Hort Innovation

Final Report

Improved soilborne disease diagnostic capacity for the Australian Vegetable Industry

Project leader:

Michael Rettke

Delivery partner:

South Australian Research and Development Institute

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Improved soilborne disease diagnostic capacity for the Australian Vegetable Industry VG15009

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Level 7
141 Walker Street
North Sydney NSW 2060

Telephone: (02) 8295 2300

www.horticulture.com.au

Content

Summary

Soil-borne diseases are a major constraint for the Australian vegetable industry. This project developed DNA based tests for quantifying key pathogens and evaluated their use as a tool for pre-plant risk assessment, monitoring of inoculum levels, and symptom testing. The DNA tests are available through SARDI's PREDICTA research service.

Focus of DNA test development and validation was clubroot, pathogens causing cavity spot, root knot and root lesion nematodes in order to develop testing services of relevance to brassica and carrot growers.

A new DNA based preplant soil test has been developed and validated to assess the risk of clubroot in brassica crops. Pre-plant soil DNA concentration of *Plasmodiophora brassicae* is linked to the risk of yield loss of susceptible brassica crops. The severity of clubroot observed ranged from no measurable crop loss to crop failure. Environmental factors such as warm growing conditions, low soil pH, soil compaction and inadequate drainage can elevate the risk of clubroot and need to be taken into account when interpreting DNA soil testing results. This test to assess the risk of clubroot is ready for use and pre-commercial adoption has commenced with brassica growers.

Two new DNA based preplant soil tests have been developed and evaluated for assessing the risk of cavity spot in carrot crops. In Australia, *Pythium sulcatum* is the most common cause of cavity spot. The preplant test developed indicated the presence of *P. sulcatum* in a paddock and its presence was associated with a risk of cavity spot occurring. Multiple soil samples, even in small paddocks of 2 ha, are required for adequate detection of this pathogen prior to planting.

A test was also developed for *P. violae*, considered the major cause of cavity spot overseas, but only previously reported in a few locations in Australia. Testing undertaken in this project established *P. violae* is more widespread than thought, particularly in cool climate production areas of Australia. Results suggest the threshold for disease risk posed by *P. violae* in susceptible varieties may be below the level of detection of the DNA test. Pre-plant soil testing failed to detect *P. violae* in a number of paddocks in which a small percentage of carrots developed symptoms of cavity spot. Testing of soil after harvest from areas where cavity spot occurred detected the pathogen, but at low levels.

Evaluation of DNA tests for measuring pre-plant root knot and root lesion nematode populations has been conducted with growers and agronomists. This has primarily been in carrot, soil grown greenhouse crops, field grown capsicums and sweet potatoes. Testing in the project has highlighted presence of nematodes at potentially damaging levels in a number of other crops including brassicas. The DNA based tests are species specific with similar or better detection levels when compared with traditional nematode counts. Sampling methods are critical when assessing the risk of nematode damage, especially for root knot nematode where thresholds for sensitive crops are close to the level of detection.

Preplant soil testing and crop assessments were conducted in South Australia, Queensland, Victoria, Tasmania and Western Australia as part of this project. Carrots and brassicas were the primary focus, with testing also conducted in capsicum, green bean, sweet corn and sweet potato production systems.

Further information on how DNA testing can assist in managing the risk of soilborne diseases of vegetables is available in the guide to Testing for Soilborne Pathogens of Vegetables.

Keywords

Soilborne disease; Vegetables; Diagnostics; DNA; PREDICTA

Introduction

Soil-borne diseases are a major limiting factor for the Australian vegetable industry and are both costly and difficult to manage. Previous research highlights that practical and economic methods of disease control are limited once the crop has been established. Knowing the disease risk prior to planting provides the grower with the knowledge to make informed decisions while the full range of available options can be implemented including the option of not planting, if risk cannot be managed.

In a review of soilborne disease management in Australian vegetable production, McMichael (2012) suggested that growers at present do not have sufficient knowledge of some risk factors and influences on them; nor of the most timely and economic responses appropriate for their farming system. It was stated that risk assessment must underpin planting decisions and in the absence of informed risk assessment, vegetable growers are limited in their capacity to choose and integrate the most appropriate and reliable management options for their farming system. Various disease risk prediction tools have been developed for vegetable crops, however most involve detailed knowledge of risk factors and cropping history, or involve time consuming and expensive testing regimes, leading to low uptake by growers. With the advent of soil DNA testing, fast, robust, multi target quantification of pathogen levels is possible. Access to this technology has opened up new opportunities at both an on-farm level and for research uses that were previously not feasible.

Wilson (2015) conducted a project (VG13077 - Soil-Borne Disease Management Diagnostics) to identify and prioritise research and development projects that will develop cost-effective quantitative diagnostics tools for improved prediction of the risk of major soil-borne diseases for the Australian vegetable industries. As part of this project target pathogens were identified as being feasible and economically justifiable to develop DNA based soil tests to quantify the pre-plant risk of soil-borne disease.

Recommendations for the development of specific pathogen tests in a future project as reported were:
In year 1 - *Meloidogyne* spp.; In years 1 – 3 - *Pratylenchus* spp., *Rhizoctonia solani* (various AG groups), *Verticillium dahliae* and *Plasmodiophora brassicae*, In years 3 – 5 - *Sclerotinia sclerotiorum*, *S. minor*, *Olpidium virulentus*, *Pythium aphanidermatum*, *P. sulcatum*, *P. violae*, *Botrytis cinerea* and *Thielaviopsis basicola*).

Providing a useful pre-plant testing service for the risk of soil-borne diseases requires: the capacity to reliably detect inoculum of the target pathogen(s) in the soil at levels below the threshold for economic damage; understanding of the role of environmental (including soil health) and host factors in disease expression; establishing risk categories for test results; and providing options to manage the quantified risk.

The aim of this project was to use DNA technology to establish relationships between levels of specific pathogens at or before planting and the amount of disease that develops in crop. Key targets for test development and evaluation were the pathogens that cause clubroot of brassicas, cavity spot of carrots, along with root knot nematodes, root lesion nematodes and Rhizoctonia which impact a wide range of vegetables. Information generated will contribute to underpinning development of a commercial service, including a guide to growers and their advisors on how to sample and use the service to develop management strategies to minimize the risk of soil-borne diseases affecting vegetable crops.

Methodology

Soil-borne diseases are a major constraint for the Australian vegetable industry. This project developed new DNA based tests and evaluated them along with pre-existing DNA based tests as a tool for pre-plant risk assessment of soilborne diseases, monitoring of inoculum levels, and symptom testing.

SARDI delivers commercial tests to the grains industry (PREDICTA B) and potato industry (PREDICTA Pt). These services deliver validated tests for soilborne pathogens of winter cereals and potatoes. In addition there are a range of DNA tests not yet fully validated, for example tests for free living nematodes, and these are offered as research test panels. A new test panel named HORT Veg was developed for use in this project, and as a starting point to establish a commercially available pathogen testing service for the vegetable industry. Tests included in the panel were selected in consultation with growers, agronomists and horticultural plant pathologists. Findings of a vegetable disease and crop prioritisation process conducted in Horticulture Innovation project VG15010 was utilised in compiling the panel along with the recommended pathogen targets set out in VG13077 that guided development of this project. Initially the HORT Veg panel included 20 pathogen tests, and through this project's timeframe was expanded to 26 pathogen tests.

Plasmodiophora brassicae, *Pythium violae* and *Pythium sulcatum* were identified in the project proposal for test development. Assays were developed using TaqMan® MGB assays (real-time qPCR assays) in line with other assays included in SARDI's PREDICTA services. Developed assays were checked for specificity against a DNA collection which included target and non-target organisms. To confirm the specificity observed with DNA from pure cultures, assays were then used to assess field soil and plant tissue samples. Finally, the next generation sequencing (NGS) methodology developed by SARDI MDC to validate new qPCR assays was used to verify assays specifically to detect their respective target in selected set of field soil samples, and that no cross reactions were observed. Calibration standards and PCR reagents were then prepared, and the tests incorporated into SARDI's PREDICTA delivery platform to enable routine assessment of samples.

Preplant soil testing was conducted in paddocks, centre pivots and greenhouses of growers in South Australia, Queensland, Victoria and Tasmania. This included samples prior to 40 carrot plantings, 55 brassica plantings, 120 greenhouse crops (mainly capsicum), 9 field grown capsicum plantings, 21 sweet potato plantings, 9 green bean plantings, 10 sweet corn plantings. Carrot and brassica crops were the main focus of field validations. Standard protocols were established for each of the crops and growing situations. Soil sampling strategies all involved collection of 30 cores (15cm deep by 1cm diameter). Samples taken prior to carrot, brassica, green bean and sweetcorn crops were tested with the HORT Veg panel, with samples prior to capsicum, eggplant, cucumber and sweet potato tested with the Potato research panel. In paddocks to be planted with carrots or brassicas, a free-living nematode panel test, a soil nutrition test and a soil respiration test were conducted.

For carrots, cavity spot and other soilborne diseases and disorders were assessed on 100 carrots systematically sampled from each soil sampling area, after being lightly washed. After assessment, peel was removed from 20 carrots for pathogen DNA testing. Representative symptoms requiring diagnosis (including for cavity spot) from plantings in South Australia and Victoria were tested either by traditional diagnostic techniques and/or pathogen DNA testing. Techniques were developed during the project for DNA testing of symptoms and from bulked peel samples. To assess if preplant soil pathogen testing was providing an indication of disease risk, the relationships between preplant soil pathogen levels, incidence and severity of symptoms and pathogen levels in the peel of harvested carrots were examined.

For brassicas, plant growth and the incidence of soilborne diseases was observed during crop growth (100 plants) and the incidence and severity of clubroot assessed during or at the end of the crop cycle. Clubroot assessment was conducted by digging up plants and assessing the root system on 50 to 100 plants (20 in Victorian sites). After assessment, a subsample of the stem and root system was removed from 10 representative plants for pathogen DNA testing. Representative symptoms requiring diagnosis from samples in South Australia were tested either by traditional diagnostic techniques and/or pathogen DNA testing. Techniques were developed during the project to refine the DNA testing methods for symptoms and peel samples. To assess if preplant soil pathogen testing was providing a useful indication of disease risk, the associations between preplant soil pathogen levels, incidence and severity of symptoms and pathogen infection levels in roots of plants were examined.

In greenhouse grown crops, soil fumigation was normally conducted after pre-plant soil testing. For this reason assessments were focused on refining sampling after fumigation to confirm nematode populations were

effectively reduced; monitoring buildup after 3 months as an in-crop monitoring technique; end of crop testing to assess nematode populations going into the following crop. Similarly, soil fumigation was practiced on most field grown capsicum and sweet potato crops.

For bean and sweet corn crops the emphasis was on destructive in-crop assessments of plant growth, root and plant health, and the incidence and severity of soilborne diseases for which DNA assays are available for the pathogens that cause them. When present, the occurrence of soilborne diseases was recorded. Selected samples from areas of paddocks with reduced growth and or poor root health were sampled and pathogen DNA tested.

Pot trials were conducted in Tasmania to investigate capability of *Pythium sulcatum* test to quantify levels in a series of samples with increasing amounts of added inoculum, and to then investigate relationship of inoculum level with incidence of cavity spot on carrots.

At each of two carrot sites productivity parameters were mapped against pre-plant soil test results from a series of over 40 small plots. At site 1, the target pathogens were *Pythium sulcatum* and *Pratylenchus thornei*. At the second site the target pathogen was *Pythium violae*.

At two brassica sites the incidence and severity of clubroot was mapped against pre-plant soil test results for *Plasmodiophora brassicae*. At site 1, a series of 20 small plots were assessed and at site 2, testing was conducted within 48 large plots.

To determine the sampling intensity required to detect root knot nematodes when present at low levels, a sampling trial was conducted in 6 paddocks in Tasmania. In each paddock, 4 separate 1ha areas were re-sampled 8 times, with soil samples DNA tested to quantify levels of *Meloidogyne fallax* and *M. hapla*.

In addition to the project managed field validation program, the project investigator worked with commercial growers and their agronomists wishing to utilize pathogen DNA testing. This has included testing in a wide variety of vegetable crops. Assessing inoculum levels of root knot nematodes, root lesion nematodes and *Plasmodiophora brassicae* prior to planting has been the largest area of interest. This one on one interaction provided valuable insight into how growers and agronomist are likely to use the testing and what aspects are important for the technology to be adopted.

Outputs

Service establishment (Refer to Appendix 1 for further details)

- New test panel (HORT Veg) established specifically for use by the vegetable industry.
- Existing PREDICTA Pt service modified so can be used for wider range of solanaceous crops and other crops, particularly where root knot nematodes are the primary target of testing.
- DNA testing methods for assessing pathogen levels in carrot peel, sweet potato peel, and vegetable root systems established, with assays available for testing of samples by researchers and industry.
- Tests are available to growers through SARDI's PREDICTA research testing services.

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

New DNA tests developed for priority vegetable pathogens

- DNA test designed for *Plasmodiophora brassicae* (causal agent of clubroot of brassicas) and developed as soil 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 tests designed for *Pythium sulcatum* and *Pythium violae* (causes of cavity spot of carrots) and developed as soil test in SARDI PREDICTA format, capability to detect pathogens in soil and plant tissue samples confirmed, with assays available for routine assessment of samples.

Thresholds and sampling to improve detection

- Thresholds for *P. brassicae* soil DNA test established to assess the risk of clubroot in brassicas crops prior to planting.
- Soil sampling requirement to test for the presence of *Pythium sulcatum* in a paddock prior to planting established. Detection of *P. sulcatum* prior to planting associated with an increased risk of cavity spot caused by this pathogen.
- Soil sampling methodologies evaluated and confirmed for assessing risk in crops to economic damage from root lesion nematodes.
- Soil sampling methodologies evaluated and refined for assessing risk in crops sensitive to economic damage from low levels root knot nematodes.

Challenges to adequate detection identified

- Challenge of detecting at the low levels of inoculum in soil that may pose a disease risk identified. *Pythium violae* detected in soil after harvest, where cavity spot occurred, but pre-plant risk assessment for *P.violae* remains a challenge.

Service delivery

Testing of samples and interpretation provided to assist other vegetable research and development activities:

- VG15010 and VG16068 - monitoring of demonstration and trial sites being conducted;
- VG15066 - identification and quantification of pathogen DNA levels in carrot samples;
- PW17001 - identification and quantification of pathogen DNA levels in sweet potato samples;
- pre-plant testing of soil samples to characterize pathogen inoculum levels at sites; in crop testing to monitor increases in pathogen activity; specific testing within trial sites to support observations of disease incidence and the effects of varying soil treatments and rotation cropping options. Refer to link for example of use;

https://www.soilwealth.com.au/resources/articles-and-publications/can-calcium-cyanamide-cacn2-fertiliser-affect-pythium-spp-and-other-soilborne-diseases-in-carrots-findings-of-an-onfarm-demonstration/

- Testing of samples and interpretation provided to assist agronomists and agribusinesses undertaking research and on-farm trials; characterize pathogen inoculum levels at sites; monitor changes in pathogen levels; specific testing within trial sites to support observations of disease incidence and the effects of varying soil treatments and rotation crops.
- Testing of samples conducted with vegetable consultants and agronomists to accelerate future uptake on grower properties. This involves one on one support from the project investigator in relation to test capabilities and choice, sampling strategies, sample handling, interpretation and use of results.

Training and awareness

Growers, agronomists and service providers in the vegetable industry have attended PREDICTA Pt training workshops allowing them to access PREDICTA services. These training courses are focused on using the technology in the potato industry. Ten of the participants (around 10%) who have been accredited are focused on other vegetable crops. They have attended to gain knowledge on accessing the testing service, soil sampling requirements and interpretation of DNA test results. Post course assistance is provided to these agronomists to assist them access tests, refine sampling and interpret results specific to vegetable crops.

Michael Rettke and Kathy Ophel Keller participated in the Soil Wealth Soilborne Diseases Masterclass program ran by Applied Horticultural Research/ RMCG. Workshops were held in Ipswich QLD on 16-17 August 2016, Devonport TAS on 30-31 August 2017 and Mawson Lakes SA on 3-4 September 2018. The Master Class program was part of the national Soil Wealth – Integrated Crop Protection program for the vegetable industry funded by Hort Innovation. Michael's and Kathy's presentations and involvement focused on the role of soil DNA and traditional pathology diagnostics in managing soilborne diseases – how is it being used and what can it do for you?

Michael Rettke delivered a Webinar on the role of soil DNA testing in managing the risk of soilborne diseases on 2 August, 2018. The Webinar was part of the Soil Wealth – Integrated Crop Protection program funded by Hort Innovation and is available soil wealth website.

https://www.soilwealth.com.au/resources/webinar-recordings/the-role-of-soil-dna-testing-in-managing-the-risk-of-soilborne-diseases-how-is-it-being-used-and-what-can-it-do/

Publication (Attached in Appendix 2)

Testing for Soilborne Pathogens

BRASSICA – CARROT – CAPSICUM – SWEET POTATO - ONION

Outcomes

Vegetable growers have access to a dedicated test panel (HORT Veg) for the quantification of pathogen levels in the soil as a direct outcome of VG15009. New tests for the pathogens that cause clubroot of brassicas and cavity spot of carrots are included along with 23 other assays for pathogens that cause soilborne disease of vegetables. HORT Veg testing is most suited to use in carrot, brassica, sweet potato and capsicum production, and has more limited applications for other vegetable crops.

At the commencement of VG15009 the use of pathogen DNA testing in the vegetable industry was limited to a few agronomists utilizing the PREDICTA Pt service (established for potatoes) to test for root knot and root lesion nematodes. Establishment of the HORT Veg panel and enhancements to the PREDICTA Pt service have expanded use of the technology in the vegetable industry.

The HORT Veg panel is now run on a monthly basis (or as demand dictates), providing vegetable growers with access to the benefits of pathogen DNA testing. In combination with the project field validation program, project staff have supported commercial growers, their agronomists and agribusinesses to use the service. This has fostered their knowledge and experience in monitoring, understanding and managing risk of soilborne diseases in vegetable crops, particularly in carrot, brassica and soil grown greenhouse crops.

As a result agronomists who have been exposed to the service have grown in confidence in the use of pathogen DNA testing to assess risk of specific soilborne diseases, for example utilizing *Plasmodiophora brassicae* test for assessing the risk of clubroot of brassicas and root knot nematode tests for assessing risk in a range vegetable crops.

Towards the end of the project individual carrot and brassica growers have requested access to testing which has been facilitated where possible by project staff, usually in cooperation with agronomists. There is also interest from lettuce growers, though the range of pathogen tests applicable to lettuce is currently limited.

There has also been increased interest from agronomists not involved in the project, wishing to learn about and access pathogen DNA testing for vegetable crops. This has mainly been to assess pre-plant risk of soilborne disease in brassicas, carrots, sweet potatoes and greenhouse crops (capsicums, cucumbers). This is evidenced by an increased number of agronomists seeking PREDICTA Pt accreditation specifically for use on vegetable crops, with 10 so far having attained accreditation.

In the timeframe of this project a total of 566 samples have been tested for 32 clients relating to vegetable crops, not including samples tested from non-levy crops or as part of this project or collaborative projects. Client instigated samples relate to a wide range of applications by growers, agronomists, agribusiness and small number samples from non-project linked research institutions. Knowledge and capacity developed in this project has directly contributed to enabling these clients to access and benefit from pathogen DNA testing.

Examples of how these early adopters have used the technology:

Carrot growers

- Assessing the risk of root knot and root lesion nematodes prior to planting to choose paddocks, or to manage the risk appropriately by applying treatments or a period of fallowing.
- Monitor pathogen levels in the cropping system to confirm effectiveness of strategy in maintaining low levels of inoculum.
- Determining the species of root knot and root lesion nematode that has caused problems to inform future management decisions.
- Confirming presence or not of *Pythium sulcatum* or *P. violae* in suspected symptoms of cavity spot.
- Investigating causes of forking and tapered crowns, symptoms that can be caused by numerous pathogens or non-pathogen related soil conditions, with cause often difficult to diagnose. In some cases testing has identified the probable cause, whereas in other cases it has changed the focus of investigation to other potential causes.

Brassica growers;

- Assessing the risk of clubroot prior to planting to choose paddocks for summer production, when risk of loss in crop productivity from clubroot is highest.
- Confirming presence of pathogen in paddocks on newly purchased and/or leased land where history of disease is unknown or uncertain.

Greenhouse growers;

- Quantify populations of root knot nematode pre and post soil treatments.
- Monitoring nematode populations during crop to better understand build up and decline under various management practices including in-crop treatments.
- Understanding unintended consequences of chemical treatments on biological control of nematodes and population increases.

Agribusiness and research providers;

- Assist in identifying sites with appropriate level of inoculum in the soil to conduct soilborne disease trials.
- In-crop testing to monitor changes in pathogen activity.
- Specific testing within trial sites to support observations of disease incidence and the effects of varying treatments.
- Calibrating disease tolerance and resistance of vegetable varieties against inoculum load.
- Scoping the range of pathogens present at a trial site.
- Quantifying infection levels of pathogens in root samples from plants with non-specific symptoms.

Testing of samples has assisted collaborative vegetable research and development projects to meet their objectives in a similar manner to the uses described above. Use of pathogen DNA testing has proven valuable in selecting sites for disease management trials, supporting visual and crop productivity measurements and diagnosis of soilborne disease problems, particularly where more than one pathogen was involved. These uses have assisted a number of collaborative projects to achieve their objectives.

Sampling is one of the most critical aspects of using pathogen DNA testing technologies to assess inoculum level in the soil within agricultural production systems. Vegetable cropping systems vary enormously with the crop, region and scale of production across Australia. Through the validation program in this project and discussions with agronomists and growers, critical information has been gained on the most appropriate soil and plant tissue sampling methodologies to apply in vegetable production systems. Underlying principles already established for sampling in other industries underpin sampling strategies. However, specific trials conducted in this project have been important to refine sampling requirements for key pathogens in intensive vegetable production systems. For large paddocks and center pivots the guidelines established for use in potatoes have been confirmed for a wider range of pathogens. Having data and experience on which to advise agronomists on the most appropriate sampling strategies going forward is an important outcome for the integrity of a future commercial service for the vegetable industry.

Based on discussions with agronomists, exposure to this technology across the wider vegetable industry is still relatively limited. There are still many growers who are unaware of the use of the technology and the value it may be able to add to their enterprise.

This is the start of a process to establish an equivalent service for the vegetable industry to other PREDICTA services currently provided to the potato and broadacre cropping industries. It is too early to assess adoption rates and benefits subsequently arising from adoption on a wider scale. Soilborne disease can cause crop failure, resulting in substantial financial loss to the grower and the inability to supply markets. Adoption of PREDICTA testing can assist growers to avoid these losses.

Monitoring and evaluation

This project has achieved its major objective; establishing a pathogen DNA test panel (HORT Veg) specifically for use in vegetable crops. At project completion the capacity to deliver testing to the vegetable industry through the HORT Veg research service has been realized. The technical knowledge generated in this project relating to the available tests for soilborne pathogens across a range of crops and situations has enabled progression to pilot testing stage. In this phase of development, a period of monitoring and troubleshooting of client usage and outcomes is critical before a decision is made in consultation with the vegetable industry to launch a commercial service.

Three new assays were identified for development in this project, namely *Plasmodiophora brassicae*, *Pythium sulcatum* and *Pythium violae*. These have been successfully developed as soil tests in SARDI PREDICTA format, with assays available for routine assessment of soil samples. Most of the focus in this project has been on brassica and carrot crops, coupled to validation of the new assays developed. Results indicate that the assay for *Plasmodiophora brassicae* will be a useful pre-plant test to assist growers manage the risk of clubroot, an important soilborne disease of brassica crops in Australia. A period of pilot testing is important prior to the commercial launch of a clubroot testing service, enabling continued evaluation over a range of regions, varieties and situations. For example validation of *Plasmodiophora brassicae* test is yet to be confirmed in Western Australian brassica production areas, though clubroot is known to be a problem in this region.

