. In other paddocks, the number of detections out of 32 samples tested was 5 or less and the highest detected level was 71 pg / g sample DNA of *Streptomyces* txtA gene. Levels of inoculum in these paddocks were at the limit of detection of the *Streptomyces* txtA gene DNA test.

Based on statistical analysis of the data using a non-parametric approach, 27 samples would be required to have an 80% confidence level of detecting the pathogen if present. To achieve a 95% confidence level of detection would require 50 samples to be tested. Four samples (standard protocol for PREDICTA Pt testing in paddocks over 10 ha) is not enough to be confident of detecting pathogenic *Streptomyces* when present at low levels in a paddock.

To test consistency of detection in a paddock with known higher levels of pathogenic *Streptomyces* another

paddock was sampled. Sampling was the same as above except the four separate 1 ha areas were each sampled 4 times, giving a total of 16 samples for the paddock. The detection rate, range in level and average level of *Streptomyces* txtA gene DNA detected by the 4 samples in each 1 ha area are reported in Table 10. Based on consistency of detection levels the data suggests 4 samples in a 1 ha area was adequate to detect and provide a reasonable estimate of inoculum level in the selected paddock.

Table 9: Detection rate, range and average level of *Streptomyces* txtA gene detected by repeated soil sampling (8 times) in the same 1 ha area at 4 locations in each of 8 paddocks (A to H).

Paddock	Sampling area	Soil Inoculum (pg DNA / g soil (*Streptomyces* txtA gene))		
		Positive detections out 8	Range of levels detected	Average
A	1	0	0 – 0	0
	2	0	0 – 0	0
	3	0	0 – 0	0
	4	2	0 – 71	10
B	1	1	0 – 1	0.1
	2	0	0 – 0	0
	3	0	0– 0	0
	4	1	0 – 1	0.1
C	1	1	0 – 2	0.2
	2	0	0 – 0	0
	3	2	0 – 5	0.6
	4	0	0 – 0	0
D	1	0	0 – 0	0
	2	0	0 – 0	0
	3	0	0 – 0	0
	4	0	0 – 0	0
E	1	1	0 – 1	0.1
	2	0	0 – 0	0
	3	0	0 – 0	0
	4	0	0 – 0	0
F	1	0	0 – 0	0
	2	0	0 – 0	0
	3	0	0 – 0	0
	4	0	0 – 0	0
G	1	1	0 – 5	0.6
	2	0	0 – 0	0
	3	1	0 – 14	2
	4	2	0 –35	4
H	1	4	1 – 6	2
	2	0	0 – 0	0
	3	0	0 – 0	0
	4	0	0 – 0	0

Table 10: Detection rate, range and average level of *Streptomyces* txtA gene detected by repeated soil sampling (4 times) in the same 1 ha area at 4 locations in paddock with high inoculum level of *Streptomyces* txtA gene in South Australia.

Site	Sampling area	Soil Inoculum (pg DNA / g soil (*Streptomyces* txtA gene))		
		Positive detections out 4	Range of levels detected	Average
D	1	4	53 – 103	79
	2	3	0 – 51	16
	3	4	27 – 130	89
	4	4	36 – 90	74

Evaluation of tests for other pathogens not developed in this project

Tests for *Pythium* clade I, *Pythium* clade F, *Macrophomina phaseolina*, *Botrytis cinerea* and *Sclerotinia sclerotiorum/minor* were used to test samples from specific regions and growers where the pathogens are suspected to cause tuber rots. The potential need for these tests was identified as part of expanding the service into additional growing regions.

Leak rot

Leak rot of potatoes is caused by *Pythium* spp., normally occurring late in the growing season and when potatoes harvested under hot conditions and not cooled promptly. *Pythium* clade I test detects multiple species including *Pythium ultimum*, *P. debaryanum*, *P. splendens* and *P. heterothallicum*. The main species that causes leak rot is *P. ultimum*. Leak rot type symptoms of potatoes have been associated with *P. debaryanum* and *P. splendens*, but *P. heterothallicum* has not been associated with causing leak rot or other diseases of potatoes. Leak rot may also be caused by *P. aphanidermatum*, which is not detected by the *Pythium* clade I test.

At a property where substantial tuber rotting caused by *Pythium* spp. had occurred in a paddock the levels of inoculum in the soil ranged from 2.4-3.0 log DNA (*Pythium* clade I), compared to other paddocks cropped at the same time on the property that had levels from below detection to 1.3 log DNA (*Pythium* clade I). These results were consistent with paddock history and confirmed presence of elevated pathogen levels in a paddock more than 12 months after an infected crop had been harvested.

