

**Pilot Project - Diagnostic
tests for soilborne
pathogens, International
Collaboration**

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Development Institute (SARDI)

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Final Report

Pilot Project - Diagnostic tests for soil-borne pathogens, International Collaboration

PT08048 (October 31, 2009)

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South Australian Research and Development Institute



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HAL Project PT08048

Pilot Project - Diagnostic tests for soil-borne pathogens, International Collaboration

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This is a final report detailing a pilot project for an international collaborative program on DNA diagnostics for soil-borne pathogens of potato.

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TABLE OF CONTENTS

Media Summary.....	2
Technical Summary	3
Introduction.....	4
Methods.....	5
Results and Discussion	6
Technology Transfer.....	7
Recommendations.....	8

Media Summary

An international collaboration effort has been established between Australia, United Kingdom, New Zealand and South Africa to validate the use of DNA diagnostic tests for key soil-borne pathogens of potatoes. A pilot project has established collaboration with a new research program funded by the Potato Council, United Kingdom. The UK research is carried out by Scottish Crops Research Institute, Scottish Agricultural College and Central Science Laboratory in York.

The Australian research builds on work funded within the Australian Potato Research Program (APRP). The APRP developed DNA tests for *Spongospora subterranea* (which causes powdery scab), *Streptomyces scabies* (common scab), *Meloidogyne fallax* and Rhizoctonia diseases of potato. The tests can quantify the pathogens in soil and seed.

The collaborative research program will determine if tests to quantify pathogens in soil can be useful as risk management tools for growers in both countries. Related research is being undertaken in New Zealand and South Africa and international efforts will be coordinated by this program, which will continue as part of phase 2 of the Australian Potato Research Program. The pilot project has established a useful framework for collaboration using web based collaboration tools, videoconferencing and shared protocols and approaches.

Technical Summary

This project was a pilot project for an international collaboration on molecular diagnostic tools for potato soil-borne pathogens, undertaken by Australia and research organizations in the UK. The UK research program is coordinated and sub-contracted by the Potato Council-UK (Dr. Mike Storey) and involves researchers from Scottish Crops Research Institute (SCRI), Scottish Agricultural College (SAC), and Central Science Laboratory (CSL) in York.

The overall aim of this program is to build on previous research on diagnostic tools and to determine if tests to quantify pathogens in soil are useful risk management tools for growers. The focus of the Australian research will be on *Rhizoctonia solani* AG3 and AG2-1, *Spongospora subterranea* and *Streptomyces scabies*, with close linkage to a proposed project on *Meloidogyne fallax* (*M. hapla*). The focus of the UK component is *Spongospora* and *Rhizoctonia*. Test results, approaches to field validation and information on test interpretation will be closely coordinated with the international group.

The aim of the pilot project was to:

- Develop mechanisms for international collaboration
- Establish methodology for standardisation of DNA tests between laboratories.
- Collate existing data on soil sampling
- Develop protocols for field trials to determine the relationship between *Rhizoctonia* inoculum and disease expression.

Mechanisms for international collaboration have been established. A web-based collaboration tool (SharePoint) site has been established and is administered by SCRI. This site contains scientific background documents, protocols for field and laboratory work and presentations. Ultimately, the site will be a mechanism for sharing data generated within the program. A videoconference linking collaborators from the UK (SCRI, SAC and CSL), Australia (SARDI, DPIVic and TIAR), New Zealand (NZPFR) and South Africa took place in July 2009 with participants in 5 time zones, including 2 in Australia. This was highly successful and will be repeated twice per year.

Protocols for comparison of DNA tests have been circulated to all participating laboratories (SCRI, SAC and CSL in the UK, SARDI and NZPFR). A full inter-laboratory comparison will be undertaken in April 2010 and will cover the DNA tests for *Spongospora subterranea* (powdery scab), *Rhizoctonia solani* AG2-1 and AG3 as well as *Colletotrichum coccodes* (black dot).

Pathogen distribution data generated in PT04016 has been loaded on SharePoint site for International Potato Diagnostics Project. Statistical analysis has been undertaken by Jeff Peters, Central Science Laboratory, and sampling recommendations for current trials have been agreed. *Rhizoctonia* trials have been established in controlled environments and field in the UK by Scottish Crops Research Institute and have been used as the basis for Australian research.

Introduction

DNA tests have been developed to quantify key soil-borne pathogens of potato in soil. This research has been undertaken both within the Australian Potato Research Program, phase 1 (APRP1) program and internationally, most notably at Scottish Crops Research Institute (SCRI). Tests include *Spongospora subterranea* (powdery scab), *Streptomyces scabies* (common scab), *Meloidogyne fallax* and *Rhizoctonia solani* (AGs 2-1, 2-2, 3, 4, and 5).

The APRP1 program (2004-2009) focused on development of robust quantitative tests which can determine pathogen levels in soil and seed, mapping pathogen distribution to determine sampling strategies, and defining the relationship between inoculum and disease development in bioassays and in the field. The latter (field validation) was done in the recently completed APRP program, PT04016, on a limited range of fields.