The HORT Veg panel is available and is being accessed by growers, agronomists and agribusiness providers in the vegetable industry. In the timeframe of the project a total of 566 samples have been tested for 32 clients relating to vegetable crops (not including non levy crops). Mostly feedback has been positive, with test results reflecting subsequent observations of the client, thus confirming specific tests are providing useful information. This testing has revealed some weaknesses in sampling methods being used. This particularly related to testing for root knot nematode prior to planting carrots, but applies to other tests. In response trials were conducted to support advice and justification for the need for more intensive sampling. Experience indicates sampling is a subject where continued reinforcement is required, as increased sampling intensity comes at an additional sampling and testing cost. Inadequate sampling highlights the need for training and accreditation of users of the service, which is an integral part of existing PREDICTA services.

This project has involved testing on the properties of 47 growers, with testing undertaken in South Australia, Queensland, Tasmania, Victoria and to a limited extent in Western Australia. In total testing was conducted in over 250 vegetable plantings. This is in line with the overall targets for the project. Inclusion of additional crops to carrots and brassicas in the project meant the overall project scope was wide. Realistically, for tests to be understood and validated in each of the crops, more intensive specific targeted research is required. As indicated in the mid project review, given the biological complexity in demonstrating a relationship between quantitative pathogen detection and disease expression for a number of different pathogen species, crop plants and over different climactic, seasonal and environmental conditions, then it is reasonable to expect that some aspects of this project may need to be extended.

Testing in this project has provided data indicating which of the available assays offer the most benefit in the additional crops included (that is capsicums, cucumbers, green beans, sweet corn and sweet potatoes). In some cases, such as for monitoring root knot nematode populations in greenhouse crops, the use of this technology has been clearly developed and demonstrated within the timeframe of this project and adoption by sectors of the industry is occurring. Additionally, important soilborne disease for which DNA assays have not been developed were identified.

A challenge in a multi-faceted multi-crop project undertaken with multiple co-operators across Australia is consistency of assessment, even with well-developed protocols. Not achieving the same levels of adherence across providers to generate a consistent set of data can be result in the inability to utilise some of the data generated. This was the case for symptoms that are difficult to visually diagnose and where providers do not have access to the same levels of resources and equipment. This resulted in a greater reliance on sites in South Australia than originally planned.

In discussions with growers it is clear that some are interested in the results of one or a few tests. The developed HORT Veg panel provides a lot of irrelevant information for these growers, when they would be better serviced by more samples tested only for the pathogens of concern. This type of service should be considered as it provides a

grower with more detailed information on level and distribution of inoculum in the soil for the pathogen(s) of most concern for the same expenditure level per planting, compared with using a HORT Veg panel of 26 tests.

Commencing a service to provide the vegetable industry with access to a DNA based pre-plant testing service to assess disease risk is the next phase and was not envisaged within the timeframe of the project. For the key targets, clubroot of brassicas, cavity spot of carrots and root knot and root lesion nematodes across a wider range of crops, this project has built a base on which to establish a commercial service in the vegetable industry.

While some adoption of this technology is already occurring, consultation with potential users of the technology indicates that more is required to build awareness of the technology. Care needs to be taken not to develop expectations before the technology has undergone adequate pilot testing in the vegetable industry.

Recommendations

PREDICTA research service

Without further investment the Hort Veg test panel will continue to be offered through SARDI's PREDICTA research testing services to researchers and agronomists accredited to deliver PREDICTA Pt. Recommended that agronomists use this service to assist growers to minimise losses from soilborne disease by:

- Measuring inoculum levels to assess disease risk.
- Monitoring to understand pathogen level changes in cropping systems.
- Evaluation management practices and their effect on pathogen inoculum levels.
- Conducting in-crop testing of soil or plant samples to investigate causes of disease.
- Improving outcomes and knowledge gain from on-farm trials.
- Implementing better informed management decisions to manage the risk of soilborne disease

In order to maximize usefulness and uptake, investment is recommended in two further areas. The first relates to supporting on-farm adoption and providing technical knowledge around use of the technology developed so far and the second is to continue test development, as uptake relies on building a suite of tests targeting key diseases that are impacting profitability.

Provision of grower support in use of technology (achieving industry benefit)

For the pathogen DNA tests already developed and validated, the next step is supporting on-farm adoption and providing technical knowledge around their use. Preferably this would involve on-farm demonstration of test use within a systems approach to soilborne disease management. This could be accompanied by a program to assist growers use and interpretation of tests within their specific growing systems. This will provide a mechanism for more detailed pilot testing of tests included in the HORT Veg panel. Specific strategies could include provision of:

- Decision support tools for identifying and managing the risk of soil borne diseases.
- On-farm demonstrations of using pathogen DNA testing in the management of soilborne diseases.
- Support network for growers adopting soil pathogen DNA testing.
- Refinement of soil sampling methods specific to crop type and growing systems.
- On-farm demonstrations of monitoring and adjusting inputs to reduce soilborne disease risk and improve soil health.
- Linkages and support of Hort Innovation projects on improving soil health, enhancing understanding of outcomes by using pathogen DNA testing in combination with advances in soil health indices.

Initial focus should be on brassica, carrot, soil grown greenhouse crops, sweet potatoes and field grown capsicums. Programs should be specific to crops and cropping systems to enable tailored advice and support, in addition to generic advice on use of the technology.

Testing in other crops, particularly beans where soilborne disease complexes are reducing productivity require more research. Investigations currently underway in broadacre production of pulse crops may provide additional insight for the future development of soilborne pathogen testing for the green bean and pea crops.

Development of additional tests (widening application and enhancing benefit)

Discussions with growers, agronomists and researchers during the life of this project, along with observations during crop assessments as part of validation trials has confirmed a number of other pathogens causing soilborne disease issues relating to the target crops for which industry would benefit from having DNA assays suitable for assessing inoculum in the soil. These include:

Southern blight (*Sclerotium rolfsii*) - carrot, beans, capsicum and others
Rhizoctonia (*Rhizoctonia solani* AG 1) – Lettuce, carrots
Crown rot (*Fusarium avenaceum*) - carrots
Gummy stem blight (*Didymella bryoniae*) - cucumbers
Damping off/*Pythium* (*Pythium aphanidermatum*) - beans, capsicum, sweet corn
Sudden wilt (*Pythium myriotylum*) - capsicums
Beet cyst nematode (*Heterodera schachtii*) - brassicas
Reniform nematode (*Rotylenchulus reniformis*) – sweet potato
Stubby root nematode (*Paratricodorus* spp.)

The listed soilborne diseases encountered within this project are not a comprehensive or prioritised list of the soilborne pathogens of vegetable crops in Australia. Potential targets should be prioritised by an advisory team of leading growers and agronomists.

Refereed scientific publications

None to report.

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Intellectual property, commercialisation and confidentiality

Intellectual property was generated in this project relating to the calibration of disease risk thresholds in vegetable crops to new and pre-existing pathogen DNA tests. This information has been made publicly available for the benefit of the Australian vegetable 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 (Hort Veg test panel) will 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 with a high level of adoption within the Australian vegetable 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. A number of growers hosted specific trials and we are especially appreciative of the assistance they provided. During the course of the project a number of other growers have generously provided their time and expertise to assist the project.

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Appendix 1 – Development and validation of tests

DNA assays for the Vegetable industry

Compilation of test panel for vegetable crops

At project commencement SARDI had established test groups for soilborne pathogens of winter cereals and potatoes, namely the crop research and potato research panels along with specific panel for free living nematodes. Only a small number of pathogen tests on the crop research panel are relevant to vegetable production, this panel having limited applicability to the vegetable industry. Many of the pathogen DNA tests on the potato research panel are important pathogens of other vegetable crops, particularly other solanaceous crops such as capsicum and eggplant. However, for crops such as carrots, brassicas, sweet corn and beans the range of tests gives limited coverage and does not include the most important soilborne diseases of these crops.

A new test panel named HORT Veg was developed for use in this project and as a starting point to establish a commercially available pathogen testing service for the vegetable industry. Tests included in the panel were selected in consultation with growers, agronomists and horticultural plant pathologists. Findings of a vegetable disease and crop prioritisation process conducted in project VG15010 was utilized in compiling the panel along with the recommended pathogen targets set out in VG13077 that guided development of this project.

Initially the HORT Veg panel included 10 pathogen assays from the potato research panel, 2 pathogen assays from the crop research panel and 8 other available assays that had been developed in the SARDI format. As a result of development of 3 new tests in this project and a further 3 new tests that became available during the life of the project the HORT Veg panel expanded to 26 tests by completion of the project (Table 1). Assay results are reported in various units depending on the standards and conversion factors used (pg DNA /g sample, kcopies DNA / g sample or nematode equivalents / g sample). In this report data is expressed in either the log values of these units (log DNA) or nematodes / g, unless otherwise stated.

Table 1. Compilation and progression of assays included in the HORT Veg panel for application in the vegetable industry to quantify pathogen DNA levels in soil and plant samples.

DNA Assay			Target disease	Target crops
Project start	Added by project completion	Units result reported in		
Aphanomyces euteiches		pg DNA / g	Aphanomyces root rot	Beans, peas
Colletotrichum coccodes		pg DNA / g	Anthracnose	Solanaceous vegetables
Macrophominia phaseolina		kcopies DNA / g	Charcoal rot	Beans, brassicas, capsicum, carrots, melons, peas, sweet potato
	Plasmodiophora brassicae	kcopies DNA / g	Clubroot	Brassicas
Leptosphaeria maculans		pg DNA / g	Black leg	Brassicas
	Pythium sulcatum	kcopies DNA / g	Cavity spot	Carrots
	Pythium violae	kcopies DNA / g	Cavity spot	Carrots
Pythium clade F		pg DNA / g	Pythium	Vegetables
Pythium clade I		pg DNA / g	Pythium	Vegetables
Rhizoctonia solani AG 2.1		pg DNA / g	Wirestem	Brassicas
Rhizoctonia solani AG 2.2		pg DNA / g	Rhizoctonia	Carrots, sweet corn
Rhizoctonia solani AG 3		pg DNA / g	Rhizoctonia	Solanaceous vegetables
Rhizoctonia solani AG 4		pg DNA / g	Rhizoctonia	Brassicas, cucurbits, sweet corn
Rhizoctonia solani AG 8		pg DNA / g	Onion stunt	Onions
	Sclerotinia sclerotiorum/minor	kcopies DNA / g	Sclerotinia rot	Beans, brassicas, carrots, celery, peas, lettuce
	Setophoma terrestris	kcopies DNA / g	Pink/Red root rot	Onions, sweet corn, sweet potatoes, strawberries
	Thielaviopsis basicola	kcopies DNA / g	Black root rot	Beans, carrots, lettuce, cucurbits
Verticillium dahliae		pg DNA / g	Verticillium	Brassicas, lettuce
Streptomyces txtA gene		pg DNA / g	Scab	Carrots

DNA Assay			Target disease	Target crops
Project start	Added by project completion	Units result reported in		
Meloidogyne hapla		pg DNA / g		Vegetables
Meloidogyne fallax		pg DNA / g		Vegetables
M. javanica/incognita/arenaria		pg DNA / g		Vegetables
Pratylenchus crenatus		nematodes / g		Carrots
Pratylenchus neglectus		nematodes / g		Vegetables
Pratylenchus penetrans		nematodes / g		Vegetables
Pratylenchus thornei		nematodes / g		Carrots
Pratylenchus zeae		kcopies DNA / g		Sweet corn

In the timeline of this project pathogen tests have also been added to the potato research panel. This panel still remains focused on potatoes. Modification of this panel to include *Rhizoctonia solani* AG 4 as a result of information gained in this project enables this panel to be used as an alternative to the HORT Veg panel for capsicums, sweet potatoes, eggplant and cucumbers.

SARDI has a panel of DNA tests for free-living nematode (FLN) which comprises 15 assays for the FLN groups (Dorylaimida, Mononchida, Aphelenchidae, Aphelenchoididae, Cephalobidae, Panagrolamidae, Rhabditinae, Mesorhabditinae, Tylenchinae) which represent over 80% of the free-living nematodes in the soil. Work outside of this project in broadacre cropping situations has indicated that 9 of the 15 tests is sufficient to characterize FLN populations in order to monitor soil biological function. Based on the data generated in this project, these 9 tests also appear adequate for use in the vegetable industry. In soil samples tested from paddocks used for vegetable cropping, detections by the 6 assays that test for Tylenchinae group would not add value to free living nematode community analysis and interpretation.

Development of new pathogen DNA tests

Cavity spot of carrots

Pythium sulcatum and *P. violae*, are the species documented as causes of cavity spot of carrots in Australia, with *P. sulcatum* reported to be the most widespread (Davison and McKay 2001). TaqMan MGB qPCR assays for the specific detection and quantification of *P. sulcatum* and *P. violae* were designed based on sequence information available in public databases. To further assist with the development of a specific *P. violae* assay, sequence was also obtained from Australian *P. violae* isolates from carrots with cavity spot symptoms. In line with SARDI Molecular Diagnostics Centre strategy to achieve high sensitivity, both assays were designed in the Internal Transcribed Spacer (ITS) region of the genome, which is in multiple copies. The TaqMan Molecular Groove Binder (MGB) probe chemistry was also used to further improve both sensitivity and specificity.

Two tentative assays were designed for *P. sulcatum* and *P. violae*, respectively and assessed for their sensitivity using DNA extracted from pure cultures. For each target, the most sensitive assay (Table 2) was selected, and tested for specificity using an extensive collection of DNA from the target and closely related species, including *P. sulcatum, P. violae, P. echinulatum, P. helicoides, P. heterothallicum, P. inflatum, P. irregulare, P. mamilatum, P. middletonii, P. ostracoides, P. paroecandrum, P. prolatum, P. ultimum, P. radiosum, P. rostratum* and *P. vexans.*

Table 2. Sequences (5' to 3') of primers and TaqMan MGB probes for the detection and quantification of *Pythium sulcatum* and *Pythium violae*.

	Pythium sulcatum	*Pythium violae*
Forward primer	CTTGGGCGCCTCACTTGT	CGTGAACCGTCAAGTAATAGATTCA
Reverse primer	AAAAAGCGCCTGCGAGATAA	CACAAGACCAACCTTCGTTCCT
Probe (FAM/MGB)	ACAAACTGTCCCAAATTG	CCTCTGCTGTGGCTGT

Each assay specifically and efficiently detected pure DNA from their respective target and did not detect any of the other species assessed, indicating that both assays are specific. To confirm the specificity observed with DNA from pure cultures, the *P. sulcatum* and *P. violae* assays were used to assess field samples. Samples included soil from carrot paddocks, both at harvest and out of crop as well as soil attached to carrots at harvest and carrot peel. Results aligned with the paddock cavity spot history and symptoms observed, with levels ranging from below detection to 2.8 and 3.1 log DNA for *P. sulcatum* and *P. violae*, respectively. Higher levels were found at harvest and lower levels out of crop. Individual carrot samples were found infected by one or the other species, not both, unless at low levels. The next generation sequencing (NGS) methodology developed by SARDI MDC to validate new qPCR assays was used to verify the *P. sulcatum* and *P. violae* assays. Results obtained with the same field sample DNA indicated that both assays specifically detect their respective target in soil samples; no cross reactions were observed with the set of samples analysed.

Calibration standards and PCR reagents were then prepared, and the test incorporated into SARDI's PREDICTA

delivery platform to enable routine assessment of samples.

Clubroot of brassicas

Clubroot is caused by the obligate pathogen *Plasmodiophora brassicae*. After a review of published assays and phylogenetic information the TaqMan MGB assay published by Deora et al. (2015) was selected for evaluation to quantify *P. brassicae* in soil (Table 3). TaqMan MGB is SARDI MDC's preferred format for quantifying DNA by qPCR. Also, Deora's assay is designed in the Internal Transcribed Spacer (ITS) region of the genome; such assays are generally very sensitive, an essential trait to detect very low concentrations of target organisms in DNA extracted from soil.

Table 3. Primer and probe sequences used in the TaqMan assay for detection and quantification of *P. brassicae* (Deora et al., 2015).

	Sequence (5' to 3')
Forward primer	CCT AGC GCT GCA TCC CAT AT
Reverse primer	CGG CTA GGA TGG TTC GAA A
Probe (FAM/MGB)	CCA TGT GAA CCG GTG AC

The assay efficiently detected DNA extracted from two Australian isolates of *P. brassicae*, and did not detect *Spongospora subterranea* f. sp. *subterranea* DNA, the most closely related species to *P. brassicae* in Australia.

DNA extracted from 24 soil samples from a range of paddocks with and without a prior history of clubroot were then assessed. The results aligned with the paddock history with levels ranging from below detection to 500 µg *P. brassicae* DNA/g soil.

The NGS method to validate new qPCR assays indicated that the forward primer is specific and does not cross react with non-target microorganisms. The reverse primer did not yield usable sequence data and no conclusion can be made; the run was not repeated.

Overall, published and newly obtained results suggest that the *P. brassicae* assay is sensitive and specific. Therefore, calibration standards and PCR reagents were prepared, and the test incorporated into SARDI's PREDICTA delivery platform to enable routine assessment of samples.

Validation of pathogen DNA tests

Brassicas – Assessing risk of clubroot

Pre-plant risk assessment in commercial crops

Validation was conducted by using the *Plasmodiophora brassicae* DNA test to quantify the inoculum level in the soil prior to planting, followed by assessing the incidence and severity of clubroot symptoms in crops grown at the sampled locations. Sampling locations were located on properties of 11 growers and in 55 plantings, with sites in South Australia, Queensland, Victoria and Tasmania. All crops included were considered susceptible to clubroot. Crops were predominately broccoli, broccolini and cauliflower, with a few wombok crops.

Soil for pathogen DNA testing was sampled using a PREDICTA Pt corer (15cm depth by 1cm diameter). Each preplant soil sample was a composite of 30 cores taken in a W or zigzag pattern across the sampling area. Sampling areas were restricted to a single planting and targeted at 1ha units. In production systems where crops are grown in irrigated bays, the bay became the sampling area and was usually less than 1ha.

In Southern production regions trials were focused on summer plantings when temperature conditions are conducive to clubroot symptoms occurring.

Plant growth was assessed 4 to 6 weeks after transplanting according to size of plants using the following rating

scale: 0 = Normal 90 -100%, 1 = 80-90 % normal, 3 = 50-80 normal, 5 = < 50% normal, 7 = dead. The incidence of black leg and wirestem were recorded. The incidence of clubroot was assessed at the completion of harvest by pulling 50 to 100 plants (20 at Victorian sites) and assessing the roots. Severity of clubroot symptoms was assessed according to the following ratings; 0 = nil, 1 = slight swelling confined to lateral roots, 3 = moderate swelling on lateral or tap roots, 5 = severe swelling, 7 = severe swelling (wilt/dead). Severity rating index of clubroot was calculated using the formula: [1*(number of plants in #1 rating category) + 3*(number of #3 rating) + 5*(number of #5 rating) + 7*(number of #7 rating)] / [(number of plants assessed)*7)]. At Queensland sites clubroot was assessed at the incrop assessment only, as no clubroot was observed at validation sites. At each sampling location root systems of 10 plants were subsampled by cutting a strip of stem and root tissue from the side of the plant for pathogen DNA testing.

The incidence and severity of clubroot, the main soilborne disease of brassicas targeted by the project, ranged from no measurable crop loss to causing crop failure. Results indicate that pre-plant soil DNA concentration of *P. brassicae* provides a useful indication of the risk of severe clubroot (Figure 1). Severity of symptoms increased with increasing soil inoculum. A high incidence of infection can occur in paddocks with low levels of soil inoculum in the soil prior to planting (Figures 2 and 3). High rates of infection causing minor symptoms of clubbing on the roots may not adversely affect productivity of the current crop, but can generate a high inoculum level for subsequent crops. When *P. brassicae* is detected in a paddock, at any level, appropriate measures are required to lower the risk of clubroot in the crop to be planted, and to reduce the buildup of inoculum for future crops. Root systems of brassicas can be infected by *P. brassicae* as indicated by pathogen DNA testing before visual symptoms are apparent (Figure 4).

Proposed thresholds when interpreting the pre-plant soil DNA test results for *P. brassicae* in relation to the risk of clubroot causing productivity loss in brassica crops are provided in Table 4.

Inoculum level of *P. brassicae* was the main factor associated with the incidence and severity of clubroot observed. The levels of other pathogens and free-living nematodes detected by pre-plant DNA testing and soil nutrient and respiration levels tested prior to planting were not significant factors contributing to the severity of clubroot observed.

Environmental factors such as warm growing conditions, low soil pH, soil compaction and inadequate drainage can elevate the risk of clubroot and need to be taken into account when interpreting pathogen DNA soil testing results to assess the risk of clubroot.

Table 4: Proposed thresholds when interpreting the pre-plant soil DNA test results for *Plasmodiophora brassicae* in relation to the risk of clubroot causing productivity loss in brassica crops. (Sufficient samples must be taken in a paddock using appropriate soil sampling protocol).

Risk Category	DNA test result *Plasmodiophora brassicae*	
	Kcopies DNA/g soil	log DNA
Low	< 2	< 0.5
Medium	2 - 40	0.5 – 1.6
High	> 40	> 1.6

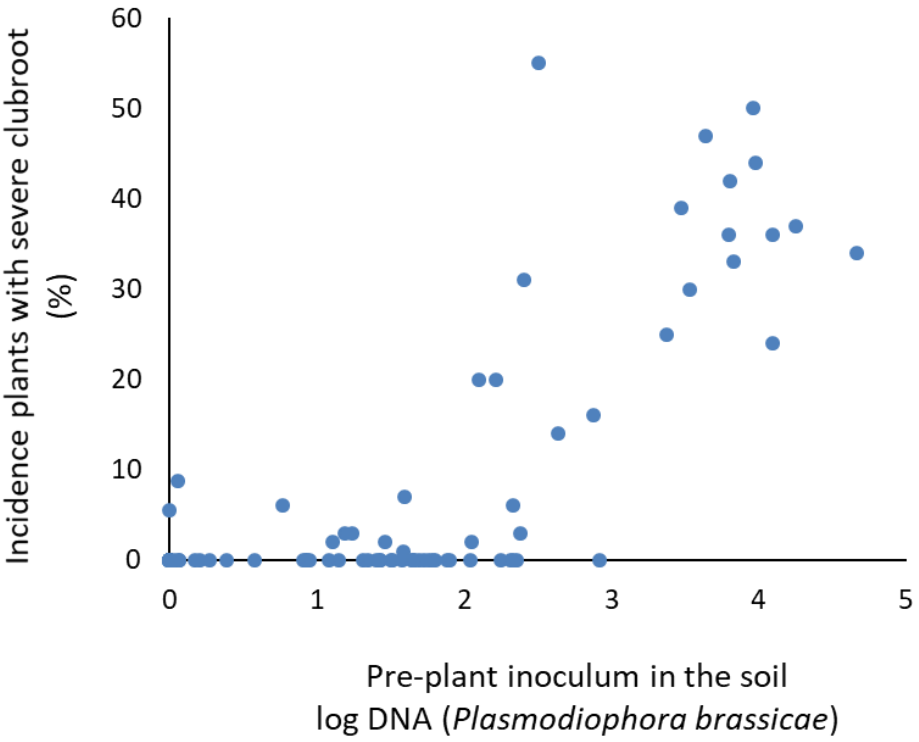

Figure 1: Relationship between the level of *Plasmodiophora brassicae* inoculum in the soil prior to planting and incidence of brassica plants with severe root clubbing impacting crop growth. (Data from South Australia, Victoria, Tasmania, Queensland).