Tissue from rotted tubers was routinely tested as part of the validation of the pink rot test. Presence of pathogens that cause pink rot was confirmed at high levels with the *Phytophthora* EDC DNA test in most of these samples. However, some primary symptoms were atypical of pink rot, and suspected to be caused by *Pythium* spp.. In these samples, *Pythium* clade I was detected at high levels.

Along with collection of data in this project, retesting of stored DNA was conducted on samples collected in South Australia during the 2012/13 season. In that season a 1 ha validation site was noted as having *Pythium* rots. *Pythium* clade I was high in the preplant soil sample from this area (2.9 log DNA) and in the composite peel sample of harvested tubers (2.5 log DNA). Overall preplant testing in 1 ha areas was a poor indicator of risk of infection of daughter tubers, Figure 45. However, when average test results of four samples in a paddock are used, high pre-plant inoculum levels in the soil are associated with higher infection levels of harvested tubers, Figure 46. Further work is required to validate against incidence of leak rot, confirm *Pythium* spp. involved, determine if a more species-specific test is beneficial, refine sampling requirements and develop categories for disease risk.

A test for *Pythium* clade F which includes *Pythium irregulare, P. sylvaticum, P. debaryanum, P. spinosum, P. paroecandrum* and *P. mamillatum* (developed with support from MLA) has also been used throughout this project. Interpreting these results is complicated due to the number of species detected, and their differing pathogenicity on potatoes. *Pythium* clade F was often detected in soil prior to planting and in seed and harvest peel samples. No clear associations or relationships were observed between these levels and tuber symptoms or yield loss in this project.

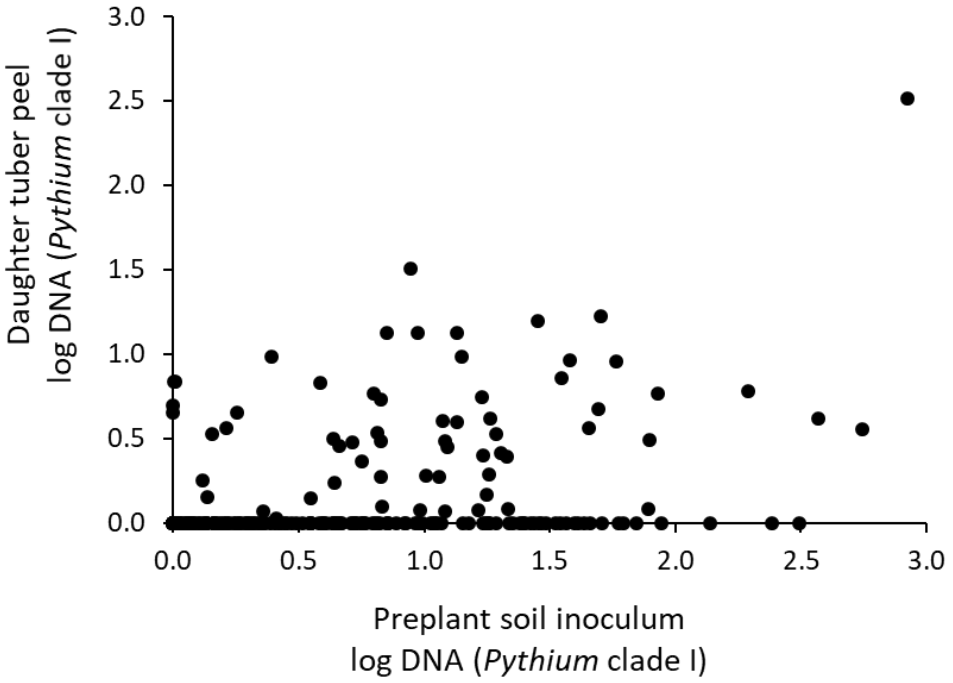

Figure 45: Relationship between inoculum level of *Pythium* clade I in the soil prior to planting and infection level of *Pythium* clade I of harvested daughter tubers. (Data from individual 1 ha areas).