To validate these tests for grower use, and to draw on the research being done by international groups, the next phase of the APRP program will aim to:

- Standardise DNA test results
- Determine the role of seed and soil-borne inoculum
- Determine key drivers of disease development for *Rhizoctonia*
- Improve sampling strategies, particularly for *Rhizoctonia* in soil
- Develop a manual to support interpretation of test results

A pilot project was established in order to align the APRP2 program with the UK program, which commenced in April 2009, in order to establish a mechanism for a voluntary contribution via the UK program, establish a collaboration framework including a mechanism to share protocols, collate existing data and establish UK field trials on *Rhizoctonia* disease development. This pilot project is a precursor to a larger project, commencing November 2009 as part of APRP phase 2.

Methods

Collaboration framework

The voluntary contribution mechanism was established with Potato Council UK. Funding for the Potato Council project 'Informing management of potato diseases through epidemiology and diagnostics: international collaboration' will be used as a voluntary contribution, matched by the Australian government via HAL, and the matched funds used to resource the Australian portion of the project.

A web-based collaboration tool (SharePoint) was established, administered by SCRI. This site contains sections for scientific references, project reports and presentations as well as protocols for field trials and laboratory techniques. The site will be the major mechanism for sharing data generated within the program.

Formal project meetings will be held by videoconference twice per year.

Standardisation of research approaches

DNA testing: It is important to standardise DNA test results between Australia, New Zealand and UK so that we can communicate clearly about DNA levels within the program. This will occur by undertaking comparative testing on spiked and naturally infected soils. Central Science Laboratory and SARDI staff have determined a 2 stage process for comparative testing which will be undertaken in APRP2.

Sampling: Previous research has focused on pathogen distribution and sampling strategies have been developed. There is concern that Rhizoctonia AG-3 DNA is poorly detected in soil. This may reflect that AG3 is at a very low level in many soils or that the sampling strategy or timing of sampling is not appropriate for AG3 detection. This project will collate existing spatial data and recommend further sampling validation to be undertaken in the UK and Australian sections of the full project.

Field trials: There are major gaps in our understanding of Rhizoctonia disease development which make it difficult to link soil inoculum to disease development. This project will undertake research in the UK and Australia to determine the key factors which drive the relationship between Rhizoctonia soil inoculum and disease development in the field. A common core trial methodology will be developed in the pilot project, and the first year trials will be undertaken in the UK to determine the key environmental drivers of disease to define soil inoculum thresholds for Rhizoctonia.

Results and Discussion

A videoconference linking collaborators from the UK (SCRI, SAC and CSL), Australia (SARDI, DPIVic and TIAR) and New Zealand (NZPFR) took place on July 1. The video-conference crossed 3 continents and 4 time zones. The main objective of the conference was to summarise available information on *Rhizoctonia* epidemiology and to agree on assessment and detailed methodologies for future field trials. Despite the time zone differences, the video meeting was highly successful and spanned about 3 hours. It was a cost-effective mechanism for discussing the project and involved full project teams, which was appreciated by all involved.

Protocols for comparison of DNA tests have been circulated to all participating laboratories (SCRI, SAC and CSL in the UK, SARDI in Australia and NZPFR in New Zealand). There are two comparative tests planned. A full inter-laboratory comparison will be undertaken in April 2010 and an initial laboratory reproducibility test or 'preliminary ring test' will be carried out in 2009 as part of PT09023.

The aim of the inter-laboratory comparison is to benchmark results from each participating laboratory. This will be completed for one pathogen, prior to the full inter-laboratory comparison being carried out and is designed to aid the interpretation of results obtained during the 2010 ring tests. The 2010 ring tests will cover *Spongospora*, *Rhizoctonia* AG2-1, AG3 as well as *Colletotrichum coccodes*.

Pathogen distribution data generated in APRP1 has been loaded on SharePoint site for International Potato Diagnostics Project. Statistical analysis is underway by Jeff Peters, Central Science Laboratory, and sampling recommendations for current trials have been agreed.

Rhizoctonia trials have been established in controlled environments and field in the UK by Scottish Crops Research Institute. Protocols and disease rating systems have been included on the SharePoint site. Where possible, methodologies will be standardised. However, the UK project will have a slightly different emphasis than the Australian program with a greater focus on fresh potatoes. This will mean that some specific methodologies will need to be adapted to each region.

Technology Transfer

There has been no technology transfer from the pilot project. The proposal for the main APRP2 project on diagnostics, PT0923, and VC contributions from Potato Council and Horticulture New Zealand have been finalised.

Recommendations

- The voluntary contribution mechanism with international funding agencies is a useful mechanism for funding international research projects. The voluntary contribution via a funding agency such as Potato Council UK is a clearer mechanism for achieving collaboration and alignment of R & D effort.
- Web and video-based collaboration tools are cost-effective means for collaboration which have an additional advantage of involving the broader research team, not only the team leaders. They are not a complete substitute for face-to-face meetings but are a useful adjunct.
- Establishment of agreed methodologies, tailored to growing season and research emphasis in the different jurisdictions, is important in establishing a common framework in which to discuss research results.