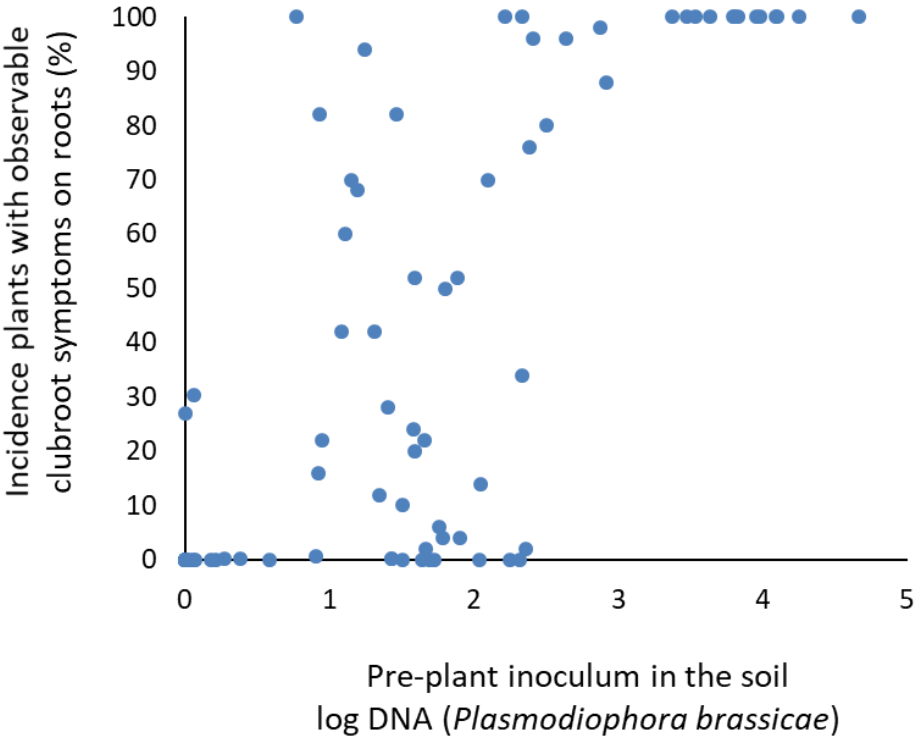

Figure 2: Relationship between the level of *Plasmodiophora brassicae* inoculum in the soil prior to planting and incidence of brassica plants with observable clubroot symptoms on roots. (Data from South Australia, Victoria, Tasmania, Queensland).

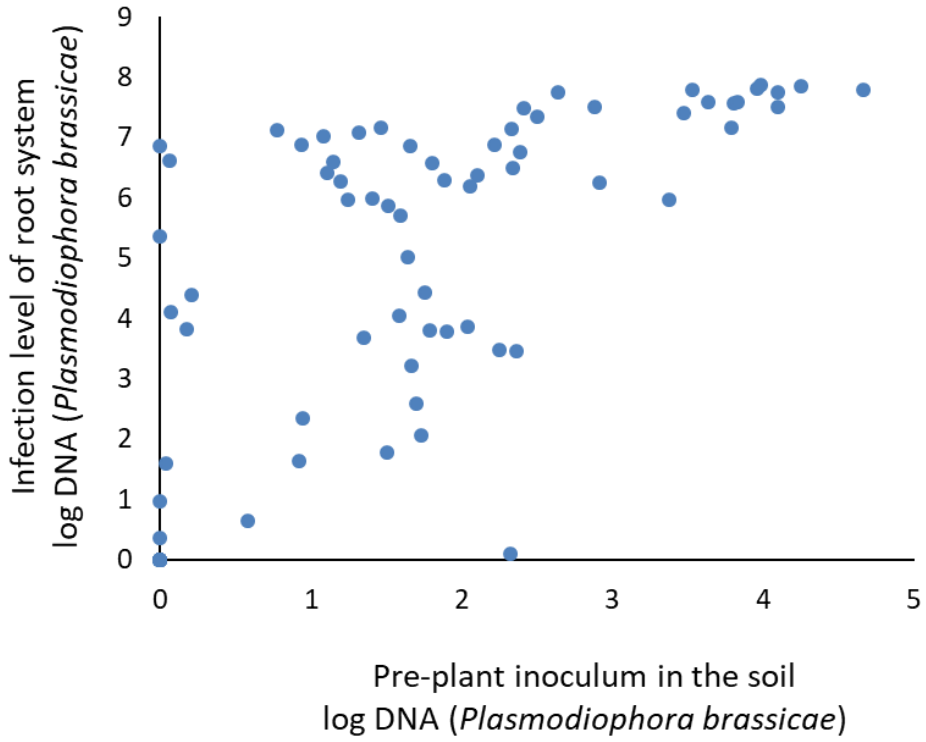

Figure 3: Relationship between the level of *Plasmodiophora brassicae* inoculum in the soil prior to planting and infection level of brassica root systems. (Data from South Australia, Victoria, Tasmania, Queensland).

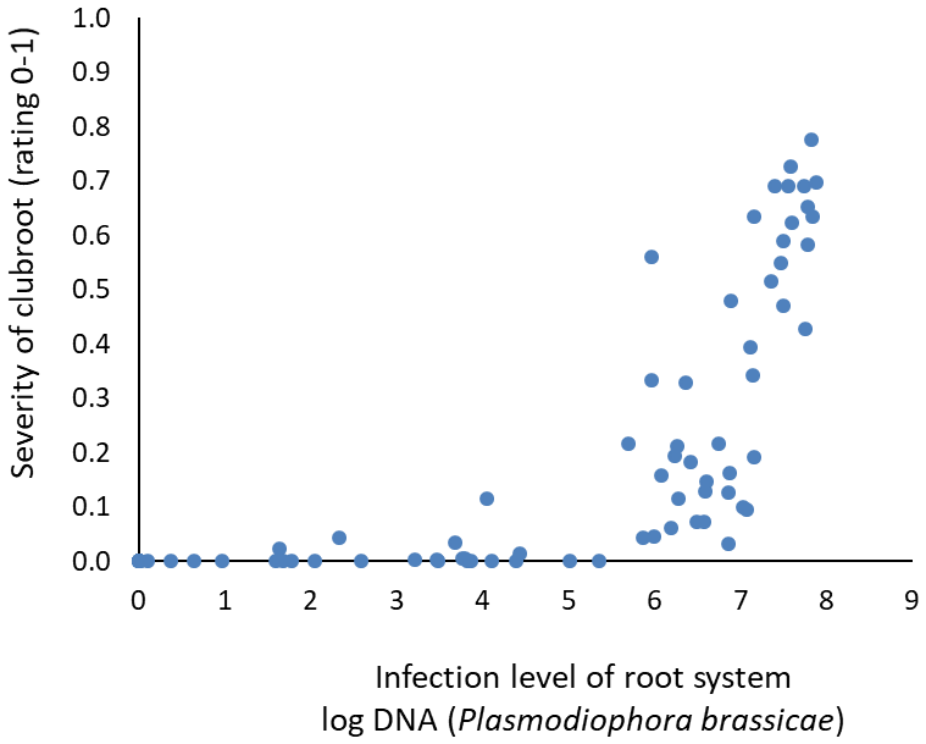

Figure 4: Relationship between the infection level of brassica root systems by *Plasmodiophora brassicae* and severity of clubroot symptoms. (0 = no observable symptoms, 1 = severe clubbing causing plant wilting or death).

Tasmania - plot trial

A commercial crop of broccoli was grown on the long-term rotation trial site located at Forthside experimental station in Tasmania (Sparrow 2015), managed by the University of Tasmania. Soil samples for pathogen DNA testing are taken annually in each of 12 sections of the paddock prior to the establishment of Caliente, ryegrass or bare fallow rotation crop strategies. Each section is divided into 4 subplots that are soil sampled individually. Pathogen DNA testing in June 2016 detected *Plasmodiophora brassicae* in 25 of 48 plots with levels ranging up to 1.6 log DNA. Highest detections were all located in sections of the paddock where Caliente cover crops were grown as the annual cover crop, particularly in one section. Detections of *Plasmodiophora brassicae* in sections where ryegrass or bare fallow are used were less frequent and at low levels.

Five plants from each of four sub plots in each section were assessed for incidence and severity of clubroot at harvest in April 2017. Of the 240 plants assessed 17 had symptoms of clubroot, with 15 being from the section where *Plasmodiophora brassicae* was detected at the highest levels.

Assessment of root symptoms demonstrated correlations between disease incidence and average *Plasmodiophora brassicae* DNA level of 4 soil samples taken 6 months before planting in each of the 12 sections (Figure 5). Symptoms of clubroot in the late summer autumn crop of broccoli were not severe, but post crop soil testing demonstrates the substantial increase in soil inoculum levels resulting from the broccoli crop.

In this trial soil pathogen testing was done well in advance of planting of the broccoli crop, with cover crops of ryegrass and Caliente along with bare fallow areas practiced in between as per annual operation of the long term rotation trial.

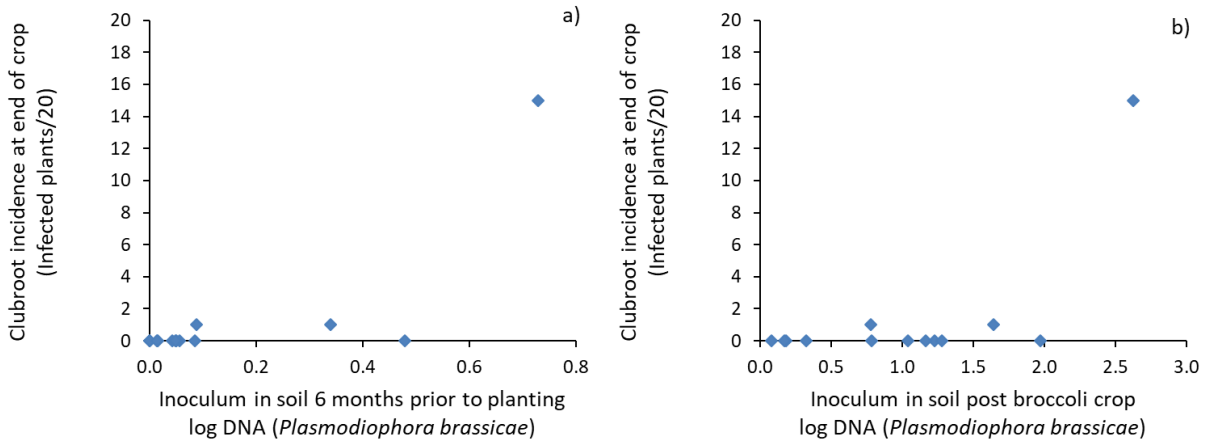

Figure 5: Relationship between incidence of clubroot in a broccoli crop with inoculum level of *Plasmodiophora brassicae* in the soil a) 6 months prior to planting crop and b) post crop after residue incorporated in the soil.

South Australia – plot trial

A trial was conducted in a long growing season brassica crop (approximately 16 weeks). Twenty plots were located along a single 200m long planting bed. Each plot was 5 m long, with 3 rows planted per bed. Following transplanting, soil samples (approximately 500g consisting of 28 cores 15cm depth by 1cm diameter) were taken from between transplants in each plot for pathogen DNA testing. A second sample was taken for pH, nutrient analysis and soil respiration rate.

Along with the primary pathogen target *P. brassicae*, soil samples were tested for 22 other pathogens. Levels of *P. brassicae* DNA in the soil at transplanting ranged from 0.1 to 2.4 log DNA. This variation was primarily associated with variation in brassica cropping history across the site due to a different planting configuration of the paddock in the past.

Trial plots were transplanted on 28 February 2018. Daily average soil temperature in the 2 weeks after

transplanting was consistently above 17°C. Soil pH (water) varied from 6.7 to 7.6 across the site.

Plants were sampled 6 weeks after transplanting (6 plants per plot) and at the end of the cropping cycle on 21 June 2018 (18 plants per plot). Incidence of clubroot assessed at the end of the cropping cycle was greater and more severe in plots that had the higher levels of *P. brassicae* at transplanting (Figure 6). A similar relationship was found when assessed at 6 weeks after transplanting (data not presented).

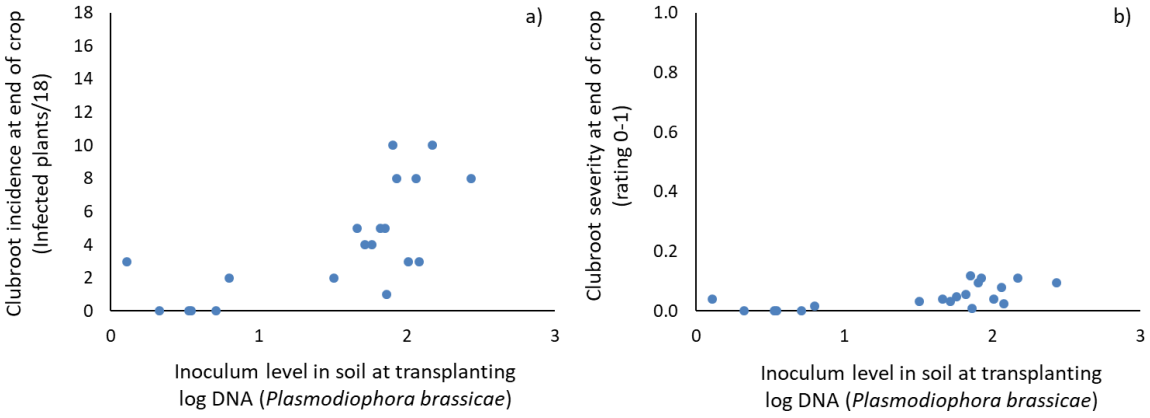

Figure 6: Relationship between inoculum level of *Plasmodiophora brassicae* in the soil at transplanting and a) incidence and b) severity of clubroot symptoms on roots of a brassica crop at completion of the cropping cycle.

Symptoms of clubroot observed on the roots of plants in this trial were minor, not causing any measurable loss in productivity of the crop. Plant weight was assessed at 6 weeks after transplanting and again at the end of the cropping cycle. No relationship was found between plant weight at either assessment and the levels of *P. brassicae* in the soil at transplanting or the incidence or severity of clubroot. This would be expected as the crop was an autumn winter crop. After the initial 2-4 week period when infection occurred, soil temperatures would have been below 17°C which would have reduced disease development.

Other factors

Soil pH ($CaCl_2$) at the site ranged from 6.2 to 7.0 at the time of transplanting. Infection of plants by *Plasmodiophora brassicae* occurred across the range of pH conditions measured. Differences in soil nutrient levels at the time of transplanting were not associated with any clear trends in the incidence and severity of clubroot. Inoculum level of *P. brassicae* was the main factor associated with the incidence and severity of clubroot observed in this trial.

Other pathogens

Reduced early growth as measured by average plant weight 6 weeks after transplanting was associated with higher levels of *Pythium* clade F measured in the plant root system by DNA testing (Figure 7). There was a weak association with level of *Pythium* clade F in the soil at transplanting and reduced average fresh weight of plants. It is unclear if the increased levels of *Pythium* spp. measured in the soil at transplanting is contributing to the reduced plant weight or a result of other limiting factors in the soil for root growth at those plots being conducive to greater infection by *Pythium* spp. Early plant growth was noticeably reduced and inconsistent at the plots with reduced biomass. Differences in plant health and plant size were not evident at the end of the crop.

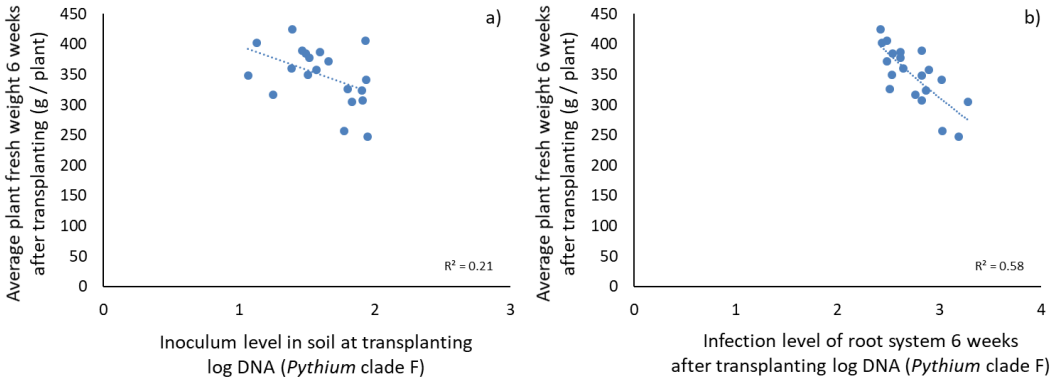

Figure 7: Relationship between fresh weight of plants 6 weeks after transplanting and a) inoculum level of *Pythium* clade F in the soil at transplanting and b) infection level of brassica root systems by *Pythium* clade F 6 weeks after transplanting.

No other obvious soilborne disease was observed to cause significant symptoms or loss in productivity within the trial area. A number of pathogens that are known to infect brassicas including *Rhizoctonia solani* AG 2.1, *Macrophominia phaseolina* and *Verticillium dahliae* were detected in the soil prior to transplanting. These pathogens were detected by DNA testing of root systems tested at 6 weeks after transplanting and at the end of the crop. Though no disease symptoms or loss of productivity were attributed to the infection of plants by these pathogens in this trial, the level of root infection tended to increase with soil inoculum level of *Macrophominia phaseolina* and *Verticillium dahliae* in the soil at transplanting. Infection level of root systems was not correlated with the soil inoculum level for *Rhizoctonia solani* AG 2.1 in individual plots at transplanting. *Streptomyces* txtA gene was detected in the soil at 9 of 20 plots and was detected in the root systems of plants from 16 of 20 plots at the end of the crop.

Low levels (less than 0.5 nematode / g soil) of the root lesion nematodes *Pratylenchus neglectus*, *P. crenatus* and *P. thornei* were detected at transplanting. At the end of the crop populations of *P. neglectus* infested root systems at much higher levels (0 to 260 nematodes / g dry root tissue) than *P. crenatus* and *P. thornei* (0 to 5 nematodes / g dry root tissue).

Brassicas – Other pathogens detected at validation sites

Along with the primary pathogen target *Plasmodiophora brassicae*, soil samples taken prior to planting at brassica validation sites were tested for 22 other pathogens. Not all of these pathogens are considered pathogenic to brassica crops. A breakdown of the rate of detection of these pathogens in soil samples is provided (Table 5) along with a breakdown of the rate of detection of pathogens in stem/root samples. Testing of stem/root systems was not conducted at all validation spots.

Table 5: Rate and maximum level of pathogens detected at brassica validation sites.

Pathogen	Pre-plant soil DNA testing (109 Samples)		Root system DNA testing (83 samples)[#]	
	Detected (%)	Maximum log DNA/g soil	Detected (%)	Maximum log DNA/g dry root
Aphanomyces euteiches	13	1.0	22	2.6
Colletotrichum coccodes	38	2.8	27	3.0
Macrophominia phaseolina	78	2.6	63	3.6
Plasmodiophora brassicae	67	4.7	81	7.9
Leptosphaeria maculans	4	0.9	4	0.9
Pythium sulcatum	7	0.9	7	1.0
Pythium violae	14	0.9	18	1.2
Pythium clade F	87	2.7	90	4.9
Pythium clade I	100	3.2	100	5.3
Rhizoctonia solani AG 2.1	70	3.2	80	5.7
Rhizoctonia solani AG 2.2	0	0	0	0
Rhizoctonia solani AG 3	5	1.2	1	0.8
Rhizoctonia solani AG 4	15	3.5	8	3.1
Rhizoctonia solani AG 8	11	1.6	0	0.0
Verticillium dahliae	44	2.3	35	3.4
Streptomyces txtA gene	12	2.2	28	4.0
Meloidogyne hapla	1	1.3	1	1.5
Meloidogyne fallax	2	0.9	4	1.2
M. javanica/incognita/arenaria	2	0.9	4	3.2
	Detected (%)	Maximum nems/g soil	Detected (%)	Maximum nems/g dry root
Pratylenchus crenatus	40	0.2	45	4
Pratylenchus neglectus	33	5.0	69	596
Pratylenchus penetrans	4	1.8	4	55
Pratylenchus thornei	29	1.6	20	5

[#] Root samples were not tested from all validation spots in Queensland

Rhizoctonia

Five anastomosis groups (AG's) of *Rhizoctonia solani* were included in testing with four of them detected in pre-plant soil samples (AG 2.1, AG 3, AG 4, AG 8). *R. solani* AG 2.2 was not detected in soil prior to planting or on root samples from assessed crops (Table 5).

R. solani AG 2.1, AG 2.2 and AG 4 are known pathogens of brassica crops, causing wirestem on seedlings and associated with *Leptosphaeria maculans* in the disease complex stem canker.

R. solani AG 2.1 was detected in 70% of soil samples tested, indicating it is widespread in brassica production areas. Rate and level of detection in Queensland was lower than in South Australia, Victoria and Tasmania. *R. solani* AG 2.1 was detected in 80% of stem/root systems tested, including a percentage where it was not detected in soil samples and vice versa. Infection level of root systems was not strongly correlated with the soil inoculum level of *Rhizoctonia solani* AG 2.1 at individual validation spots. The indication of inoculum level was improved when 3 or more samples were taken in a paddock.

Some incidence of wirestem was recorded, particularly in Tasmania with 1 to 3% of plants assessed with symptoms in 4 of 5 paddocks assessed. *R. solani* AG 2.1 was the predominant AG detected on stem/root systems tested from these paddocks. In 5 of 15 paddocks in Queensland, the field reported incidence of wirestem was from 1 to 4% of plants assessed. Symptoms were not tested to confirm cause.

R. solani AG 3 was detected in 5 soil samples prior to planting, but was not detected in stem/root samples of these crops when assessed. A single low detection was found on stem/root systems from a site where *R. solani* AG 3 was not detected in the soil prior to planting. This detection suggests that some brassica crops are possibly weak hosts of *R. solani* AG 3.

R. solani AG 8 was detected in 11 soil samples prior to planting, but was not detected in stem/root samples of brassica crops assessed, which indicates the crops were not hosts of this AG group.

Blackleg

Black leg caused by *Leptosphaeria maculans* is a major disease of brassicas. *L. maculans* was detected in 4% of soil samples tested prior to planting and in 3 of 83 stem/root samples tested from validation sites (Table 5). Previous research (Hall 2012) found soil testing to assess the inoculum level of *L. maculans* not to be a reliable indication of the risk of blackleg occurring in brassica crops. Disease is spread by airborne spores and can travel long distances to infect crops.

Verticillium

Verticillium dahliae was detected in 44% of soil samples tested prior to planting and in 29 of 83 stem/root samples tested from validation sites (Table 5). Level of infection of the root system tended to increase with increasing level of inoculum in the soil prior to planting (Figure 8). As many sites were affected by clubroot, it is not possible to isolate what impact infestation of root systems by *V. dahliae* had on plant growth. *V. dahliae* is known to be a pathogen of brassicas.

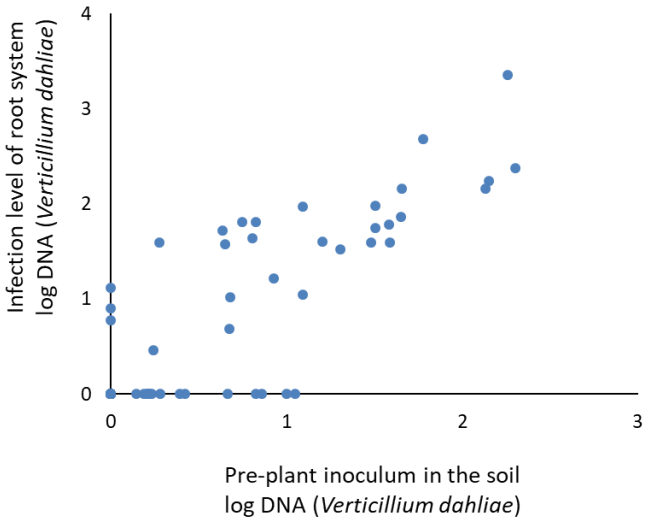

Figure 8: Relationship between the level of *Verticillium dahliae* inoculum in the soil prior to planting and infection level of brassica root systems. (Data from South Australia, Victoria, Tasmania, Queensland).