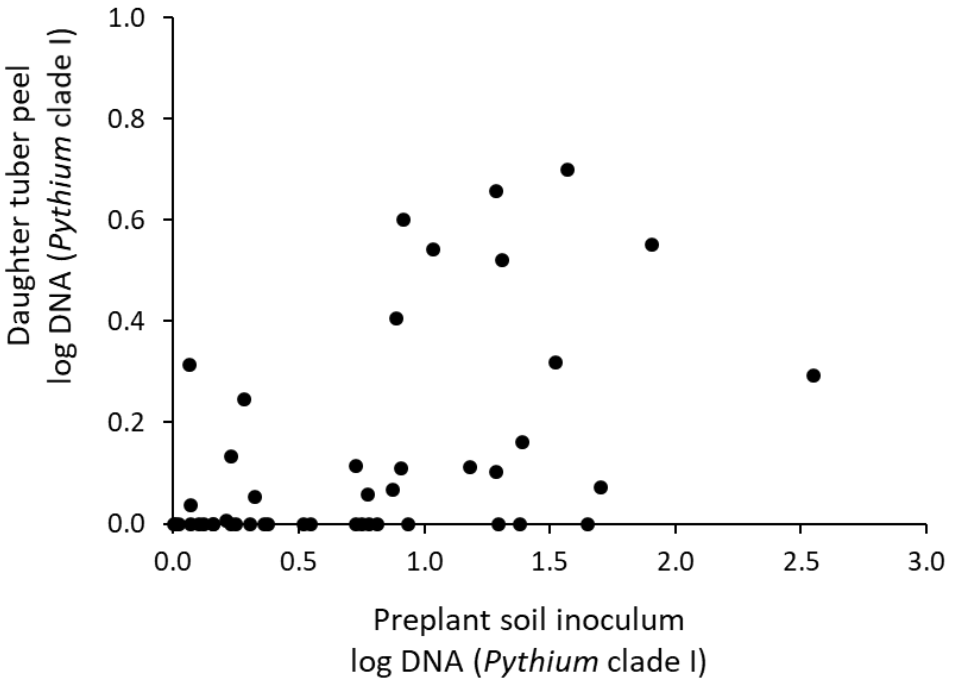

Figure 46: Relationship between inoculum level of *Pythium* clade I in the soil at time of planting and infection level of *Pythium* clade I in harvested daughter tubers. (Data is average of four 1 ha areas in each paddock).

Charcoal rot

Macrophomina phaseolina causes charcoal rot in many crops, including potatoes. A test for *Macrophomina phaseolina* (developed with support from GRDC) has been run throughout this project and was specifically requested by potato growers on several occasions. The pathogen is commonly detected in soil samples from potato paddocks in warm production regions; it is less common in cool temperate regions. The data obtained shows both pre-plant soil inoculum levels and seed inoculum levels are linked to levels on tuber peel at harvest, Figure 47 and Figure 48 respectively. When seed with high levels of inoculum were planted into soils with moderate to high levels inoculum, infection levels on daughter tubers were higher than when clean seed was planted. Both soil and seed inoculum appear to be contributing to tuber infection levels.

Charcoal rot on potato tubers is not well documented in Australia, but this data indicates that soil and seed inoculum are likely to be useful indicators of disease risk. Limited testing of root systems detected *Macrophomina phaseolina* in samples collected from paddocks where inoculum was present. Insufficient data was generated to make conclusions on the effect of *Macrophomina phaseolina* infection on yield of potatoes.

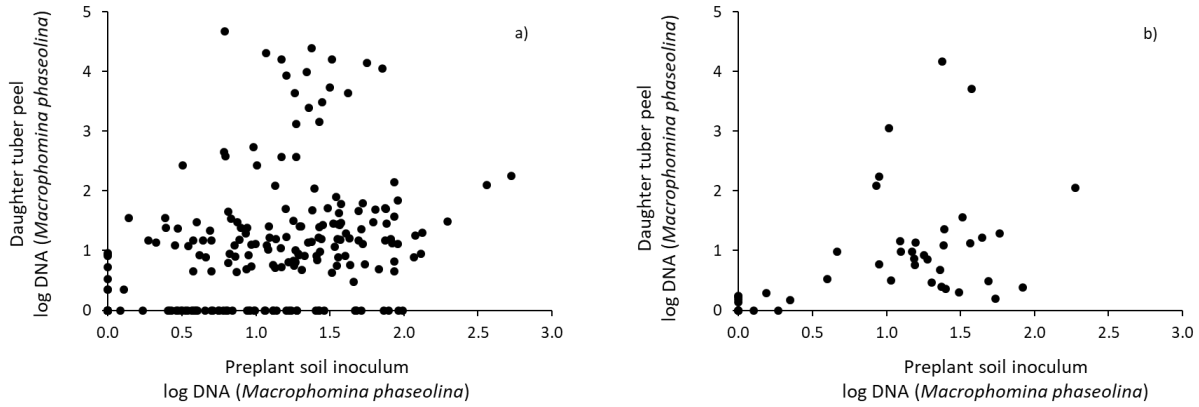

Figure 47: Relationship between inoculum level of *Macrophomina phaseolina* in the soil prior to planting and infection level harvested daughter tubers, a) data from individual 1 ha areas, b) average of four 1 ha areas in each paddock.