Pythium

Pythium clade tests are not species specific, with each test detecting a range of pathogenic *Pythium* spp.. Species detected by clade F assay include *P. irregular, P. sylvaticum, P. debaryanum, P. spinosum, P. paroecandrum* and *P. mamillatum*. Species detected by clade I assay include *P. ultimum, P. splendens* and *P. heterothallicum*. As such interpretation of these tests is more difficult than for tests that only detect 1 pathogen species. Level of infection of brassica stem/root systems was not correlated with level of *Pythium* clade F DNA detected in the soil prior to planting, whereas increasing levels of *Pythium* clade I DNA in the soil prior to planting were correlated with increasing levels of DNA in the stem/root systems of brassicas grown in those soils (Figure 9). High levels of *Pythium* clade I were often associated with severe clubroot symptoms (Figure 10). Data indicates when present, at least one of the species in this clade can build to high levels in association with development of clubroot symptoms.

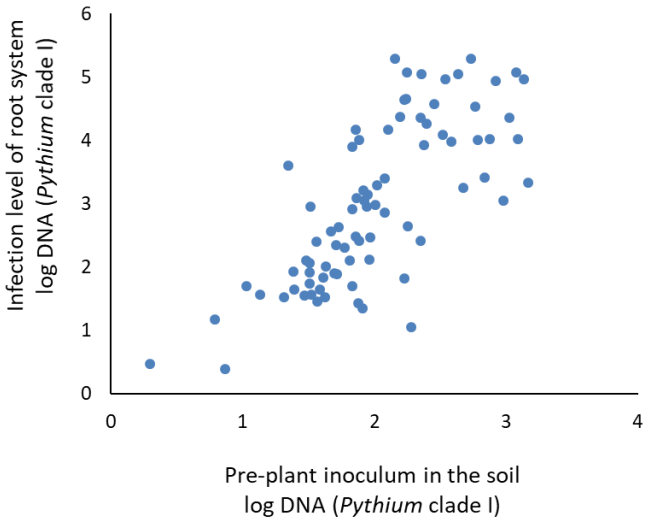

Figure 9: Relationship between the level of *Pythium* clade I DNA in the soil prior to planting and infection level of brassica root systems. (Data from South Australia, Victoria, Tasmania, Queensland).

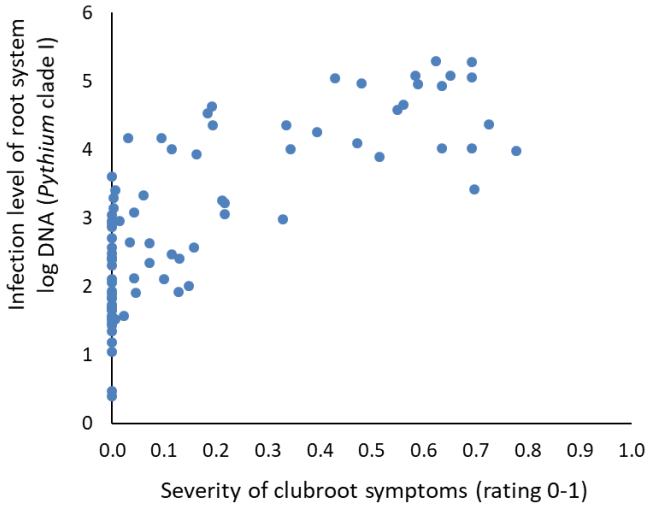

Figure 10: Association between severity of clubroot symptoms observed and infection level of brassica root systems by *Pythium* clade I. (Data from South Australia, Victoria, Tasmania, Queensland).

Colletotrichum

Colletotrichum coccodes was detected in 38% of soil samples tested prior to planting and in 22 of 83 stem/root samples tested from validation sites (Table 5). Most detections were found in Tasmania, though this pathogen is widely distributed in Australia. *C. coccodes* is a major pathogen of potatoes, a crop grown in rotation with brassicas in the northern vegetable production regions of Tasmania including at the paddocks assessed. Level of infection of the root system tended to increase with increasing level of inoculum in the soil prior to planting, (Figure 11). As many sites were affected by clubroot, it is not possible to isolate what impact infestation of root systems by *C. coccodes* had on plant growth. *C. coccodes* is not considered a pathogen of brassicas.

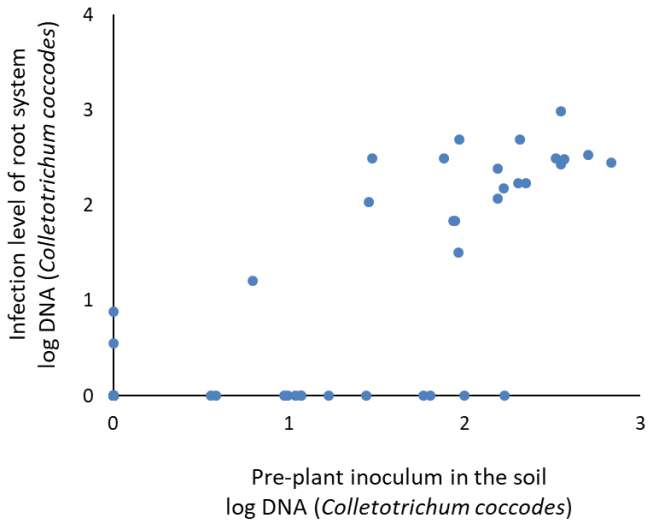

Figure 11: Relationship between the level of *Colletotrichum coccodes* inoculum in the soil prior to planting and infection level of brassica root systems. (Data from South Australia, Victoria, Tasmania, Queensland).

Charcoal rot

Macrophomina phaseolina was detected in 78% of soil samples tested prior to planting and in 52 of 83 stem/root samples tested from validation sites (Table 5). Detection of inoculum in the soil prior to planting was associated with infection of the root system (Figure 12). As many sites were affected by clubroot, it is not possible to isolate whether there was any impact from infestation of root systems by *M. phaseolina* on plant growth.

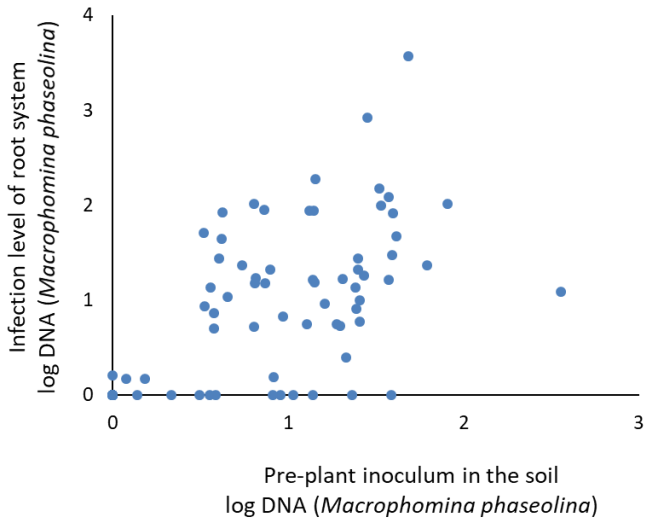

Figure 12: Relationship between the level of *Macrophomina phaseolina* inoculum in the soil prior to planting and infection level of brassica root systems. (Data from South Australia, Victoria, Tasmania, Queensland).

Aphanomyces

Aphanomyces euteiches was detected in 13% of soil samples tested prior to planting and in 18 of 83 stem/root samples tested from validation sites (Table 5). Level of infection of the root system was not correlated with level of inoculum detected in the soil prior to planting. *A. euteiches* is not considered to be a pathogen of brassicas.

Streptomyces

Streptomyces txtA gene was detected in 12% of soil samples tested prior to planting and in 23 of 83 stem/root samples tested from validation sites (Table 5). Level of infection of the root system was not correlated with level of inoculum detected in the soil prior to planting. *Streptomyces* spp. is not considered to be a pathogen of brassicas.

Root lesion and root knot nematodes

Five species of root lesion nematodes were included in testing. *Pratylenchus neglectus*, *P. crenatus* and *P. thornei* were each detected in 29% or more soil samples, with *P. penetrans* detected in 4 soil samples (Table 5).

Infestation levels in stem/root samples reached higher levels for *P. neglectus* than other species, with 15 of 83 samples having greater than 100 nematodes / g of dry root. Preplant levels ranged from below detection to 5 nematodes / g soil. As many sites were affected by clubroot, it is not possible to isolate what impact infestation of root systems by *P. neglectus* had on plant growth. Multiple soil samples per paddock are required to assess risk of nematodes. *P. neglectus* was not detected in the soil prior to planting at 53 of the 83 sampling sites that stem/root samples were tested from. Of these 53 sites, *P. neglectus* was detected in the stem/root systems from 32 sites, normally at low levels unlikely to impact crop growth. In 3 cases levels above 100 nematodes / g dry root were detected. Of the 30 sites where *P. neglectus* was detected in the soil prior to planting, levels above 100 nematodes / g dry root occurred at 12 of them, with the highest level detected 596 nematodes / g dry root. At these levels it is possible crop growth is being affected.

P. penetrans was detected in 4 soil samples, with the highest population 1.8 nematodes / g. All 4 soil samples were from Tasmania and planted with cauliflowers. Levels of *P. penetrans* measured in the stem/root samples from paddocks where *P. penetrans* were detected in at least 1 soil sample ranged from below detection to 55 nematodes /g. Based on testing for other crops, *P. penetrans* is widespread in other brassica growing regions of Australia, particularly in Western Australia.

P. thornei and *P. crenatus* were detected in a high proportion of stem/root samples of brassica plants from paddocks where detected in the soil prior to planting and rarely at locations where they were not detected prior to planting. Infestation levels of stem/root samples were low (5 or less nematodes / g dry root). This suggests that the brassica crops assessed were poor hosts of these species with little or no multiplication occurring on them.

At sites tested in this project, *P. neglectus* and *P. penetrans* were detected at much higher levels than *P. thornei* and *P. crenatus* in the root systems of brassicas. Populations of root lesion nematodes in the soil prior to planting were relatively low. At sites with high populations of *P. neglectus* and *P. penetrans*, much higher levels of root infestation are likely to occur, with the potential to cause loss of productivity.

Limited data was generated on root knot nematodes as they were only present at a very small number of the validation sites assessed.

Carrots – Assessing risk of cavity spot

Pre-plant risk assessment in commercial crops

Validation was conducted by using the *Pythium sulcatum* and *P. violae* DNA tests to quantify the level of inoculum in the soil prior to planting, followed by assessing the incidence and severity of cavity spot symptoms in crops grown at sampled locations. Sampling locations were located on properties of 7 growers and in 40 plantings, with sites in South Australia, Queensland and Victoria. Varieties have been introduced in recent years that are less susceptible to cavity spot. In regions where losses due to cavity spot has been a substantial problem in the past, which included regions targeted for validation sites, crops grown were mostly these varieties.

Soil was sampled for DNA pathogen testing using a PREDICTA Pt corer (15cm depth by 1cm diameter). Each preplant soil sample was a composite of 30 cores taken in a W pattern across the sampling area. Sampling areas were restricted to within a single planting where possible. Normally at least two sampling areas were located in a single planting and 4 in a paddock. Exception was where paddocks were less than 4 ha in size. In production systems with irrigated bays, sampling was conducted across bays in an approximately 100m by 100m area (1ha). In center pivot irrigated crops at least 4 sampling units were tested per pivot.

The incidence of cavity spot on carrots was assessed prior to commercial harvest. Carrots were systematically sampled at 10 locations throughout each assessment unit, with 10 carrots dug out at each location. Where multiple rows were planted per bed carrots were sampled from the outer rows. Carrots were lightly washed before being assessed for the incidence of soilborne disease symptoms, including cavity spot, crown rot, scab, galling, stumping, forking, root protrusions, misshapen and tapered crowns. Severity of cavity spot was assessed according to the following ratings; 0 = Not observed, 1 = (1 lesion <1 cm^2), 3 = (2 lesions <1 cm^2 total area), 5 = (3 lesions or >1 cm^2), 7 = (>3 lesions or > 2 cm^2).

During assessment 2 representative carrots out of each 10 assessed were selected and peel from each side of the 20 carrots was removed including the end of the tap root for pathogen DNA testing.

Measurements of carrot size and yield were undertaken in South Australia, but are not reported as they are predominantly impacted by planting density and earliness of harvest to meet particular market requirements.

In Australia, *P. sulcatum* has been the most commonly reported cause of cavity spot, with *P. violae* only previously detected in a few locations in production regions along the river Murray.

P. sulcatum was detected in 53 of 141 soil samples from validation sites to be planted with carrots, with detection in South Australia, Victoria and Queensland. Separate soil testing detected *P. sulcatum* in soils from carrot production areas in Western Australia and Tasmania. This pathogen is widely distributed across the major carrot production areas of Australia and was confirmed in symptoms of cavity spot from all states.

P. violae was only detected in 2 of 141 soil samples from validation sites to be planted with carrots, with single detections in Queensland and South Australia. Separate soil testing subsequently found *Pythium violae* to be widespread in carrot production areas of northern Tasmania. Testing of samples from carrots with cavity spot symptoms, particularly from South Australia, confirmed *P. violae* was present at sites where it was not detected by pre-plant soil testing.

Many of the cavities observed on carrots were not caused by either *P. sulcatum* or *P. violae.* Many of the symptoms investigated were caused by insect damage, physical restrictions to growth and in some cases other pathogens. Testing of symptoms has improved diagnosis of typical cavity spot caused by *P. sulcatum* and *P. violae*. For some samples visual diagnosis is still difficult.

Pythium sulcatum

Multiple soil samples, even in small paddocks of 2 ha, are required for adequate detection of this pathogen prior to planting (Figure 13). When 3 or more samples were tested prior to planting, DNA testing of the soil indicated the presence of *P. sulcatum* in a paddock and its presence was associated with a risk of cavity spot occurring. A single soil test was inadequate to assess the level of inoculum of *P. sulcatum* in a paddock, or in the 1 ha area it was taken. *P. sulcatum* was present in harvested peel from individual sampling spots where levels were below detection in the soil prior to planting. A minimum of 3 samples should be taken and preferably 4 or more per paddock.

Incidence and severity (Figure 14) of cavity spot symptoms caused by *P. sulcatum* were relatively minor at validation trial sites, when compared with the high incidence of severe symptoms that can occur from this pathogen. Only one sampling spot had more than 5% of carrots with symptoms caused by *P. sulcatum* rated as 5 or higher, and would likely be rejected. Ideally a number of sites included in the validation trials would have developed more severe symptoms. Sites were included in regions and paddocks where cavity spot has occurred before. Inoculum levels at validation sites in the soil prior to planting were low, with the highest detection 0.9 log DNA. Detection of *P. sulcatum* at these levels was associated with risk of cavity spot occurring. It is possible if higher inoculum levels had been present, a higher incidence and greater severity of cavity spot may have occurred. Alternately, if more susceptible varieties were grown, or conditions were very conducive for development of disease more severe symptoms may have occurred.

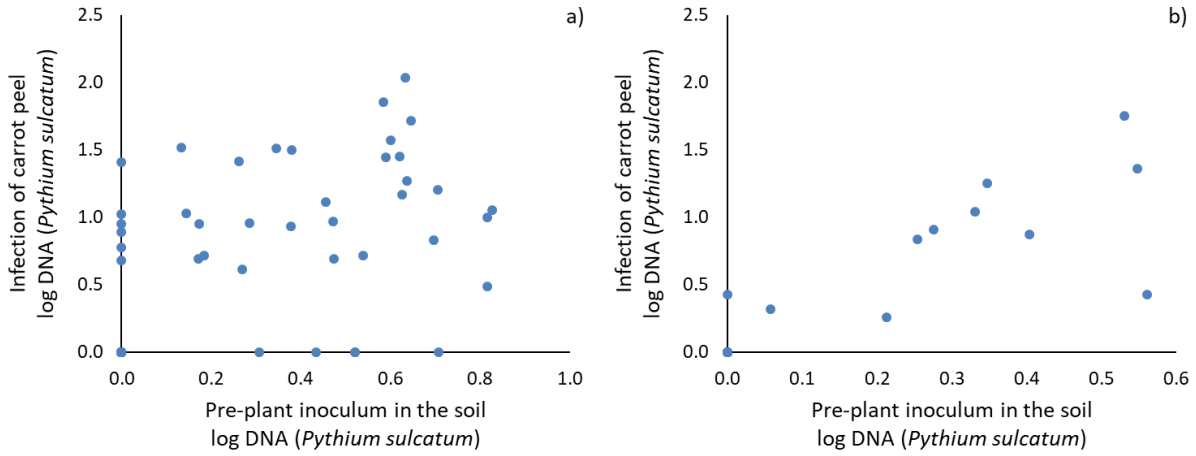

Figure 13: Relationship between inoculum level of *Pythium sulcatum* in the soil prior to planting with infection level of the peel of harvested carrots a) of individual sampling areas and b) of paddock averages. Data only included from paddocks where 3 or more samples taken prior to planting.

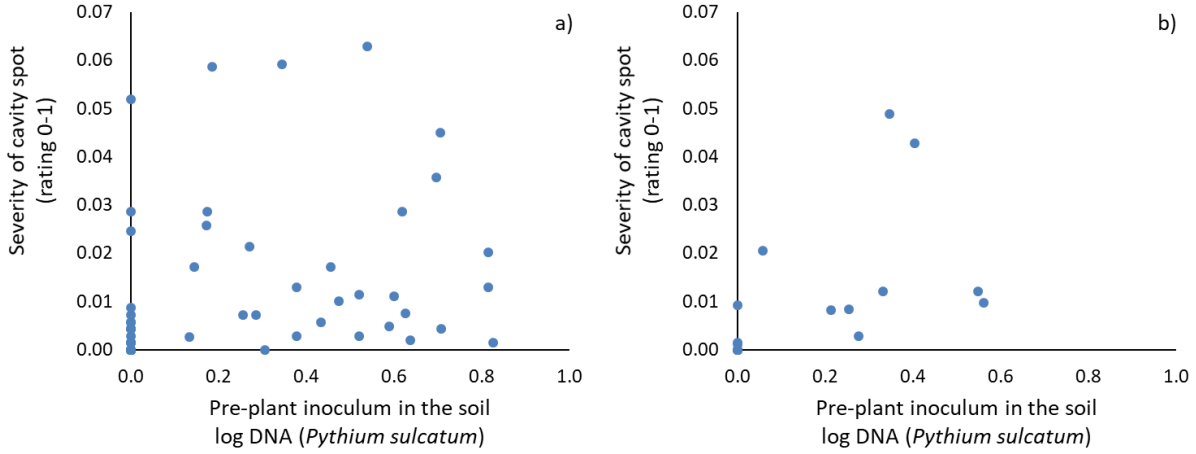

Figure 14: Relationship between inoculum level of *Pythium sulcatum* in the soil prior to planting with severity of cavity spot of harvested carrots a) of individual sampling areas and b) of paddock averages. Data only included from paddocks where 3 or more samples taken prior to planting. Data from sites with symptoms caused by *P. violae* not included. Rating scale 0 = No symptoms, 1 = All carrot with severe symptoms).

Pythium violae

Pre-plant testing did not provide an indication of the presence of *Pythium violae* in a paddock. Out of 40 plantings where soil testing was conducted, *P. violae* was detected at only 2 of them. Testing the peel of carrot samples confirmed that *P. violae* was present in at least 7 other plantings. Of these, symptoms caused by *P. violae* were confirmed from 4 plantings. All affected plantings were harvested in the late winter to late spring period. The incidence and severity of cavity spot symptoms was not commercially significant at 2 of these paddocks. At the other two paddocks 1.3% and 2.4% of carrots assessed had symptoms rated 5 or higher (would be rejected). Carrots with severe symptoms were confined to a few sampling spots within paddocks, with most of the sampling spots having no or minimal symptoms.

In the 2 paddocks where *P. violae* was detected, it was a single detection at a very low level (less than 0.3 log DNA). At one site no cavity spot was observed and at the other site cavity spot occurred, but was caused by *P. sulcatum* which was also detected in the soil prior to planting (in 4 of 4 soil tests). The carrots were harvested from this paddock were harvested in late summer in South Australia. Conditions at this time of year are favorable for *P. sulcatum* to cause cavity spot, and not for *P. violae*.

Results suggest the threshold for disease risk of cavity spot posed by *P. violae* in susceptible varieties is below the level of detection of the test, when soil is sampled from a 1ha area prior to planting.

Testing of soil after harvest from spots where cavity spot occurred in South Australia detected *P. violae* at levels of 1.5 to 1.8 log DNA. Testing across a 1ha that includes these areas, levels were much lower (0.1 to 0.7 log DNA). In paddocks in Tasmania where carrots have been grown, *P. violae* has been detected by sampling of 1ha areas at levels from below detection to 1.7 log DNA.

Pot trials - Tasmania

Three pot trials were conducted by TIA (UTAS, New Town) to establish whether, a) adding increasing amounts of inoculum could result in consistent detection of increasing *P. sulcatum* levels by DNA testing, and b) would these *P. sulcatum* levels promote cavity spot disease.

Soil inoculum levels of *P. sulcatum* detected by DNA testing indicated a robust linear relationship between inoculum added and levels of *P. sulcatum* identified across multiple pathogenic isolates and 3 trials, indicating successful soil spiking and detection (Figure 15).

Inoculation with *P. sulcatum* produced inconsistent results across the three pot trials. Significant disease was recorded in pot trial 1 (field soil) with greater than 90% of the pots treated showing cavity spot infection, the majority of carrots (variety Stefano) with multiple lesions. Pot trial 2 (potting soil) produced minor single lesion disease with 10 out of the 36 pots showing cavity spot symptoms in the carrots (variety Stefano). Negligible disease symptoms were recorded in pot trial 3 (potting soil) in carrots (variety Chantenay). No consistent significant relationship was determined between inoculum levels and disease produced. Although a trend of increasing disease with increasing inoculum levels was recorded, further work is required in this area of threshold development. Environmental, seasonal, irrigation, agronomic and cultivar effects may have played a significant role in impacting disease symptom expression in pot trial 1 and or the lack of significant disease expression in pot trials 2 and 3.

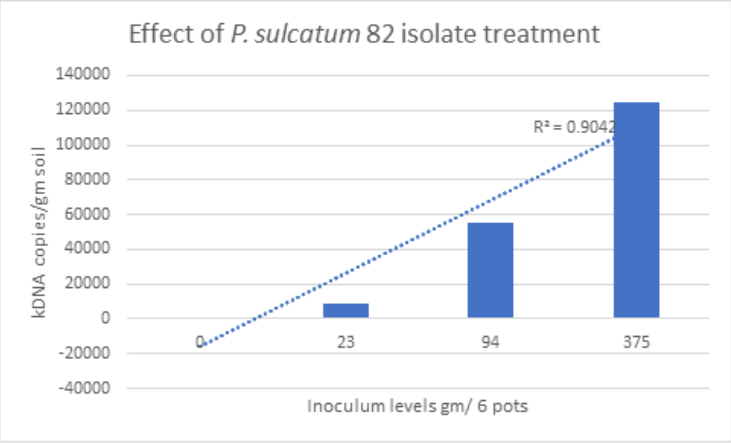

Figure 15: Typical consistent linear relationship between inoculum added and that detected by *Pythium sulcatum* DNA test.

Plot trial – South Australia

A paddock was selected based on pre-plant soil DNA testing that had inoculum of *Pythium sulcatum* (cause of cavity spot) and *Pratylenchus thornei* (root lesion nematode). The trial was split across two plantings located in the same paddock, P1 planted on 8 January 2018 and P2 planted on 1 February 2019 with carrots (variety Avano). Paddocks were maintained at a high moisture level throughout the crop. Twenty plots were located along a single planting bed in each planting. Each plot was 3 m long, with 4 double rows planted per bed. At time of seedling emergence soil samples (approximately 500g consisting of 28 cores 15cm depth by 1cm diameter) were taken from beside rows in each plot for pathogen DNA testing. A second sample was taken for soil nutrient analysis. Along with the primary pathogen targets *P. sulcatum* and *P. thornei*, soil samples were tested for 21 other pathogens. Levels of *P. sulcatum* DNA sampled from individual plots in P1 ranged from below detection to 0.8 log DNA and in P2 from below detection to 0.7 log DNA at the time of planting. Levels of *P. thornei* sampled from individual plots in P1 ranged from 0.1 to 1.8 nematodes/g soil and in P2 from 0.2 to 4.5 nematodes/g soil at the time of planting. *P. violae* was not detected in the soil prior to planting or on peel of harvested carrots.

Soil and carrots were sampled from 3 plots in each of P1 and P2 at 6 and 12 weeks after planting for pathogen DNA analysis.

Harvest assessment was conducted on P1 on 17 April 2018 and on P2 on 15 May 2018. Incidence of cavity spot-like symptoms that would cause downgrading was less than 1% across the trial site. Furthermore, most of the cavities observed were not suspected to be caused by *Pythium* spp., which was confirmed by DNA testing of symptoms.

Levels of *P. sulcatum* detected in the peel of carrots at harvest ranged 0.5 to 1.9 log DNA. Levels did not change substantially between sampling at 6 weeks after planting and at harvest in P1. In P2 levels of *P. sulcatum* were on average 5 times higher when sampled at 6 weeks than when sampled at 12 weeks or at harvest or in P1. These early higher levels of *P. sulcatum* were not linked to increased levels of cavity spot, other defects or productivity loss in P2.

Yield of carrots was dramatically reduced as a result of reduced plant establishment in one area of P1, badly affecting approximately 4 plots and some other plots to a lesser degree. Soil nutrient analysis confirmed low electrochemical stability index (ESI) values at these locations consistent with crusting and dispersion of soil. Coupled with high temperatures at the time of germination this resulted in poor plant establishment and lower plant densities at these plots (Figure 16). Pathogen activity may have contributed to poor establishment and other growth deformities in the carrots from plots with low ESI, as the conditions were favorable for *Pythium*. Levels of *P. sulcatum* tended to be elevated at these locations.

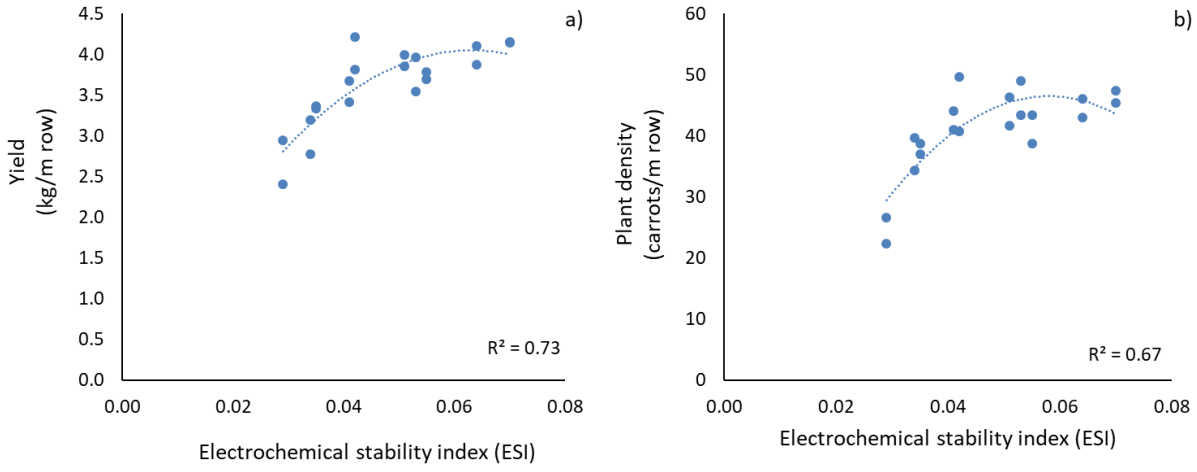

Figure 16: Relationship between electrochemical stability index of soil and a) total yield of carrots and b) plant density of carrots in planting 1.

Root protrusions and tapered crowns were the most significant defects recorded at trial site. Tapered crowns were a result of different soil conditions to root protrusions as there was an inverse relationship between incidences of the two defects.

Incidence of root protrusions on carrots ranged from 5 to 46%, though not all would result in downgrading of the carrots at packing. Increasing levels of *P. sulcatum* inoculum in the soil at planting was associated with increased incidence of root protrusions on harvested carrots (Figure 17). Variation in soil properties across the trial site was also correlated with the prevalence of root protrusions. *P. sulcatum* soil DNA concentration at the seedling stage was correlated with a number of soil chemical properties, indicating that soil type and condition influence pathogen prevalence and/or prevalence of root protrusions.

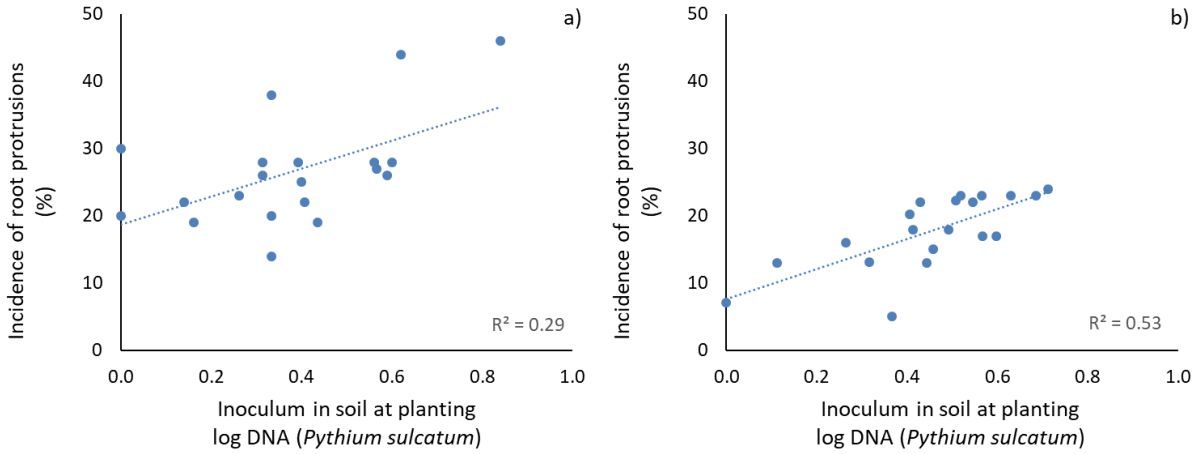

Figure 17: Relationship between inoculum level of *Pythium sulcatum* in the soil at planting and the incidence of root protrusions on harvested carrots in a) planting 1 and b) planting 2.

In both P1 and P2 there was an association between yield loss and the population of *P. thornei* in the soil at the time of planting (Figure 18). The 4 plots with poor plant establishment were omitted from the analysis. Initial *P. thornei* populations in the soil at planting tended to be highest in areas of the paddock with slightly higher clay content. Yield was not strongly associated with the variations in clay content. Some areas of higher clay content had low nematode populations, and higher yields than comparable areas with higher nematode populations. Populations of *P. thornei* in the soil increased more than fivefold between seedling emergence and harvest. Peel of carrots was infested by *P. thornei* from all plots, with levels ranging from 1 to 108 nematodes / g of dry peel. In this trial *P. thornei* when sampled at planting was associated with yield loss at relatively low populations (1 nematode /g soil).

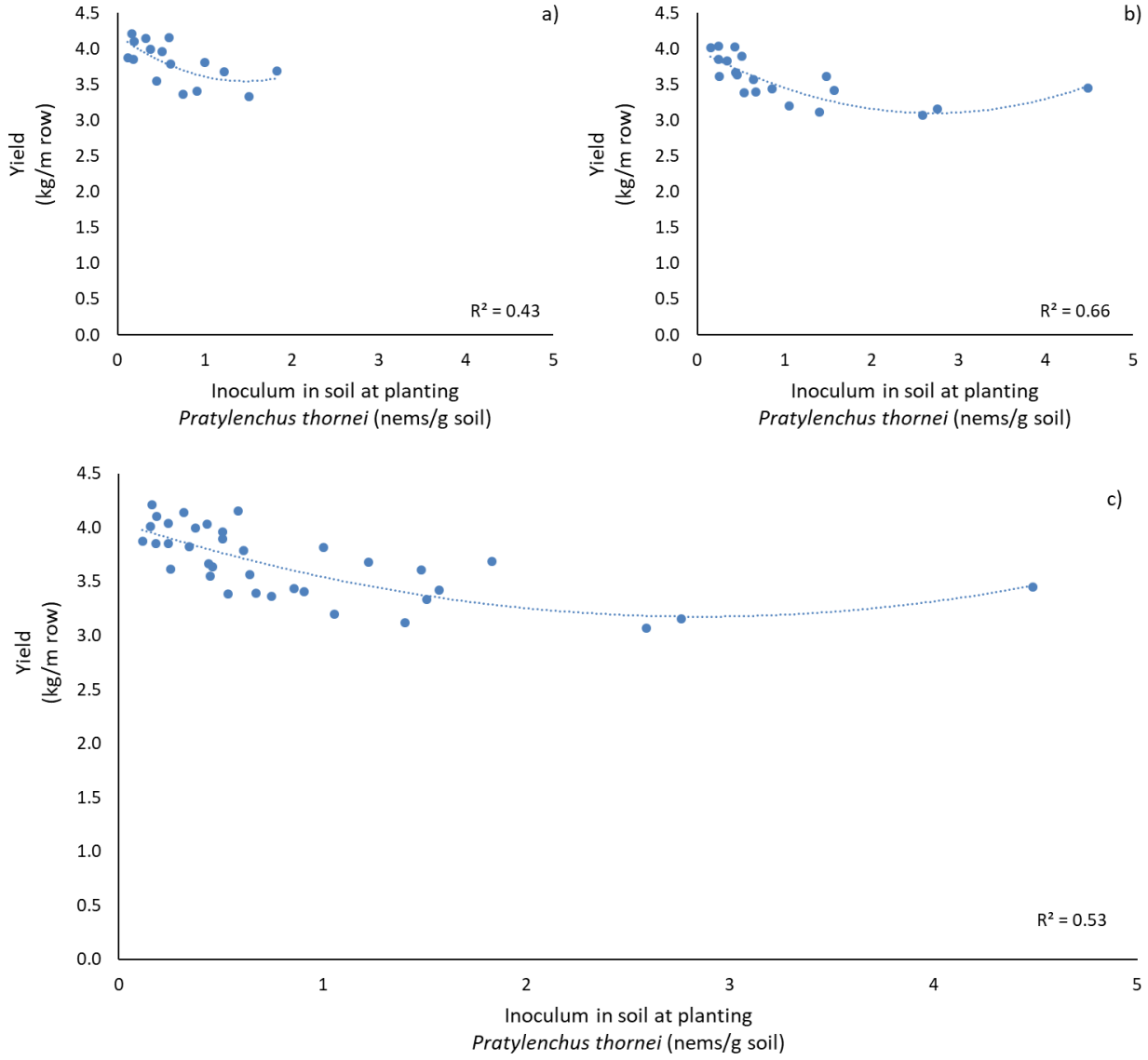

Figure 18: Relationship between population of *Pratylenchus thornei* in the soil at planting and the total yield of carrots in a) planting 1 and b) planting 2 and c) planting 1 and 2 combined.

Plot trial - Tasmania

To investigate the relationship between inoculum level of *P. violae* in the soil and incidence of cavity spot a plot trial was conducted at the Forthside rotation trial site in Tasmania. Previous soil DNA testing had detected *P. violae*. The site was planted with a commercial crop of carrots (variety Stefano) in the last week of November 2018. A more susceptible variety would have been preferred, but this was the commercial variety planted at the site. A series of 24 soil samples were taken from small plots along each of two planting beds and pathogen DNA tested. Sampling consisted of 28 cores taken from a 3m length of the planting bed. An additional 16 small plots across the site were sampled to capture variation in other pathogens at the site including root knot and root lesion nematodes.

At planting the growers standard fungicides (including Ridomil) were applied to the site except for one bed in which 24 small plots were located which was left untreated.

Monitoring of 48 plots has been conducted as part of a long term rotation trial at this site. Soil DNA levels were

sampled in each of these rotation plots 4 months prior to planting carrots.

No obvious symptoms of cavity spot occurred at the site. Results obtained from the small plots and larger rotation plots were similar, so only results from the small plots for *P. sulcatum* and *P. violae* are reported.

P. violae was detected in the soil at planting at 56 of 64 small plots at levels of up to 0.4 log DNA. At harvest *P. violae* was only detected in peel samples from 5 plots (0.3 to 1.6 log DNA) and was not confirmed as the cause of cavity spot symptoms at this site. Stefano is considered to be a less susceptible variety to cavity spot. Soil temperatures towards the end of the crop should have been cool enough for *P. violae* to be active.

P. sulcatum was detected in the soil at planting at 3 small plots at low levels (0.1 log DNA). At harvest *P. sulcatum* was detected in peel samples from 14 plots, mostly at low levels (< 1 log DNA). It was detected in the soil at one of these plots. In this section of the paddock *P. sulcatum* was detected in 4 of 4 peel samples tested at levels ranging from 1.1 to 1.7 log DNA but was not associated with development of cavity spot symptoms. Results indicated that Stefano carrots may be more susceptible to *P. sulcatum* infection than *P. violae*.

Other pathogens

Root knot nematode *M. fallax* was detected in the soil at 5 small plots at low levels (< 0.5 log DNA). *M. fallax* was not detected in the peel of any carrot samples from small plots and low detections in soil at planting were not associated with any quality or productivity loss in harvested carrots. In the larger rotation plots *M. fallax* was detected in 3 samples, with the highest detection being 1.2 log DNA. This was the only plot from which *M. fallax* was detected in the peel of harvested carrots. Three percent of carrots from this plot were stumped, compared with 1% in 3 other rotation plots and no stumping observed in other plots. The level of *Pythium violae* (1.4 log DNA) detected in the peel of harvested carrots from this rotation plot was also the higher of only two plots where *P. violae* was detected.

In past monitoring at the site, isolated detections of *Meloidogyne hapla* have occurred. In these trials *M. hapla* was not detected in the soil samples taken at planting or in peel of harvested carrots from any plot.

Two species of root lesion nematode were detected by preplant testing at this site. *Pratylenchus crenatus* was detected in soil from 41 of 48 rotation trial plots at planting with populations ranging up to 0.3 nematodes / g soil. *P. neglectus* was detected in soil from 39 of 48 rotation trial plots at planting with populations ranging up to 2.2 nematodes / g soil. In similar results to validation sites, *P. neglectus* was not detected in the peel of harvested carrots from any plots and was not strongly associated with any quality or productivity parameter measured. Levels of *P. crenatus* in the soil at planting were much lower than *P. neglectus*, but *P. crenatus* was detected in peel of harvested carrots. Populations at planting were too low to draw conclusions on the impacts of this nematode on productivity, but it is recognized as a pathogen of carrots. Findings were similar from rotation trial plots.

Carrots from the rotation trial were stored after harvest for 3-5 days prior to assessment and sampling for DNA testing. Soft watery lesions developed on up to 8% of carrots. Incidence and severity of the lesions was strongly related to the level of *Sclerotinia sclerotiorum/minor* in the peel of carrots. *S. sclerotiorum/minor* was detected in soil of 47 of 48 plots at planting with levels ranging up to 4.3 log DNA.

Carrots harvested from these trials were the first tested for *Thielaviopsis basicola* (cause of black root rot) with a new DNA test developed outside of this project. *T. basicola* was detected in peel samples from 18 of 48 plots, with some clustering of detections in sections of the paddock. Carrots were not assessed for symptoms of black root rot.

Carrots - Other pathogens and symptoms – Validation sites

Along with the primary pathogen targets of *Pythium sulcatum and P. violae*, soil samples taken prior to planting at carrot validation sites were tested for 20 other pathogens. Not all of these pathogens are considered pathogenic to carrot crops. A breakdown of the rate of detection of these pathogens in soil samples is provided (Table 6) along with a breakdown of the rate of detection of pathogens in carrot peel samples. Testing of carrot peel was not conducted at all validation spots.

The incidence of symptoms other than cavity spot were recorded at validation sites (Table7).

Table 6: Frequency of detection and maximum level detected of pathogens at carrot validation sites by DNA testing.

Pathogen	Pre-plant soil DNA testing (141 Samples)		Carrot peel DNA testing (105 samples)[#]	
	Detected (%)	Maximum log DNA/g soil	Detected (%)	Maximum log DNA/g dry root
Aphanomyces euteiches	2	0.4	1	0.4
Colletotrichum coccodes	18	2.3	2	2.1
Macrophominia phaseolina	62	2.8	47	2.5
Leptosphaeria maculans	30	3.5	1	1.0
Pythium sulcatum	38	0.9	45	2.7
Pythium violae	1	0.1	11	2.1
Pythium clade F	94	2.9	52	3.2
Pythium clade I	91	2.9	37	2.1
Rhizoctonia solani AG 2.1	37	2.8	28	3.1
Rhizoctonia solani AG 2.2	1	1.5	1	1.2
Rhizoctonia solani AG 3	7	2.6	5	3.1
Rhizoctonia solani AG 4	10	2.5	6	3.4
Rhizoctonia solani AG 8	23	2.3	0	0
Verticillium dahliae	1	0.5	0	0
Streptomyces txtA gene	4	1.5	3	2.1
Meloidogyne hapla	1	1.3	0	0
Meloidogyne fallax	0	0	0	0
M. javanica/incognita/arenaria	4	2.5	3	5.5
	Detected (%)	Maximum nems/g soil	Detected (%)	Maximum nems/g dry root
Pratylenchus crenatus	2	0.1	0	0
Pratylenchus neglectus	31	4.5	1	1.6
Pratylenchus penetrans	0	0	0	0
Pratylenchus thornei	23	8.8	21	24.6

[#] Peel samples were not tested from all validation spots in Queensland.

Table 7: Incidence of disease symptoms and disorders recorded on harvested carrots from 112 sampling spots assessed in South Australia, Victoria and Queensland.

Disease or disorder	Percentage sampling areas with detected symptoms	Average incidence	Maximum incidence
Crown rot	36	5.9	52
Scab[+]	53	4.9	60
Galling[#]	22	5.8	100
Stumped	56	2.2	15
Forking	60	2.5	23
Root protrusions	79	5.7	29
Tapered crowns	64	5.0	38
Misshapen	89	11.1	29

[+] Includes range of symptoms from light callus like growth to dark scabby lesions.

[#] Includes swelling on carrots or roots irrespective of the cause.

Root knot nematode

Galling caused by root knot nematodes was only confirmed on carrots from two paddocks located in Queensland included in validation trials. In both cases the severity of symptoms was severe, resulting in harvesting being abandoned. In both cases *M. javanica* was confirmed as the species of root knot nematode involved. Levels detected by the *M. javanica/incognita/arenaria* DNA test in two soil samples prior to planting in one paddock were high (2.3 and 2.5 log DNA), but were lower in the other paddock high (1.0 and 1.3 log DNA). Carrots are very susceptible to root knot nematode damage, with low preplant levels posing a high risk.

Incidence of galling in all other paddocks included in validation trials was confined to enlargements on the tap root of carrots. Examination and DNA testing of these samples confirmed that root knot nematodes were not the cause. These swellings on the tap root can be initiated by damage from pathogens and non-pathogenic causes.

Samples were provided of carrots with root galling symptoms by growers and agronomists from Tasmania and Western Australia. In addition to galling observed on roots, the carrots were generally stumpy and small. For samples from Tasmania, DNA testing of carrot tissue confirmed symptoms were mostly associated with damage from *M. hapla*. Symptoms included galling on fine roots and high incidence of stumpy carrots. In samples from one paddock symptoms were associated with detection of *M. fallax*, though *M. hapla* was also detected in soil and carrot tissue at a lower level. Symptoms included galling on fine roots and high incidence of stumpy carrots. *Pythium violae* was detected at levels up to 1.7 log DNA in soil samples taken post crop from this paddock, but not in tissue of the stumpy carrots tested. For samples from Western Australia, DNA testing of carrot tissue confirmed symptoms were mostly associated with damage from *M. javanica*.

No data was generated on *M. incognita* or *M. arenaria* in relation to causing damage to carrots. Neither of these species were confirmed present in soils at any of the validation sites or in soils from sites where carrots with symptoms of root knot nematode damage were received.

Species of root knot nematode present is related to the temperature zone, soil conditions and region. For example, in southern regions of Western Australia, *M. hapla* and *M. fallax* are known to be present and could pose a risk in carrot crops, while in the Granite Belt of Queensland *M. hapla* is the species known to pose a risk to carrot crops.

Root lesion nematodes

Five species of root lesion nematodes were included in testing. *Pratylenchus neglectus* and *P. thornei* were each detected in 23% or more soil samples, with *P. crenatus* detected in 2% soil samples (Table 6). *P. penetrans* was not

detected in soil samples from validation sites.

At validation sites in this project *P. thornei* was the root lesion nematode that was most frequently detected infesting carrots, as indicated by testing of peel samples (Figure 19). Effect of differences in *P. thornei* levels between validation sites on productivity is difficult to determine due to differences in growing conditions, varieties and the time at which they are harvested to meet market conditions. More specific information on the impacts of *P. thornei* was generated in a plot trial where pre-plant population varied within the one paddock.

P. neglectus was detected in 31% of soil samples, but was only detected in the peel of carrots from 1 sampling spot. This included sampling spots where no nematode treatments were applied. Populations at these sites were low (maximum of 1.3 nematodes/g soil).

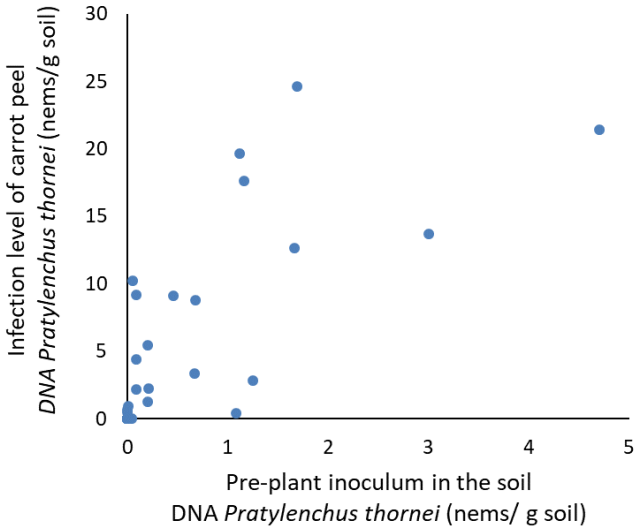

Figure 19: Relationship between the population of *Pratylenchus thornei* in the soil prior to planting and infection level of peel of harvested carrots. (Data from South Australia, Victoria, Queensland). Sites treated for nematodes were omitted from the data.

P. crenatus was detected in 2% of soil samples, but at very low levels (<0.1 nematodes/g soil). These levels are at the limits of detection and no conclusions should be drawn from the data generated. Previous research has indicated that *P. crenatus* is a pathogen of carrots (Hay et al. 2013) and this nematode is widely distributed in cool climate carrot production districts, such as growing regions of Tasmania.

No data was generated on *P. penetrans* from the validation sites, as this species was not detected in either soils or peel samples of harvested carrots. However, in samples provided by growers and agronomists from Western Australia from poor performing crops (reduced growth and small carrots) *P. penetrans* was detected in soil and peel samples of affected carrots. It is likely the poor growth was associated with infestation of the root system by *P. penetrans*, but other pathogens including *Pythium sulcatum* were also detected.

Crown rot

Carrot crown rot suspected to be caused by *Fusarium avenaceum* is not a priority of this project, but at some sites it was the main reason for rejection of carrots for retail sale. The highest incidence and most severe symptoms were observed in a paddock following production of cereals. In one sampling area 20% of carrots would have been rejected due to crown rot symptoms. Isolates were cultured from symptomatic carrots from this and other paddocks, identified by DNA sequencing, and provided to project VG15066 which was investigating factors contributing to the crown rot in carrots. SARDI does not have a DNA assay for *F. avenaceum* in the format for routine soil testing as part of PREDICTA services.