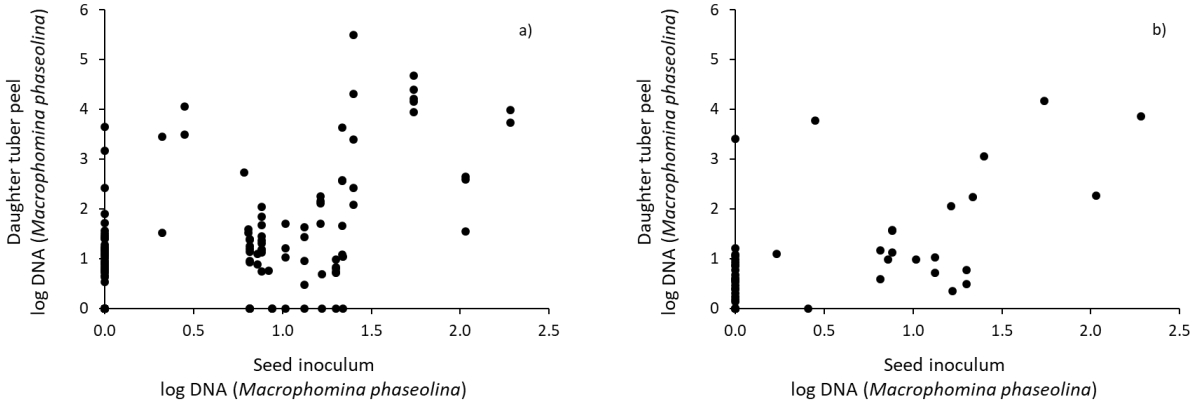

Figure 48: Relationship between inoculum level of *Macrophomina phaseolina* in the peel of seed tubers and infection level in harvested daughter tubers, a) data from individual 1 ha areas, b) average data for paddocks where same seed was planted in at least two 1 ha areas.

Sclerotinia stem rot

Sclerotinia stem rot of potatoes can be caused by *Sclerotinia sclerotiorum* and *S. minor*. A combined test for both species (developed with support from GRDC) has been run on selected samples to generate information on distribution and data within potato production systems to calculate population density categories. The pathogen was detected in soil samples from paddocks prior to potato production in all regions across Australia. As expected, detections in peel of seed and daughter tubers of the *Sclerotinia sclerotiorum/minor* DNA were rare. Limited testing of stem samples from infected crops was conducted to confirm symptoms. While some observations were made of the presence of sclerotinia stem rot in paddocks, no specific validation data was collected for the *Sclerotinia sclerotiorum/minor* test.

Botrytis

Botrytis cinerea causes grey mould, has a wide host range and is present in many agricultural production systems. It is not considered a major pathogen of potatoes in Australia but can cause leaf symptoms and tuber rots. In some southern regions*, Botrytis cinerea* was consistently detected in soil and peel samples of seed tubers and commercial crops. It is also widely detected in soil samples from these regions. Based on the limited data collected, it was not associated to tuber symptoms or rots assessed in this project.

Evaluation of transect sampling

Sampling in radial transects is the method developed for pre-plant risk assessment of onion stunt and reduced productivity in onion pivots caused by *Rhizoctonia solani* AG8 and *Pratylenchus neglectus*. Some find this method of sampling more convenient than sampling four 1 ha areas in a W pattern, which is the protocol recommended for PREDICTA Pt in potato pivots. The two techniques were compared in 4 pivots in South Australia used for potato production.

In each pivot 12 equally distributed radial transects were sampled around 4 pivots. Each of the radial transects consisted of 30 cores (15cm depth by 1 cm diameter) sampled at an even spacing, excluding the centre and outer pivot spans. This produced 3 sets of 4 samples taken in a cross pattern over the pivot area. At pivot site F a 30-core sample was taken in a W pattern from a 1 ha area mid-way along each radial transect. At other pivots a set of 4 samples was taken in a W pattern from a 1 ha area in the centre of each ¼ of the pivot. At pivot site D each of the four 1 ha areas was resampled 4 times.