Scab

Several types of scab symptoms were observed on carrots. Some carrots developed callus like scab symptoms, usually at lateral root connections to the main storage root. These symptoms would not have resulted in rejection of carrots for retail sale. A more severe symptom that would have reduced the marketability of carrots was also frequently observed from paddocks in South Australia and Victoria. The symptom developed late in crop development, particularly if harvest was delayed. Carrot scab was not a priority of this project, however isolations were cultured, mainly bacterial, from these symptoms and in some cases identified by DNA sequencing. Identified isolates were considered unlikely to cause the symptoms observed. Symptoms of the range of scab symptoms observed were tested using the DNA test for *Streptomyces* txtA gene. Results were mostly below detection, indicating that scab associated with thaxtomin A was not involved in development of the symptoms. In the two paddocks where *Streptomyces* txtA gene was detected in the soil prior to planting, it was detected in the peel of harvested carrots from those paddocks. In other paddocks where *Streptomyces* txtA gene was not detected in the soil prior to planting, it was only detected at a very low level in the carrot peel of 1 sample. Carrot scab has been reported as a problem when carrots are planted after potatoes in Tasmania. Potatoes are susceptible to common scab caused by *Streptomyces* spp.. It is possible that other forms of scab not observed at validation sites could be caused by *Streptomyces* txtA gene.

Rhizoctonia

Five anastomosis groups (AG's) of *Rhizoctonia solani* were included in testing (AG 2.1, AG 2.2, AG 3, AG 4, AG 8), Table 6. *R. solani* AG 2.1, AG 3, AG 4 and AG 8 were detected in 7 to 37% of soil samples prior to planting. No specific measurements on the impact of these pathogens was undertaken to determine if they were causing productivity loss. With the exception of *R. solani* AG 8 which is not known to be a pathogen of carrots all were commonly detected in peel samples of harvested carrots.

R. solani AG 2.1 was most common AG detected (37% of soil samples tested). Infection level of carrot peel was not strongly correlated with the soil inoculum level of *Rhizoctonia solani* AG 2.1 at individual validation spots. Testing 3 or more samples in a paddock provided a good indication of presence of *R. solani* AG 2.1 in a paddock.

Infection level of carrot peel was not strongly correlated with the soil inoculum level for either *Rhizoctonia solani* AG 3 or AG 4. Data indicates that 3 samples was not sufficient to provide an indication of presence of these pathogens in a paddock prior to planting.

At one site *Rhizoctonia solani* AG 2.2 was identified by DNA tests as the probable cause of severe rot in carrots in wet, sometimes waterlogged, areas of the paddock. *R. solani* AG 2.2 was below the level of detection in all samples taken from this paddock prior to planting. The total area observed to be impacted by *R solani* AG 2.2 within the paddock was small. *R. solani* AG 2.2 was rarely detected in pre-plant soil samples prior to carrot or other crops in this project by DNA testing. *R. solani* AG 2.2 was only detected in the peel of carrots at one other location, which was the only location it was detected by pre-plant testing at a carrot validation site.

Forking and root protrusions

The incidence of forking and root protrusions appeared to be independent of each other, high levels of one not necessarily being associated with high levels of the other.

Forking can be caused by anything that damages the growing tap root early in the crop, including a range of pathogens, but also many nonpathogenic causes such as poor soil structure, soil compaction or pest damage. There were seven paddocks in which forking levels exceeded 5%. Soil conditions would have contributed to forking in at least 3 of these paddocks. Of pathogens that were tested, *Pythium sulcatum*, *Macrophomina phaseolina* and *Pratylenchus neglectus* had elevated levels in either preplant soil or harvest peel testing at some paddocks with high levels of forking. While theses pathogens were present at high levels in some paddocks they may or may not have been associated with any of the forking observed. Incidence of forking tended to be higher in paddocks where the soil respiration rate measured prior to planting was low (Figure 20). Soils with low respiration rate were not confined to a particular soil type, occurring in light sandy soils with low organic matter

and more fertile loam soils.

Unlike forking, not all root protrusions result in rejection of carrots at packing, as small protrusions are simply knocked off. There were seven paddocks in which incidence of root protrusions on carrots exceeded 10%. Soil conditions may have contributed to the incidence of root protrusions in these paddocks. Elevated pathogens levels, either measured in the soil prior to planting or in peel of harvested carrots, were often present in paddocks with a high incidence of root protrusions on harvested carrots. *Pythium* spp. (including *Pythium sulcatum*) and root lesion nematodes were the pathogens most associated with a high incidence of root protrusions. While theses pathogens were present at high levels in some paddocks they may or may not have been associated with any of the root protrusions observed.

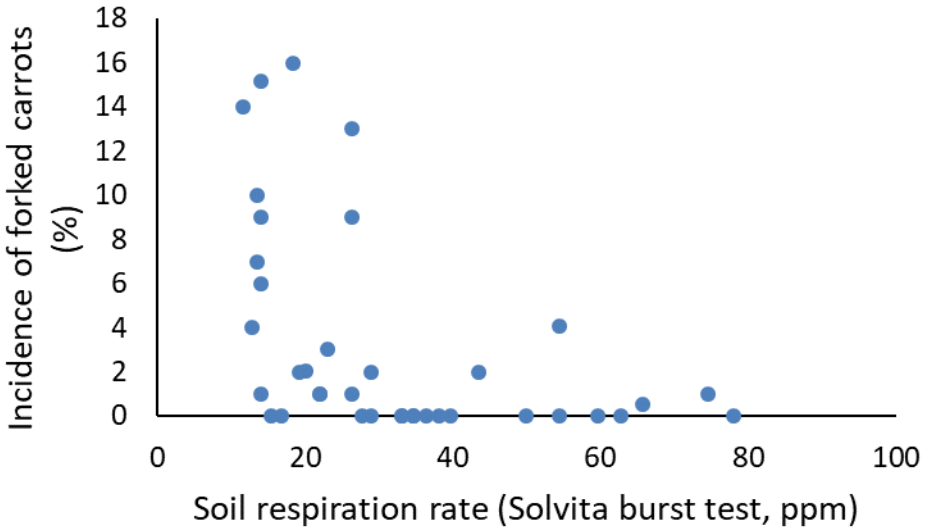

Figure 20: Association between soil respiration rates measured prior to planting and incidence of forking of harvested carrots. (Data from South Australia, Victoria, Queensland).

Pythium

Pythium clade tests are not species specific, with each test detecting a range of pathogenic *Pythium* spp.. Species detected by clade F assay include *P. irregular, P. sylvaticum, P. debaryanum, P. spinosum, P. paroecandrum* and *P. mamillatum*. Species detected by clade I assay include *P. ultimum, P. splendens* and *P. heterothallicum*. As such interpretation of these tests is more difficult than for tests that only detect 1 pathogen species. *Pythium* species from both clades were detected in over 90% of paddocks prior to planting. Level of infection of peel taken from harvested carrots was not correlated with average level of *Pythium* clade I DNA detected in the soil of a paddock prior to planting, whereas increasing levels of Pythium clade F DNA in the soil prior to planting were associated with increasing levels of DNA in peel of harvested carrots grown in those paddocks (Figure 21). Species specific tests are likely to be required to understand relationships between possible symptoms such as reduced emergence and crop establishment or increased incidence of root protrusions and the level of soil inoculum.

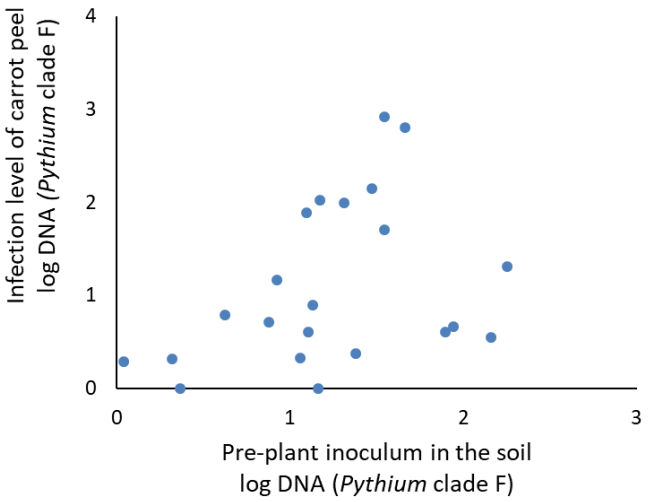

Figure 21: Relationship between the level of *Pythium* clade F DNA in the soil prior to planting and infection level of peel of harvested carrots. (Data from South Australia, Victoria, Tasmania, Queensland).

Charcoal rot

Macrophomina phaseolina was detected in 62% of soil samples tested. Testing indicated that increasing level of inoculum in the soil prior to planting of a paddock was associated with increased infection level in the peel of harvested carrots (Figure 22). *Macrophomina phaseolina* has been linked overseas with forking in carrots. Higher than normal levels of forking were recorded in some paddocks with high pre-plant inoculum levels of *Macrophomina phaseolina*, but this was not confirmed as the cause of this forking. Forking of carrots can be caused by anything that damages the tap root during early crop development and there may have been other causes in these paddocks.

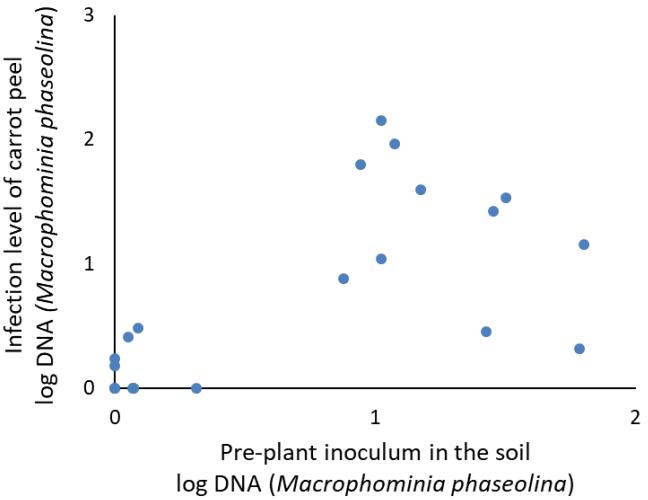

Figure 22: Relationship between the paddock average level of *Macrophominia phaseolina* DNA in the soil prior to planting and infection level of peel of harvested carrots. (Data from South Australia, Victoria, Queensland).

Soil grown greenhouse crops

Greenhouses located on the Northern Adelaide Plains used for capsicum production were the main focus of testing, with some houses included that also produced cucumbers and eggplants. A few houses had grown tomatoes and other crops. Pre-plant soil testing was conducted prior to 120 crops on the properties of 15 growers. Soil was sampled for DNA pathogen testing using a PREDICTA Pt corer (15cm depth by 1cm diameter). Each preplant soil sample was a composite of 30 cores taken along planting rows in each bay of individual greenhouses. Sampling in the dry area outside the crops root zone was avoided as it will underestimate nematode populations in a greenhouse. Testing was focused on root knot nematodes, which are significant soilborne pathogens impacting soil grown greenhouse production of capsicums, cucumbers and eggplants. As the potato research panel was utilized for testing, information was also obtained on other pathogens including *Rhizoctonia solani* and *Colletotrichum coccodes*. Testing was conducted prior to soil treatments, which is standard practice for the control of nematodes and other soilborne pathogens prior to planting greenhouse crops. Post fumigation, incrop and post crop testing was conducted on selected greenhouses to evaluate effectiveness of controls and monitor changes in nematode populations.

The combined test for *Meloidogyne javanica/incognita/arenaria* detected nematodes in 105 of 120 pre-plant soil tests conducted (Table 8). A high proportion of houses had high to very high populations of root knot nematodes prior to planting.

Table 8: Breakdown of the levels of root knot nematodes detected in greenhouses using the combined DNA test for *Meloidogyne javanica, M. incognita* and *M. arenaria* on the Adelaide plains (120 samples tested).

Meloidogyne javanica/incognita/arenaria Detection range	Percentage of pre-plant soil samples tested
Below detection	13
< 1 log DNA	10
1 – <2 log DNA	11
2 to <3 log DNA	33
>3 log DNA	33

Individual species tests for *M. javanica, M. incognita* and *M. arenaria* were also conducted. As these tests are less sensitive than the combined test, often only the combined test gives a positive detection of nematodes when levels are low. Based on samples where the individual tests detected nematodes, *Meloidogyne incognita*, recognized as the important species that impacts productivity of capsicums, was the most common species. *M. incognita* was detected in 54 % of samples compared with *M. javanica* (30% samples) and *M. arenaria* (17% samples). Some houses had all three species present. Some houses used exclusively for capsicum production had populations of *M. javanica* and *M. arenaria*. Testing of healthy capsicum root systems with small galls confirmed they were hosting *M. arenaria* and *M. javanica*. In terms of impact on capsicum crops *M. incognita* was the main species detected at high levels in capsicum roots and associated with severe galling and disease symptoms on plants. Within an individual greenhouse the populations of root knot nematodes in the soil change over time with the cropping cycle and management practices. However, the species present in a greenhouse tends to remain the same, even if at times after soil treatments populations may drop below the level of detection.

Meloidogyne hapla was detected in 7% of greenhouses, its presence associated with cropping history and type of houses, such as past open air field production vegetables or production of tomatoes. Root lesion nematodes (*Pratylenchus crenatus and P. penetrans*) were not detected in any of the 120 preplant samples. *P. neglectus* was present in 2 samples (< 0.1 nematode /g soil).

Colletotrichum coccodes was detected in 88% of pre-plant soil samples, often at high levels. *Rhizoctonia solani* AG 2.1 and AG 4 were detected in 33 and 23% of preplant soil samples respectively.

Monitoring root knot nematode levels

Population of root knot nematodes in the soil is frequently high at the end of a greenhouse cropping cycle and needs to be reduced prior to planting. In the example provided (Figure 23) fumigation was applied to reduce nematode population. When traditional tray extraction and counting was conducted on soil samples after fumigation, nematodes were rarely detected, indicating an absence of healthy J2 stage nematodes in the soil samples. When DNA testing is conducted it frequently indicates a large drop in populations, but detects a residual level of DNA in the soil samples. This does not indicate failure of the fumigation treatment. This residual DNA has been detected in many houses after fumigation. Normally DNA of a dead pathogen is broken down quickly (Pierre et al. 2018), however the reduced biological activity in the soil after fumigation appears to slow down this process until biological activity increases again. In the example the level of DNA in the soil continued to decline for least 49 days. Testing indicates that there is always some nematodes still present and levels generally build up to high levels by the end of a cropping cycle. In some growing systems this buildup was reduced by in-crop management practices.

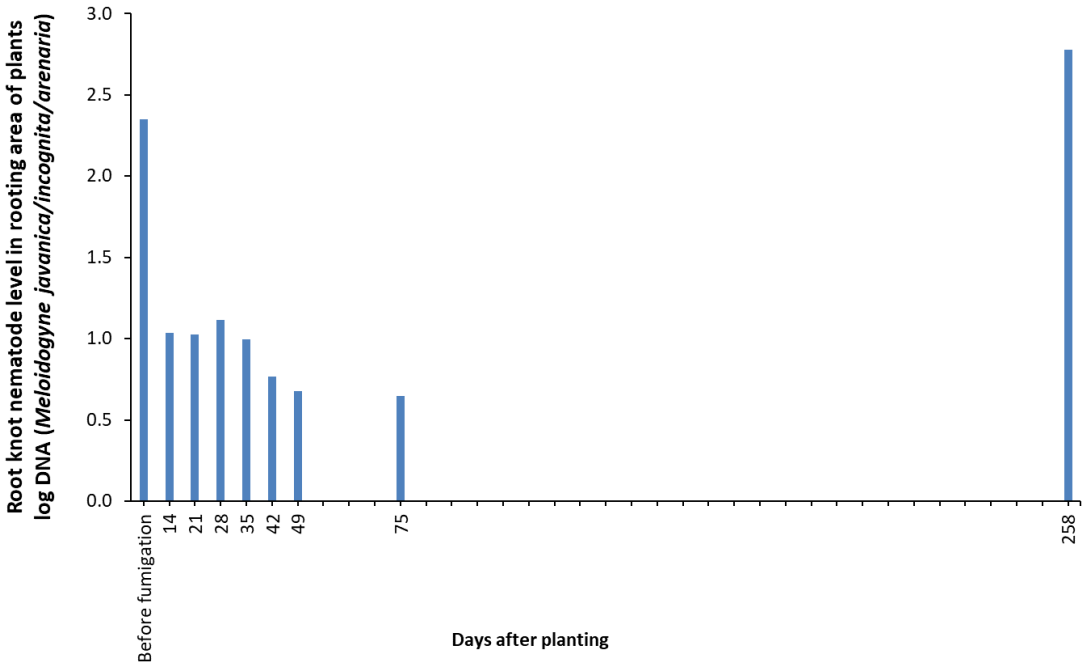

Figure 23: Example of monitoring the population of root knot nematode levels through a typical cycle in greenhouse used for capsicum production from before fumigation to the end of the crop.

Cultivation, fallowing and non host cover crops can be used to reduce nematode population between greenhouse crops. For many growers the window between crops is not sufficiently long to achieve the necessary reduction in levels between crops by these strategies. If a sufficient time window is available, it is important to test that these strategies have effectively reduced the risk of nematodes. Failure to adequately control nematodes in a susceptible greenhouse crop will result in reduced productivity and crop life. In more severe cases, particularly with sensitive crops such as cucumber, crop failure. An example of monitoring a section of a greenhouse where nematode population had been controlled by a soil treatment compared to where it had not been treated and had not dropped sufficiently by the time of planting is provided (Figure 24). Testing allows growers to make informed decisions and monitor effectiveness of strategies to manage the risk of root knot nematodes prior to and during the life of a greenhouse crop.

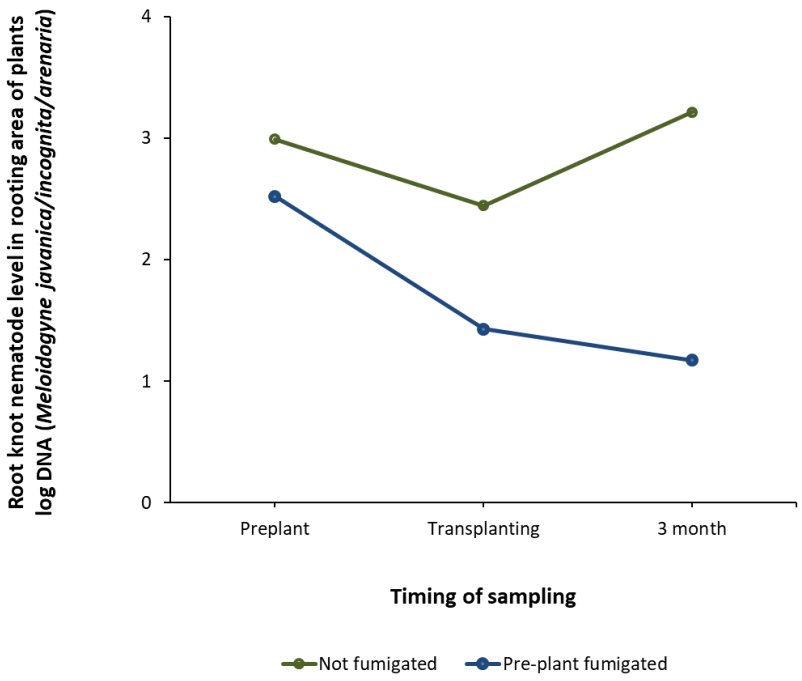

Figure 24: Monitoring of population levels of root knot nematodes in a section of greenhouse where nematodes adequately reduced prior to planting compared with section of greenhouse where crop impacted by buildup of root knot nematodes 3 months into its crop cycle.

Field grown capsicums

Testing was conducted in the Bundaberg district of Queensland. Samples were tested from 9 plantings on the properties of 2 growers. Soil for pathogen DNA testing was sampled using a PREDICTA Pt corer (15cm depth by 1cm diameter). Each preplant soil sample was a composite of 30 cores taken in a W or zigzag pattern across the sampling area, with 2 samples taken in each of 6 paddocks and 1 sample in the other 3 paddocks. Sampling areas were restricted to a single planting and targeted at 1ha units. Samples were tested using the Potato research panel.

The combined test for *Meloidogyne javanica/incognita/arenaria* detected nematodes in 7 of 9 paddocks (9 of 15 soil samples tested). High levels (2.8 to 4.0 log DNA) of *M. javanica/incognita/arenaria* were detected in both areas sampled of two paddocks. In the other 5 paddocks where *M. javanica/incognita/arenaria* was detected levels were low (< 1 log DNA). In 2 of these paddocks *M. javanica/incognita/arenaria* was only detected in 1 of two areas sampled.

Individual species tests for *M. javanica, M. incognita* and *M. arenaria* were also conducted. As these tests are less sensitive than the combined test, often only the combined test gives a positive detection of nematodes when levels are low. In the two paddocks with high levels the individual test for *M. incognita* confirmed this was the dominant species present. *M. javanica* was detected in a single sample of a paddock with low levels. *M. arenaria* which is known to be present in the Bundaberg region was below the level of detection in all preplant soil samples. The cool climate species *M. fallax* and *M. hapla* were not detected.

Paddocks were fumigated after pre-plant testing was conducted. Plant growth along with the incidence and severity of nematodes was assessed 5-8 weeks after planting. Overall the incidence and severity of galling was low. In the two paddocks where *M. javanica/incognita/arenaria* was detected at high levels prior to planting the incidence and severity of galling was slightly higher (10-12% plants with minor root galling) compared with paddocks with low preplant levels (0-10%). High levels of *M. incognita* were confirmed by DNA testing in plants with root galling.

Beans

Testing was conducted in green bean crops in the vegetable growing areas of Lockyer Valley and Gympie. Samples were tested from 9 paddocks on the properties of 3 growers. Crops were grown in rotation with sweetcorn and brassicas or following a brassica biofumigant crop in between bean crops.

Soil for pathogen DNA testing was sampled using a PREDICTA Pt corer (15cm depth by 1cm diameter). Each preplant soil sample was a composite of 30 cores taken in a W or zigzag pattern across the sampling area. Sampling areas were restricted to a single planting and targeted at 1ha units. Samples were tested using the HORT Veg panel.

Macrophomina phaseolina was detected in all pre-plant soil samples tested, with levels ranging from 0.5 to 1.4 log DNA. *Pythium* clade I was detected in all pre-plant soil samples tested, with levels ranging from 0.4 to 2.3 log DNA. Other pathogens detected in preplant soil samples that cause soilborne disease of beans were *Rhizoctonia solani* AG 2.1, *Aphanomyces euteiches*, *Pythium* clade F, *Sclerotinia sclerotiorum/minor* and *Pratylenchus neglectus*.

Crops were assessed for uniformity of growth between flowering and poding. Five of the bean crops were uniform and not showing obvious signs of soilborne disease. In four paddocks, size of 10 -24 percent of plants was visually reduced (10% or more) of which 1- 8 percent were reduced by more than 20%. Composite root samples of plants showing reduced growth with compromised root systems were collected from areas within 4 of the 9 paddocks.

At two paddocks in the Lockyer Valley *Macrophomina phaseolina* (cause of charcoal rot) was detected at high levels in the four composite root samples tested. Other pathogens detected in the root samples from these two paddocks were *Rhizoctonia solani* AG 2.1 and *Aphanomyces euteiches* (1 sample), *R. solani* AG 4 (1 sample from each paddock) with *Pythium* clade I, *Sclerotinia sclerotiorum/minor, Thielaviopsis basicola, Setophoma terrestris* and *Pratylenchus neglectus* detected in all samples.

At two paddocks in the Gympie region *Thielaviopsis basicola* (cause of black root rot) was detected at high levels in the three composite root samples tested. Other pathogens detected in the root samples from these two paddocks were *Aphanomyces euteiches* (1 sample) and *Pythium* clade F, *Pythium* clade I, *Sclerotinia sclerotiorum/minor* and *Setophoma terrestris* detected in all samples. Disease complexes are commonly associated with root rots in bean crops.

There is insufficient data to draw conclusions on the relationships between inoculum in the soil detected prior to planting and impacts on root health and productivity in bean crops. A more detailed study relating pathogen inoculum levels in soil, pathogen infection levels in root systems with disease incidence and lost productivity would be required. Previous work was conducted in Tasmania of a similar nature using a more limited suite of DNA tests for soil testing and traditional pathology for symptom identification (Pung 2010). Testing in this project provides an example of the range of DNA assays now available coupled with methods to quantify infection levels of root systems to assist investigate soilborne disease complexes of beans in the future.

Sweet potato

Testing of soil samples was conducted in paddocks to be planted with sweet potato crops in Bundaberg region. Samples were tested from 21 paddocks on the properties of 7 growers.

Soil for pathogen DNA testing was sampled using a PREDICTA Pt corer (15cm depth by 1cm diameter). Each preplant soil sample was a composite of 30 cores taken in a W or zigzag pattern across the sampling area, with 2 samples taken in 15 of the 21 paddocks. Sampling areas were targeted at 1ha units. Sampling was conducted prior to soil fumigation treatments, which is commonly applied for the control of nematodes and other soilborne pathogens prior to planting sweet potatoes. Testing was focused on root knot nematodes, which are significant soilborne pathogens impacting sweet potatoes with samples tested using the Potato research panel.

The combined test for *Meloidogyne javanica/incognita/arenaria* detected nematodes in 15 of 21 paddocks (23 of 36 soil samples tested). In 8 of 9 paddocks where two samples were taken and *M. javanica/incognita/arenaria* detected, it was detected in both samples. Individual species tests for *M. javanica, M. incognita* and *M. arenaria* were also conducted. As these tests are less sensitive than the combined test, often only the combined test gives a positive detection of nematodes when levels are low. In the 6 paddocks where individual tests detected nematodes in soil prior to planting, *M. javanica* was confirmed present. *M. incognita* was confirmed present in two paddocks of the same grower. *M. arenaria* which is known to be present in the Bundaberg region was below the level of detection in all preplant soil samples. The cool climate species *M. fallax* and *M. hapla* were not detected.

Sampling at harvest was conducted in 8 paddocks, with a total of 20 samples tested. *Meloidogyne javanica* was

the only species detected in the peel of tuber samples. This was consistent with testing of peel samples that had symptoms of root knot nematodes provided by agronomists from paddocks in the Bundaberg region. Symptoms included galling and cracking. *M. javanica* was the main species of root knot nematode associated with symptoms. In some tubers *M. incognita* was present, but at much lower levels. *M. arenaria* was not detected in the peel of the samples tested.

Some tubers submitted had a condition called dirty eye (deepened eyes with a dark discoloration that sometimes extends into the tuber flesh). When symptoms were tested, they were associated with high levels of *M. javanica*, but this does not confirm that this is the cause or the only pathogen involved causing this symptom.

Sweet corn

Limited testing was conducted in sweetcorn crops grown in vegetable growing areas of South-east Queensland. Samples were tested from 10 paddocks on the properties of 2 growers. Crops were grown in rotation with brassicas and beans.

Soil for pathogen DNA testing was sampled using a PREDICTA Pt corer (15cm depth by 1cm diameter). Each preplant soil sample was a composite of 30 cores taken in a W or zigzag pattern across the sampling area, with 2 samples taken in each paddock. Sampling areas were restricted to a single planting and targeted at 1ha units.

Crops were assessed for uniformity of growth between 3 and 6 weeks after planting. All crops were uniform and not showing obvious signs of soilborne disease on the roots of 100 plants examined at each sampling location. As crops were not showing signs of soilborne disease, limited pathogen DNA testing of root samples was conducted.

Macrophomina phaseolina (cause of charcoal rot) was detected in 19 of 20 pre-plant soil samples at levels 0.3 to 1.8 log DNA and detected in the 4 root samples tested.

Pythium clade F was detected in both samples of 7 paddocks at levels of 0.2 to 1.9 log DNA and was not detected in the other 3 paddocks. Samples from the same paddock had similar levels. In the paddock *Pythium* clade F detected in one soil sample, level in the other was the lowest detected (0.2 log DNA).

Pythium clade I was detected in 19 of 20 pre-plant soil samples at levels 0.3 to 3.1 log DNA. Samples from the same paddock had similar levels.

Both *Pythium* clade F and clade I were detected in the 4 root samples tested. All were from paddocks where detected in soil prior to planting.

Three species of root lesion nematodes were detected in the soil prior to planting. *Pratylenchus neglectus* was detected in both samples of 4 paddocks (0.3 to 1.3 nematodes / g soil). *P. thornei* was detected at very low levels (< 0.2 nematodes / g soil) in 4 samples across 3 paddocks and *P. zeae* was detected in a single sample (0.9 nematodes / g soil). As levels in the soil prior to planting were low or below detection, no conclusions should be drawn on the potential for root lesion nematodes to reduce productivity from these sites.

Rhizoctonia solani AG 4 was detected in 8 of 20 samples and in 5 of 10 paddocks. *R. solani* AG 4 was detected in roots of samples from paddock where detected in soil prior to planting, but not in roots of samples from paddock where not detected in soil prior to planting.

Setophoma terrestris was not tested for in all paddocks. In the 6 paddocks where tested, it was detected in all pre-plant soil samples at high levels (2.1 to 2.5 log DNA) and was detected on roots of the 4 samples tested.

Results indicate that the above pathogens are present and infecting root systems. While crops assessed in the SE region of Queensland were not showing symptoms of soilborne disease at the time of assessment, some loss in productivity may be occurring from pathogens such as *Macrophomina phaseolina*, *Setophoma terrestris, Pythium* spp. *and Rhizoctonia solani* AG 4.

Sampling

Root knot nematodes

Economic thresholds for root knot nematodes, based on Whitehead tray extraction method, are available for most crops, to calibrate the DNA tests. For some crops such as carrots, sweet potato and greenhouse grown vegetables, detection at low population levels (<10 nematodes / 200g) is required. Intensity of field sampling, and not sensitivity of the DNA test, is the limiting factor. To be confident that the risk in an area is low, sampling intensity must be sufficient that additional samples would also (with high probability) not detect nematode populations above the economic threshold. In greenhouses, a high intensity of sampling can be economically utilized to provide an indication of the risk posed by root knot nematodes. However, in field grown crops, where paddock sizes of up to 50 ha are grown, and the return per hectare is lower, a strategic sampling strategy is required.

Soil samples were collected using the standard protocol for PREDICTA Pt testing in 6 paddocks in Tasmania with low levels of the root knot nematodes *Meloidogyne hapla* and/or *M. fallax*. Soil for pathogen DNA testing was sampled using a PREDICTA Pt corer (15cm depth by 1cm diameter). Each pre-plant soil sample was a composite of 30 cores taken in a W or zigzag pattern across the sampling area. 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 *M. fallax* and *M. hapla* detected by the 8 samples in each 1 ha area are reported (Table 9). Taking one sample per paddock is not adequate, as there is an unacceptable risk of failure to warn of a nematode risk. Analysis of the data indicates a minimum of 4 samples per paddock should be taken, and more than 4 samples; in paddocks over 4 ha with a high value crop or where the paddock is heterogeneous.

Table 9: Range in levels of *Meloidogyne fallax* and *M. hapla* detected by repeated soil sampling (8 times) in the same 1ha area at 4 locations in each of 6 paddocks.

Paddock	Sample	*Meloidogyne fallax* (log DNA)			*Meloidogyne hapla* (log DNA)		
		Number samples below detection	Range of levels detected	Average	Number samples below detection	Range of levels detected	Average
A	1	0	0.7 – 1.7	1.2	7	0 – 0.4	0.1
	2	0	0.4 – 1.3	1.1	7	0 – 0.3	0.0
	3	0	1.1 – 1.6	1.4	8	0 – 0	0.0
	4	0	1.0 – 1.9	1.4	8	0 – 0	0.0
B	1	0	1.7 – 2.3	2.0	6	0 – 1.3	0.2
	2	0	1.8 – 2.2	2.1	4	0 – 1.2	0.3
	3	0	1.4 – 1.9	1.8	0	0.8 – 1.6	1.2
	4	0	1.6 – 2.1	1.8	2	0 – 1.9	1.0
C	1	0	0.6 – 1.4	1.0	8	0 – 0	0.0
	2	0	0.4 – 1.1	0.9	8	0 – 0	0.0
	3	1	0.0 – 1.2	0.8	8	0 – 0	0.0
	4	0	0.7 – 1.5	1.1	8	0 – 0	0.0
D	1	0	0.5 – 1.7	1.2	8	0 – 0	0.0
	2	0	0.9 – 2.0	1.4	8	0 – 0	0.0
	3	0	1.3 – 2.5	2.0	7	0 – 0.2	0.0
	4	0	1.4 – 2.2	1.8	8	0 – 0	0.0
E	1	0	0.4 – 2.1	1.5	0	0.3 – 2.1	1.2
	2	0	0.4 – 2.5	1.4	0	0.4 – 2.2	1.5
	3	0	0.4 – 2.1	1.1	0	0.5 – 1.7	1.1
	4	1	0.0 – 1.9	1.1	0	0.5 – 2.4	1.5
F	1	0	1.0 – 2.1	1.4	1	0 – 1.3	0.6
	2	0	1.2 – 2.2	1.5	3	0 – 1.1	0.5
	3	0	1.3 – 1.9	1.5	3	0 – 0.8	0.4
	4	0	2.7 – 3.0	2.9	0	1.5 – 2.6	2.2

Calibration of DNA tests to traditional counts

Root knot nematodes

Comparative counts have been conducted on field samples in a number of trials in this project and other projects (including MT090067, SRA project 'Molecular assay of major soil-borne pathogens for better exploitation of commercial varieties') to establish the relationship between DNA test results for root knot nematodes *Meloidogyne fallax*, *M. hapla*, *M. javanica/incognita/arenaria* and traditional tray extraction methods. The two methods are not directly comparable, as manual counts rely on the extraction of J2's, whereas DNA measures all stages of the nematode present. Depending on the field data used, values of 5 to 17pg DNA / g soil sample were generated as being comparable to 10 nematodes / 200g using tray extraction. For practical interpretation, values for DNA testing quoted in pg DNA / g soil for *M. fallax*, *M. hapla*, *M. javanica/incognita/arenaria* can be regarded as equivalent to nematode population based on Whitehead tray extraction method reported as nematodes / 200 g soil.

There is a poor correlation between DNA testing and manual tray extraction at low nematode populations (<10 nematodes / 200g soil sample), due mainly to sampling variation. For crops and situations where any detection of root knot nematode is considered a risk, sampling intensities must be sufficient to be confident of detecting root knot nematodes if present. These findings are not limited to DNA soil testing, as the detection limit for the DNA based test is as good as or better than that for traditional tray extraction.

Root lesion nematodes

Test results for the HORT Veg and other SARDI delivered PREDICTA testing services are already reported in nematode per g equivalents based on established calibrations for *Pratylenchus crenatus, P. neglectus, P. penetrans and P. thornei*.

P. zeae is currently reported in Kcopies DNA/g soil. A conversion factor has been developed for *P.* zeae in SRA project 'Molecular assay of major soil-borne pathogens for better exploitation of commercial varieties'. To convert Kcopies DNA/g soil to nematode per g equivalent divide by 28.

Appendix 2: Publication - Testing for soilborne pathogens

Testing for soilborne pathogens

BRASSICA - CARROT - CAPSICUM - SWEET POTATO - ONION

PREDICTA
DNA Soilborne disease tests

How PREDICTA® can support your crop monitoring and management decisions

Hort Innovation

Government of South Australia
Primary Industries and Regions SA

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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EDITOR: Belinda Cay and Alistair Lawson (AgCommunicators)

DESIGNER: Jane McLean Design

Disclaimer
The information in this manual is only intended to be general background technical information to support the awareness of **PREDICTA®** testing in the vegetable industry. It is not intended to be exhaustive and readers may need to refer to other technical literature and information and/or seek independent professional advice.

While the authors have made every effort to ensure the information contained within is accurate and up to date at the time of preparation, they do not make any representation or give any warranty as to the accuracy, currency, reliability or completeness of the information.

Contents

Key contact:

MICHAEL RETTKE
0401 122 124
michael.rettke@sa.gov.au

Overview

Soilborne diseases are a major limiting factor for the Australian vegetable industry and are costly and difficult to manage. Previous research has highlighted that practical and economic methods of disease control are limited once a crop has been established. Knowing the disease risk prior to planting allows growers to make informed crop management decisions.

The SARDI Molecular Diagnostic Centre has developed PREDICTA®, a DNA-based soil testing technology that can accurately quantify the level of DNA of specific disease causing pathogens in a sample of soil. The testing service allows growers to identify which soilborne pathogens pose a significant risk to their crops so steps can be taken to minimise production losses.

The current suite of tests available for vegetable growers is most suited to use in brassica, carrot, capsicum, sweet potato and onion production. While testing can support other vegetable crops, the applications can be more limited and is dependent on the diseases present in the soil. The SARDI Molecular Diagnostic Centre support team is available to answer any questions on the application and potential of testing.

Know before you plant

- Pre-planting soilborne disease assessment is critical to make informed decisions, implement cost-effective disease control strategies and reduce potential losses.
- Crop loss from soilborne disease can be substantial. Soilborne diseases can cause crop failure resulting in substantial financial loss as well as failure to supply the market.
- Knowing the level of risk and being able to monitor changes in this risk through cropping cycles provides the greatest opportunity to manage soilborne diseases successfully.

Soilborne pathogens and diseases

Soilborne diseases can have a serious impact on vegetable quality and yield. Productivity losses can occur through:

- Lower field yield and/or reduced packout
- Limited water and nutrient uptake
- Increased input requirements
- Increased weed seed set through reduced crop competition
- Increased crop damage from some herbicides
- Reduced cropping options

The pathogens that cause soilborne diseases survive in the soil, waiting to infect the root system of vegetable crops. For some pathogens, the quantity of inoculum in the soil is strongly related to the risk of disease occurring.

Definitions

PATHOGEN – organism (e.g. plant parasitic fungus, bacteria or nematode) that infects plant to cause disease

INOCULUM – parts of pathogen that reside in the soil and can infect plants

DISEASE – expression of symptoms that negatively affect yield and/or quality of vegetable crop (i.e. symptoms caused by the pathogen)

Did you know?

There are four main factors that determine the development of each soilborne disease.

- Pathogen level
- The crop or variety grown
- On-farm management practices, and
- The environment.

The grower has the ability to vary agronomic practices, but unfortunately, they have less control on the environment.

PREDICTA® fast facts

Did you know PREDICTA® testing can …

- ✓ Detect low levels of specific pathogens in soil (or other sample types)
- ✓ Quantify specific pathogens in a sample
- ✓ Test multiple pathogens on the same sample
- ✓ Deliver sample results relatively quickly, compared to traditional inoculum measurement techniques
- ✓ Quantify pathogen levels both prior to planting and at any stage of the cropping cycle

PREDICTA® allows growers and advisers to:

- ✓ Conduct pre-plant assessments of disease risk
- ✓ Conduct in-crop testing of soil or plant samples
- ✓ Monitor and understand pathogen level changes in cropping systems
- ✓ Evaluate management practices and their effect on pathogen inoculum levels
- ✓ Investigate causes of disease
- ✓ Select on-farm trial sites
- ✓ Improve outcomes and knowledge gain from on-farm trials
- ✓ Implement better management decisions, such as crop type and variety selection
- ✓ Minimise losses from soilborne disease

PREDICTA® pathogen testing

Growers can now use PREDICTA® to understand pathogen levels.

PREDICTA® testing can provide an indication of the risk or probability of some soilborne diseases occurring. This is possible for pathogens where the inoculum level in the soil – measured as the concentration of DNA by PREDICTA® – is strongly linked to the likelihood of disease occurring. Higher levels of DNA indicate higher levels of disease risk.

PREDICTA® test results can tell a grower which diseases are of greatest concern, allowing a suitable management plan to be implemented which considers variety choice, rotations, chemical management or whether or not to even plant a crop.

Testing allows growers to optimise paddock management to minimise current and future productivity losses.

Pathogen DNA tests available

SARDI offers growers a diverse range of pathogen DNA tests (TABLE 1).

TABLE 1: Pathogen DNA tests included in the vegetable testing suite offered by SARDI.

	Pathogen	Disease
Fungi	*Rhizoctonia solani* (AG2.1)	Rhizoctonia
	Rhizoctonia solani (AG2.2)	
	Rhizoctonia solani (AG3)	
	Rhizoctonia solani (AG4)	
	Rhizoctonia solani (AG8)	
	Verticillium dahliae	Verticillium wilt
	Leptosphaeria maculans	Blackleg
	Colletotrichum coccodes	Black dot
	Setophoma terrestris	Pink root
	Macrophomina phaseolina	Charcoal rot
	Sclerotinia sclerotiorum, *S. minor*	Sclerotinia rot
	Thielaviopsis basicola	Black root rot
Oomycetes	*Aphanomyces euteiches*	Aphanomyces root rot
	Pythium sulcatum	Cavity spot
	Pythium violae	
	Pythium (clade F)	Pythium (damping off)
	Pythium (clade I)	
Nematodes	*Meloidogyne fallax*	Root knot nematodes
	Meloidogyne hapla	
	Meloidogyne arenaria, *M. incognita*, *M. javanica*	
	Pratylenchus crenatus	Root lesion nematodes
	Pratylenchus neglectus	
	Pratylenchus penetrans	
	Pratylenchus thornei	
	Pratylenchus zeae	
Bacteria	*Streptomyces* txtA gene	Scab
Phytomyxea	*Plasmodiophora brassicae*	Clubroot

List is subject to change – for updated list and other available pathogen DNA tests
https://pir.sa.gov.au/research/services/molecular_diagnostics/predicta_research

New PREDICTA® tests only become available after a process of test design and verification

Additional tests available

Soil health

PREDICTA® technology can quantify the level of Free Living Nematodes (FLN) in the soil that can be used to monitor soil health.

Beneficials

PREDICTA® technology can quantify the levels of Arbuscular Mycorrhiza Fungi (AMF) and *Trichoderma* which can be beneficial to some vegetable crops.

Types of samples that can be tested

Soil

Soil is the most common sample type tested by PREDICTA®.

Soil samples weighing up to 500 grams (dry weight) can be tested.

Potting mixes and growth media have high levels of organic matter and require special processing techniques. Therefore, please notify the laboratory prior to testing if these types of samples are submitted.

Plant tissue

- Specialist testing of plant tissue samples is available.
- Testing can include plant root systems, plant stem sections and peels.

Discuss options, sample preparation and handling with the laboratory before sampling.

Sampling for PREDICTA® testing

Correct sampling is critical to obtaining meaningful results from PREDICTA® soil DNA testing.

Pathogens are not evenly distributed throughout paddocks and soil profiles. Inadequate sampling will most certainly either overestimate or underestimate the level of pathogen inoculum in a paddock.

SARDI provides training and accreditation to agronomists to collect soil samples for PREDICTA® testing using a PREDICTA® soil corer or equivalent.

PREDICTA® Soil corer

Sampling in field

General sampling guidelines

Sample handling

- If the sample is moist, keep it cool (below 10°C) until dispatch
- Deliver samples to the testing laboratory within 1-2 days
- Avoid leaving samples in plastic bags exposed to sun (i.e. on the dash board of a car or ute)

Individual samples

- Collect 30 individual cores using a soil corer with a 15cm depth by 1 cm diameter tip (sample weight should not exceed 500g if the correct corer tip used – avoid subsampling)
- Sample in W pattern across the area
- Collect samples within one hectare if the paddock is greater than one hectare in size
- Target productive cropping areas, avoiding the edge of paddocks, atypical small patches and low waterlogged areas or areas too close to trees

Number of samples required per paddock

The number of samples required per paddock depends on:

- The size and shape of the paddock
- The variability of soils and conditions within the paddock
- The uniformity of past cropping history
- The differences in past disease incidence
- The pathogen that is the main target of sampling

Sampling pattern

Open paddock or pivot

- Multiple representative samples are required per paddock
- Each sampling area is one hectare
- Sampling areas must be aligned with variations in soil conditions and past history

Permanent bays

- Each sampling area must be confined within a bay or a set of bays
- Multiple samples per paddock are required
- Sampling areas must be aligned with variations in soil conditions and past history
- Sampling areas must be aligned to planting schedule
- In cases of controlled traffic, sampling must be confined to cropped areas

Greenhouses

- Each sampling area must be confined to one greenhouse
- Normally one test per greenhouse will suffice, unless past cropping history or disease incidence varies
- Sampling must be conducted along row lines

Did you know?

Targeted samples taken from a defined area can be used if soilborne diseases are suspected to be a problem at that location.

Important note

When using PREDICTA® to enhance outcomes and findings from on-farm trials or ongoing monitoring of the cropping system, specific advice should be obtained on best sampling strategies to maximise the value of the data collected.

Disease risk thresholds

Establishment of disease risk thresholds requires field validation of the probability of disease occurring at different levels of inoculum in the soil for a specific crop and production environment.

- At low or non-detectable levels of inoculum, the risk of disease is low.
- At high levels of inoculum, occurrence and severity of disease depends on susceptibility of the variety, conduciveness of the environment and effectiveness of management options applied.
- High levels of inoculum do not mean disease will occur, but that there is a high risk if conditions are favourable for disease development.

If available, disease risk categories should be used as a general guide only. Other factors such as climate, management practices, soil type, crop type, variety, seasonal conditions and seedling health (if transplanted) should be considered in interpreting PREDICTA® results and assessing disease risk.

After repeated use of PREDICTA® tests within a cropping system, patterns observed in pathogen levels and disease occurrence can be used to refine interpretation.

Interpretation of disease risk is not available for all tests.

For some pathogens, the level of inoculum that poses a disease risk is lower than what can be detected. PREDICTA® testing is not a useful tool for assessing the risk of disease for these pathogens.

These tests can still be powerful tools for monitoring and decision making as they provide quantitative data on pathogen levels in cropping systems where infection and/or disease is occurring. Some examples of this include providing early warning of pathogen build-up and infection in a crop, assessing rotation crops as non-host break crops and evaluation of management strategies on infection levels and changes in inoculum.

What PREDICTA® testing does not do

PREDICTA® testing does not:

- Indicate the presence of pathogens that are not specifically included in testing. For example, beet cyst nematode will not be detected, as a test has not been developed for that pathogen
- Confirm whether disease will occur, as pathogen inoculum will only cause disease if conditions are favourable
- Confirm the disease will not occur, as a pathogen below detection limits may still cause disease if the conditions are favourable

What actions can be taken based on test results?

Knowing the risk of soilborne disease prior to planting maximises the range of management options available. It enables the implementation of a soilborne disease management strategy appropriate to the level of risk.

Depending on the crop, production system and environment, management options may include:

- Not planting in paddocks where risk is unmanageable
- Extending the length of the rotation
- Including non-host crops in the rotation
- Including a non-host cover crop in the rotation
- Choosing a tolerant or resistant variety
- Changing paddock scheduling to avoid growing in a high-risk timeslot
- Adjusting harvest scheduling to lower disease risk
- Applying soil amendment before planting
- Applying mulch or organic matter
- Adjusting tillage practices
- Utilising growth promotants or biological products
- Applying soil treatment
- Optimising nutrition to lower disease risk
- Optimising irrigation to lower disease risk
- Improving drainage

Disease tests

- Clubroot
- Root lesion nematodes
- Root knot nematodes
- Cavity spot
- Rhizoctonia
- Sclerotinia rot
- Pythium clade F and I
- Black leg
- Charcoal rot
- Black root rot
- Black dot
- Verticillium wilt
- Aphanomyces root rot

Clubroot

(caused by *Plasmodiophora brassicae*)

Clubroot is a devastating disease of brassicas. It can cause total crop failure if inoculum levels are high and conditions are favourable.