For pathogens where PREDICTA Pt soil results are reported with an indication of disease risk (*Spongospora subterranea*, *Colletotrichum coccodes*, *Verticillium dahliae* and *Meloidogyne fallax* both techniques were found to provide similar results, Table 11. Levels of *M. javanica/incognita/arenaria* were below detection in all samples.

Increasing the sampling intensity, compared with the standard PREDICTA Pt protocol resulted in detection of *M. fallax* in a single soil sample in two paddocks. Follow up testing in the paddock where the single *M. fallax* detection was high enough to be of concern (71 pg DNA / g soil) confirmed presence of *M. fallax* in the area where it was detected by a transect sample. The level of *M. fallax* infestation of the potato crop subsequently grown at this site was negligible.

When root lesion nematodes *P. neglectus* and *P. penetrans* were present at low population in the sampled pivots, using 4 radial transects improved sensitivity and consistency of detection, compared with sampling 4 by 1 ha areas in a W pattern. Based on data from these four pivots neither method was better than the other for detecting *Streptomyce*s txtA gene, *Rhizoctonia solani* AG3 and AG2.1.

Phytophthora erythroseptica/drechsleri/cryptogea and *Helminthosporium solani* were below the level of detection in all soil samples tested from these pivots.

The data reaffirmed the current PREDICTA Pt soil sampling protocol and supports the recommendation to test at least 4 samples per paddock when assessing the level of inoculum of soilborne pathogens in paddocks of 10 ha and above. It also indicates that sampling a pivot using four transects is an acceptable alternative to sampling four 1 ha areas in a W pattern.

Table 11: Average pathogen DNA levels of 4 samples taken in radial transects in each ¼ of the pivot compared with the recommended PREDICTA Pt sampling, i.e. 4 samples taken in a W pattern in 1 ha areas in the centre of each ¼ of the pivot. (Sampling strategies repeated 1-4 times = sets)

Pathogen	Pivot	Soil Inoculum level						
		Radial transects			1 ha W's			
		Set 1	Set 2	Set 3	Set 1	Set 2	Set 3	Set 4
Spongospora subterranea pgDNA/g soil	D	1	4	3	3	4	4	8
	F	Not tested						
	B	125	107	85	126			
	E	1934	1767	2603	1926			
Colletotrichum coccodes pgDNA/g soil	D	284	234	308	142	218	110	147
	F	4	2	BD	1	BD	0.4	
	B	142	82	187	148			
	E	241	249	277	236			
Meloidogyne fallax pgDNA/g soil	D	28	45	38	22	11	18	10
	F	BD	BD	BD	BD	BD	BD	
	B	18	BD	BD	BD			
	E	BD	BD	1	BD			
Verticillium dahliae pgDNA/g soil	D	26	26	28	14	23	21	21
	F	1	0.2	BD	0.2	0.5	BD	
	B	46	51	60	51			
	E	5	3	4	5			
Rhizoctonia solani AG2.1 pgDNA/g soil	D	27	54	92	62	102	71	132
	F	6	5	1	2	0.5	BD	
	B	6	6	BD	0.3			
	E	BD	BD	BD	BD			
Rhizoctonia solani AG3 pgDNA/g soil	D	BD	BD	BD	BD	BD	BD	BD
	F	1	1	BD	BD	19	0.3	
	B	BD	BD	BD	0.2			
	E	BD	BD	BD	BD			
Streptomyces txtA gene pgDNA/g soil	D	136	97	86	58	85	70	45
	F	BD	BD	BD	BD	BD	BD	
	B	38	28	50	40			
	E	BD	BD	1	BD			
Pratylenchus crenatus nematodes/g soil	D	0.1	0.2	0.2	0.2	0.2	0.2	0.2
	F	BD	BD	BD	BD	BD	BD	
	B	BD	BD	BD	BD			
	E	0.3	0.4	0.4	0.4			
Pratylenchus neglectus nematodes/g soil	D	0.2	0.1	0.6	BD	BD	BD	BD
	F	1.2	0.9	0.8	0.9	0.5	1.3	
	B	0.5	0.4	0.6	0.8			
	E	0.1	BD	0.1	BD			
Pratylenchus penetrans nematodes/g soil	D	0.4	0.1	0.1	0.02	0.04	0.02	BD
	F	BD	BD	BD	BD	BD	BD	
	B	3.3	3.4	4.6	3.5			
	E	BD	BD	BD	BD			

Appendix 2: Guide – Testing for Seedborne and Soilborne Pathogens