Inoculum can build to high levels in crops showing minimal symptoms and then badly affect the next crop.

PREDICTA® can quantify the level of *Plasmodiophora brassicae* inoculum in the soil which has been linked to the risk of disease (FIGURE 1). Testing prior to planting provides a useful indication of the risk of clubroot occurring in susceptible brassica crops.

There are several races of *P. brassicae* that cause clubroot. The PREDICTA® test is designed to detect all races of *P. brassicae,* but does not identify the race being detected in a sample.

Understanding risk of this disease by using PREDICTA® can assist in avoiding large economic losses.

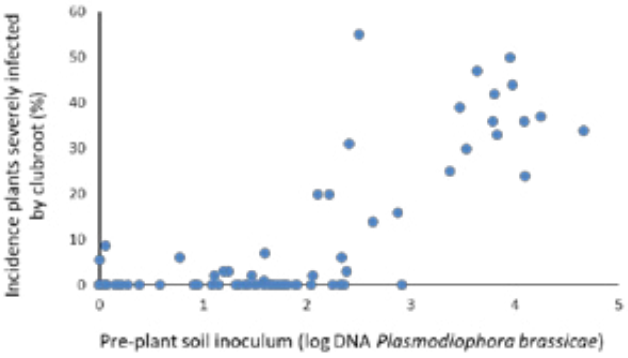


FIGURE 1: The relationship between the level of *Plasmodiophora brassicae* inoculum in the soil prior to planting and incidence of plants with severe clubroot impacting crop growth.

Root system badly affected by clubroot

Crop failure of brassica plant caused by clubroot

Crop failure of brassica crop caused by clubroot

Root lesion nematodes

(*Pratylenchus* spp.)

Root lesion nematodes are a significant horticultural pest. These microscopic plant-parasitic pests live in soil and roots, entering plant roots to feed and lay eggs. Affected plants grow poorly, crop growth is uneven and yields can be reduced. Infected plants may have chlorotic leaves, may appear wilted and roots can have dark brown/reddish lesions.

There is a strong relationship between pre-plant population of root lesion nematodes and level of infestation of root systems of susceptible crops.

PREDICTA® quantifies the population of the root lesion nematodes *Pratylenchus crenatus, P. neglectus, P. penetrans, P. thornei,* and *P. zeae*.

Quantification to a species level is important, as not all species are pathogenic to all crops. A high population of one species of root lesion nematodes may not impact a particular crop while a lower level of another species may reduce productivity of that same crop type.

Thresholds (pre-plant nematode population that causes economic loss) have been developed for most crops. These are not always defined for individual species of root lesion nematode and are best refined for the species present, specific cropping situation and varieties grown.

Thresholds for the pre-plant population of root lesion nematodes that pose an economic loss vary with crop, variety and production environment.

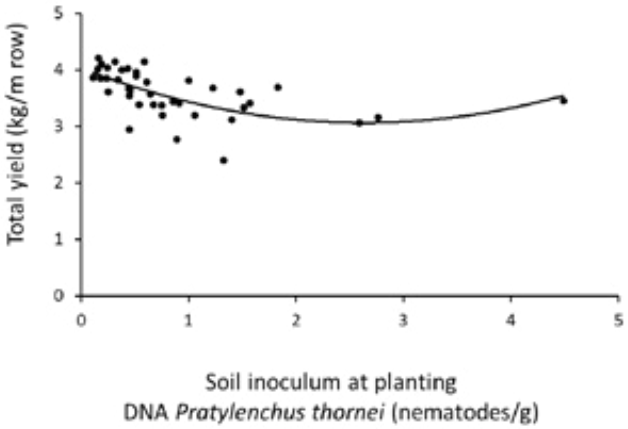


FIGURE 2: Reduced yield associated with population of *Pratylenchus thornei* at planting

Crop susceptibility, tolerance and economic damage thresholds vary with species of root lesion nematode, so management strategies should be considered for each species separately.

With the exception of *P. zeae*, root lesion nematodes are reported as nematodes per gram of soil equivalent.

There are other species of root lesion nematodes, such as *Pratylenchus brachyurus* and *P. coffeae* that are economically important nematodes of vegetable crops in regions of Australia. These nematodes will not be detected by current PREDICTA® testing.

Tolerant crops

Some crops and varieties have tolerance to specific species of root lesion nematode. Nematode populations will survive and may increase on the root systems of these crops, but productivity loss is less likely.

Non-host crops as a management tool

Non-host crops will result in a decline in the population of nematodes, as long as weed hosts are not present. These crops reduce risk to future susceptible crops.

PREDICTA® testing can be used to understand a crop's susceptibility, tolerance and impact on population build up.

Root knot nematodes

(*Meloidogyne* spp.)

Stunting and galling on carrots caused by root knot nematode

PREDICTA® quantifies the population of the root knot nematode species *Meloidogyne hapla, M. fallax, M. javanica, M. incognita and M. arenaria*.

All known species of root knot nematode in Australia are detected by the available PREDICTA® tests.

There is a strong relationship between pre-plant population of root knot nematodes and level of infestation of root systems of susceptible crops.

PREDICTA® *M. javanica/incognita/arenaria* is a combined test that detects all three species. Individual tests for these nematodes are available using the PREDICTA® potato research service, but the individual species tests are not as sensitive as the combined test and are not suitable for pre-plant risk assessment. For most crops susceptible to root knot nematode, all three species are pathogenic. Quantification to a species level is important for capsicums, as *M. incognita* is considered to be the only important species for this crop.

Nematologists have established thresholds (pre-plant nematode population that causes economic loss) for most crops. These are guides only and are best refined for the specific cropping situation, soil type, time of year and varieties grown.

PREDICTA® reports root knot nematodes in units of DNA (pg/g soil) rather than nematodes per gram of soil, so a conversion is required if comparing with established thresholds.

For highly susceptible vegetable crops including cucumbers, capsicums, sweet potato, peas and carrots, any detection of root knot nematodes is of concern. As the threshold for economic damage in these crops is low, sampling intensity must to be sufficient to be confident of detecting nematodes if they are present at a level that is economically important. Inadequate sampling technique or number of tests per paddock may result in failure to detect a nematode risk.

Tolerant crops

Some crops and varieties have tolerance to specific species of root knot nematode. Nematode populations will survive and may increase on the root systems of these crops, but productivity loss is less likely.

Non-host crops as a management tool

Non-host crops will result in a decline in the population of nematodes, as long as weed hosts are not present. These crops reduce risk to future susceptible crops.

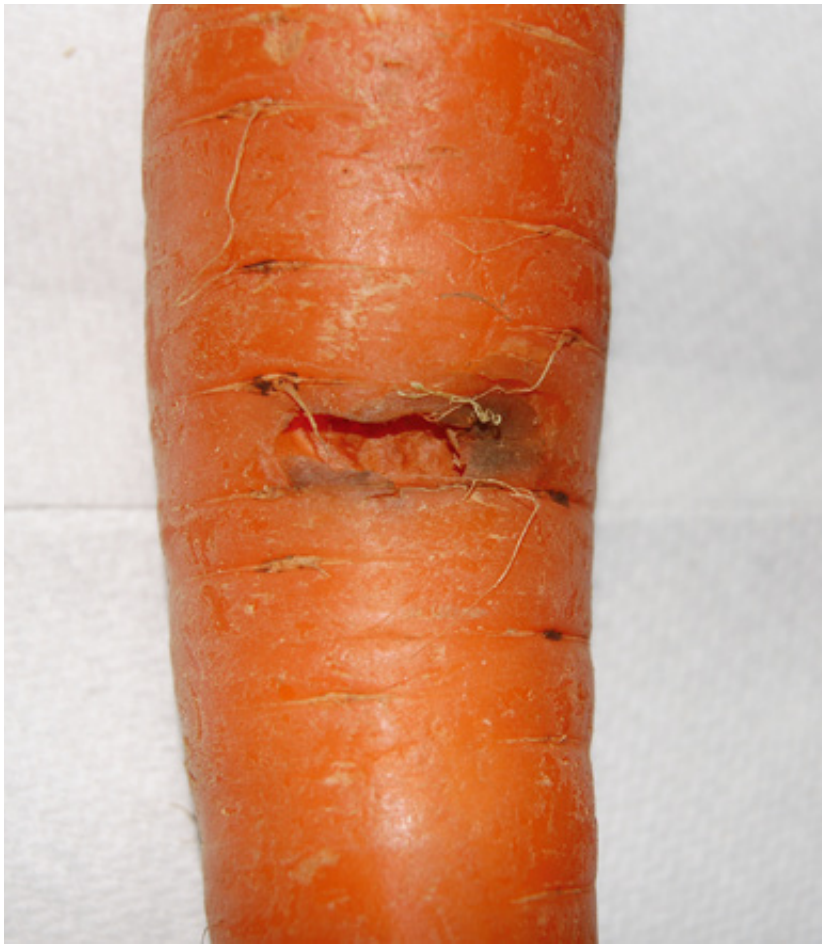
PREDICTA® testing can be used to understand a crop's susceptibility, tolerance and impact on population build up.

Cavity spot

(caused by *Pythium sulcatum* and *Pythium violae*)

Pythium sulcatum is the most common cause of cavity spot of carrots in Australia. Cavity spot caused by *P. sulcatum* is most severe in summer and autumn harvested crops. PREDICTA® testing of *P. sulcatum* DNA levels in the soil prior to planting provides a useful indication of the presence of disease risk. Multiple tests (at least three individual tests) per paddock are required to adequately test for the presence of this pathogen.

At the standard intensity of sampling recommended for PREDICTA®, pre-plant soil testing has not always detected *Pythium violae* where carrots have developed symptoms in some patches throughout the paddock. Testing of soil after harvest from areas where cavity spot has occurred has detected the pathogen, but at low levels. These results indicate the threshold for disease risk in susceptible varieties may be close to or below the level of detection. Cavity spot caused by *P. violae* is most severe in winter and spring harvested crops.



Cavity spot symptoms on carrots caused by *Pythium sulcatum*

Rhizoctonia

(caused by *Rhizoctonia solani*)

Rhizoctonia solani has many anastomosis groups (AGs). Disease of a specific vegetable crop may be caused by one or more of these AGs. PREDICTA® tests for *R. solani* are AG-specific (includes AG2.1, AG2.2, AG3, AG4, AG8).

Distribution of *R. solani* in a paddock is known to be patchy and difficult to define. Testing of multiple samples is required to detect if *R. solani* is present. Specific sampling protocols to assess the risk of onion stunt caused by *R. solani* AG8 occurring have been established.

Knowing the AGs present in a paddock is important, as crop susceptibility varies with AG. This is important for planning rotations, as many crops and cover crops will host particular AGs, though symptoms may not be obvious. PREDICTA® testing provides a means to assess which crops are resulting in a decline or an increase in inoculum levels.

Not all AGs are detected by PREDICTA®. *R. solani* AG1 is an important pathogen of lettuce and carrots, but is not currently detected by PREDICTA® tests.

Carrot rot caused by *Rhizoctonia solani* AG2.2

Carrot rot caused by *Rhizoctonia solani* AG2.2

Sclerotinia rot

(caused by *Sclerotinia sclerotiorum and S. minor*)

The level of both *Sclerotinia minor* and *S. sclerotiorum* inoculum in soil is known to be related to disease incidence, though the latter also has an airborne spore phase which can result in widespread distribution from localised inoculum points. Testing in broadacre crops has demonstrated that the combined test for *S. sclerotiorum/minor* can provide a useful indication of the presence of disease risk. Results of PREDICTA® testing has demonstrated the ability to detect these pathogens in soil samples from vegetable paddocks where relatively low levels of disease have occurred. Knowledge of which species is present is important for test interpretation and for management of sclerotinia. These pathogens have a wide host range and are important pathogens of crops including beans, brassicas, carrots, celery and lettuce. Insufficient evaluation in vegetable crops has been conducted to validate the test's use for pre-plant assessment of disease risk.

Sclerotinia rot of lettuce

Pythium clade F and I

Tests for Pythium clade F and I are not species specific, with each test detecting a range of pathogenic *Pythium* species:

- Species detected by clade F include *P. irregular, P. sylvaticum, P. debaryanum, P. spinosum, P. paroecandrum, P. mamillatum*
- Species detected by clade I include *P. ultimum, P. splendens, P. heterothallicum*.

Interpreting these tests is more difficult than interpreting tests which only detect one pathogen species. When used in a paddock over time, PREDICTA® tests can provide an indication of changes in soil biology. High levels can indicate unfavourable soil conditions for seedling establishment and plant growth, an imbalance or that other disease pressure is present.

Blackleg

(caused by *Leptosphaeria maculans*)

Soil testing using PREDICTA® to assess the inoculum level of *Leptosphaeria maculans* has not provided a reliable indication of the risk of blackleg occurring in brassica crops. Disease is spread by airborne spores and can travel long distances to infect crops. The test will reliably detect the pathogen in infected plant tissue including infected crop residues that carry over inoculum.

Charcoal rot

(caused by *Macrophomina phaseolina*)

Macrophomina phaseolina has a wide host range and is an important pathogen of vegetable crops including beans, cucurbits, sweet corn, sweet potato and strawberries.

Limited testing in strawberry crops has demonstrated that the PREDICTA® test can provide a useful indication of inoculum presence in the soil prior to planting. Insufficient evaluation has been conducted in other vegetable crops to validate the test's use for pre-plant assessment of disease risk.

Black root rot

(caused by *Thielaviopsis basicola*)

Thielaviopsis basicola can be an important pathogen of crops including beans, lettuce, carrots and cucurbits. Only a low number of tests have been conducted in vegetable growing areas, with low levels of the pathogen detected in soils prior to planting. Insufficient evaluation in vegetable crops has been conducted to validate the tests use for pre-plant assessment of disease risk.

Black dot

(caused by *Colletotrichum coccodes*)

PREDICTA® testing for pre-plant assessment of disease risk has been established for black dot of potatoes. In other vegetable crops, mainly solanaceous crops, *Colletotrichum coccodes* is frequently present, but not known to be causing significant losses. In tomatoes, the pathogen can infect the plant and then cause anthracnose on fruit. Insufficient evaluation in vegetable crops has been conducted to validate the test's use for pre-plant assessment of disease risk.

Verticillium wilt

(caused by *Verticillium dahliae*)

PREDICTA® testing for pre-plant assessment of disease risk has been established for verticillium wilt of potatoes.

Verticillium dahliae has a wide host range and can cause productivity loss in crops including brassicas, lettuce and strawberry. Insufficient evaluation in vegetable crops has been conducted to validate the test's use for pre-plant assessment of disease risk.

Aphanomyces root rot

(caused by *Aphanomyces euteiches*)

Aphanomyces root rot is significant pathogen of peas and beans. No evaluation in vegetable crops has been conducted to validate the test's use for pre-plant assessment of disease risk.

How PREDICTA® can support your monitoring and management decisions

- Crop monitoring and management
- Identification of the issue
- Monitoring cropping system
- Assessing effectiveness of management strategy

Crop monitoring and management

PREDICTA® testing of soil or root systems can quantify pathogen levels at any point of crop growth. Testing can provide insight into the cause of crop symptoms being observed, and provide an early warning for some soil borne diseases, such as root knot nematodes (FIGURE 3).

Monitoring of root knot nematode levels in greenhouse production of capsicums (expected growth period nine months).

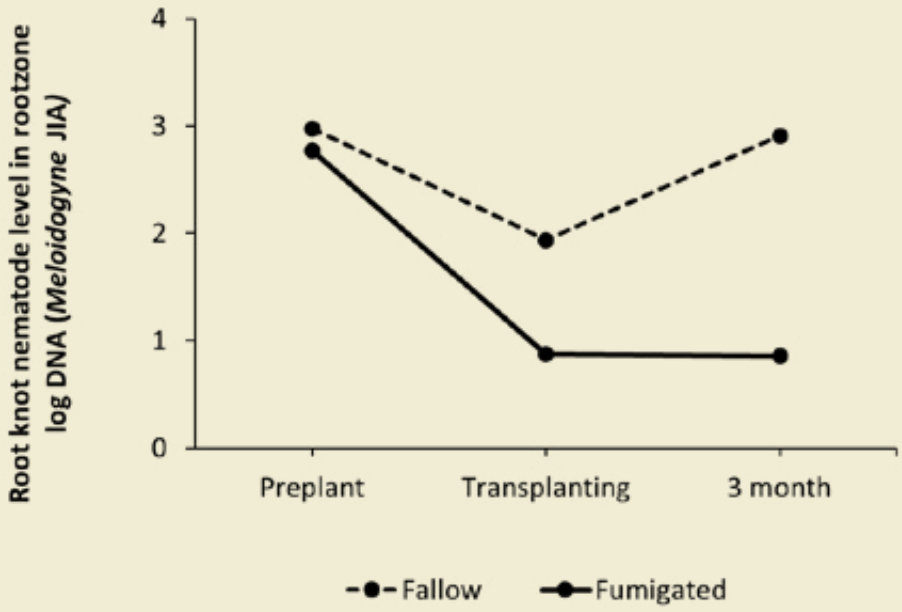


FIGURE 3: Testing indicates fallow period reduced levels of root knot nematode prior to planting, but not enough as the population has increased three months into crop. Symptoms of disease would be visible on plants and roots and crop productivity will be reduced. Testing indicates the population was reduced by fumigation and has remained low three months into crop, justifying the use of fumigation.

The greenhouse was fumigated prior to planting except for a small untreated area which relied on a period of bare fallow to reduce nematode population.

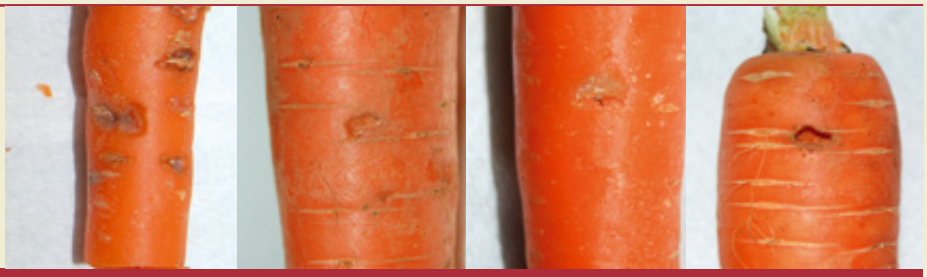
Identification of the issue

Correct diagnosis of the pathogen causing disease symptoms is central to implementing an effective management strategy. Incorrect diagnosis results in costly treatments being applied for no benefit with loss in productivity continuing.

Results of a PREDICTA® test are not a diagnosis, but when used by experienced plant pathologists they can assist diagnosis by:

- Identifying the pathogen down to a species or group when the cause of disease is known
- Confirming if the pathogen is present in disease symptoms when the pathogen is difficult to isolate
- Quantifying pathogen levels in plant tissue or soil from around diseased plants

Cavity spot of carrots can be caused by *Pythium sulcatum* or *P. violae*. Symptoms of both species can be similar. Cavities in carrots similar to those caused by *P. sulcatum* or *P. violae* can be caused by other pathogens, pest damage and physical soil constraints. In these cases, management aimed at controlling *Pythium* will not improve the outcome.

What is the cause of this cavity symptom?				
PREDICTA® testing can quantify pathogen levels in symptoms, assisting correct diagnosis				
Pythium sulcatum (kDNA / g sample)	4022	Below detection	Below detection	Below detection
Pythium violae (kDNA / g sample)	Below detection	62	Below detection	Below detection

Correct identification of the cause of cavity symptoms helps to target control options to minimise yield and quality loss. It also informs the development of future management strategies.

Important note

Presence of a pathogen does not mean it is causing disease, but the quantification of pathogen levels may indicate which ones are involved. This is particularly useful where a disease complex is suspected.

Monitoring cropping system

Rotations and cover crops are an integral part of many cropping systems. Regular PREDICTA® testing determines how effective these strategies are at managing the level of inoculum for pathogens.

Rhizoctonia solani AG2.1 has a wide host range, including vegetable and broadacre crops. It can be pathogenic on vegetables including brassicas, carrots and potatoes. Some crops will host *R. solani* AG2.1 without obvious symptoms. Monitoring provides an indication of the build-up or decline of inoculum than is occurring within a crop production system. Crop type is only one factor in inoculum change. Other factors include time of the year, nutrition, growing conditions and interactions with other soil microbes.

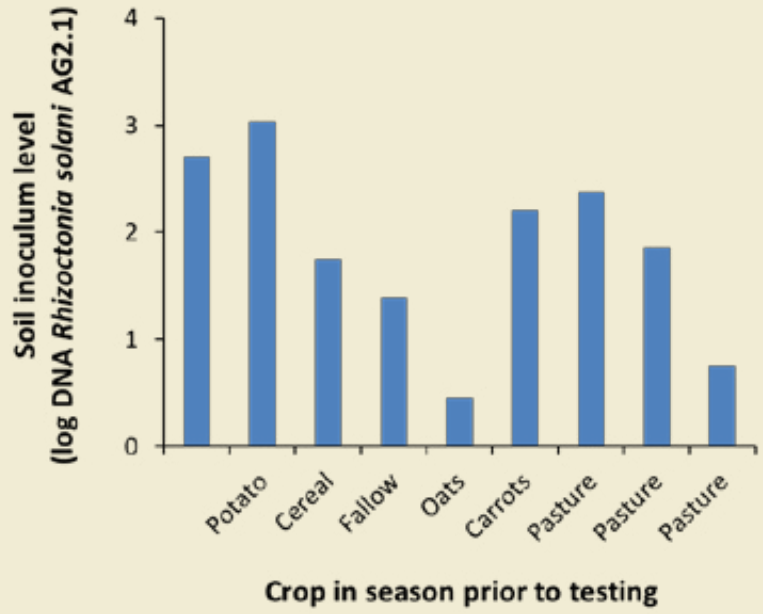


FIGURE 4: Example of annual monitoring of *Rhizoctonia solani* AG2.1 levels in a paddock.

Assessing effectiveness of management strategy

The effectiveness of treatments is best determined by quantifying the impact on yield and quality that contribute to profitability.

Sometimes disease does not occur, making it difficult to know if the strategy has had an impact. Disease symptoms may be due to a number of reasons. The treatment can have multiple effects and the reason for benefit or failure may not be clear. Using PREDICTA® to quantify pathogen levels can clarify what is happening and confirm the reason for the benefits observed.

Impact of pre-plant soil treatment on the level of *Pythium sulcatum* (cause of cavity spot) in an on-farm carrot trial was investigated.

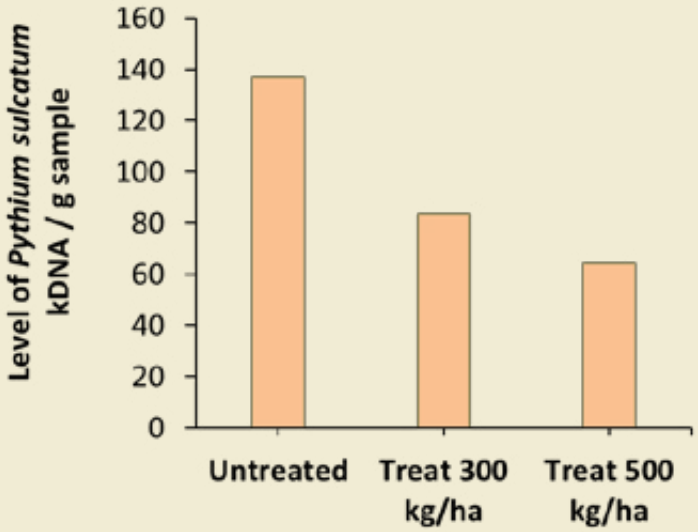


FIGURE 5: Results of peel testing at harvest indicate that *P. sulcatum* is the likely cause of minor cavity spot symptoms observed in an untreated area. Treatment reduced pathogen levels in peel of carrots indicating treatment has positive impact on cavity spot management.

Further information

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

Key contact:

MICHAEL RETTKE
0401 122 124
michael.rettke@sa.gov.au