Paddock science in the molecular age

Most growers have by now heard of PreDicta® B, the soil-testing service being increasingly used to find out what disease risks may be present in soils as crop rotations change. Few, however, would know the story behind this test; a classic tale of scientific insight, serendipity and doggedness – and how it may shape future crop management

By Brad Collis

Inside a bar-fridge-sized Perspex box, small plastic trays comprising tiny wells filled with clear liquid are robotically collected and dispensed onto another plate with smaller wells. The process is repeated many times to set up individual DNA tests to quantify specific organisms. The trays are then sealed and placed into the analysing compartment of one of four real-time polymerase chain reaction (PCR) instruments.

There is barely a sound as four units work autonomously inside a biosecure room at the South Australian Research and Development Institute (SARDI) in Adelaide. Not much to look at from the outside, they nonetheless represent the cutting edge of agriculture's struggle to keep crops one step ahead of plant diseases.

This streamlined process is the culmination of nearly three decades of research and development of DNA techniques for identifying and quantifying the presence of disease-causing pathogens in crop soil. They are at the final stage of a sampling and analysing process called PreDicta® B, an Australian technology now recognised internationally for its capacity to accurately identify and measure soil pathogens.

PreDicta® B has become the key tool for helping agronomists and growers monitor pathogens in soils – a constantly shifting threat as crop cycles and weather patterns change.

Current estimates put the value of grain yield lost to soil-borne diseases caused by microorganisms such as root lesion nematode (RLN) and Fusarium crown rot at more than $300 million a year. PreDicta® B has become the frontline weapon for reducing this toll. But, like so many technologies, the research and its eventual application in the field is such a long journey that the inspiration, historic influences and people behind the success are too easily forgotten.

INTERSECTION OF IDEAS AND TECHNOLOGY

The story of PreDicta® B is in many ways the story of the research career of SARDI's Dr Alan McKay, although there have been many collaborators along the way.

It was also the confluence of emerging DNA techniques that began in the early 1990s and the lateral thinking of several young, early-career researchers.

Dr McKay, who remains a driving force behind PreDicta® B's continuing development, began his career in nematology, working on annual ryegrass toxicity for 20 years, but was then drawn to the challenge of soil pathogens generally.

"I spent a lot of time counting nematodes and I could see the techniques were labour-intensive. This restricted progress on our ability to evaluate different agronomic practices," he recalls.

His initial interest in soil-borne diseases was influenced by his early work experience, after university, with the company ICI, which was attempting to promote minimum-tillage systems.

"On graduating from the University of Melbourne I moved to SA to work with ICI and undertook some of the early Spray. Seed®/minimum-till trials using a triple disc seeder. Well, this was my first encounter with soil-borne diseases, and the losses caused by Rhizoctonia, in particular, were impressive. While seeking more information on Rhizoctonia I got to know Dr Alan Dubé, the leader of a small group of plant pathologists in the SA Department of Agriculture, located at the Waite Research Institute. Alan was looking for someone to work on annual ryegrass toxicity. I ended up working on this for 20 years, before switching my focus to the development of DNA tests to study and manage soil-borne diseases.

"In the early 1990s, two bioassays were being used to assess the disease risk; one for cereal cyst nematode (CCN) and one for take-all. The tests took up to 10 weeks because plants had to be grown in the soil samples, then their roots examined to score the disease.

"It was around this time that there was growing interest in using DNA techniques to quantify inoculum in the soil," Dr McKay says. "That, basically, was the genesis of the idea behind PreDicta® B." SARDI was working with the SA wheat committee to develop a DNA test for Rhizoctonia. Across the road, at the Cooperative Research Centre for Soil and Land Management, fellow researcher Dr Kathy Ophel-Keller was working with Dr Albert Rovira to develop a DNA soil test for take-all.

By 1997, Dr Ophel-Keller and Dr McKay had joined forces at SARDI, a division of Primary Industries and Regions SA, and together pitched a concept to the SARDI board to launch the Root Disease Testing Service.

INVESTMENT FORESIGHT

Dr McKay recalls that the proposal was for more than $1 million to build a dedicated laboratory: "It was a lot of money and a big risk for the organisation, but fortuitously DNA was seen as a key emerging technology.

"Soil-borne diseases were also considered one of the major constraints on grain yields and everyone could see the existing testing techniques were inadequate, because you needed different protocols for different pathogens and all were slow. DNA, by contrast, offered the potential for one platform on which to test for the presence of all soil-borne diseases. So the board agreed to the funding request."

PHOTO: BRAD COLLIS

Above: Dr Kathy Ophel Keller at SARDI in 2003.

Below: A young Albert Rovira during his pioneering research into soil biology problems that later threatened to thwart the introduction of minimum tillage.

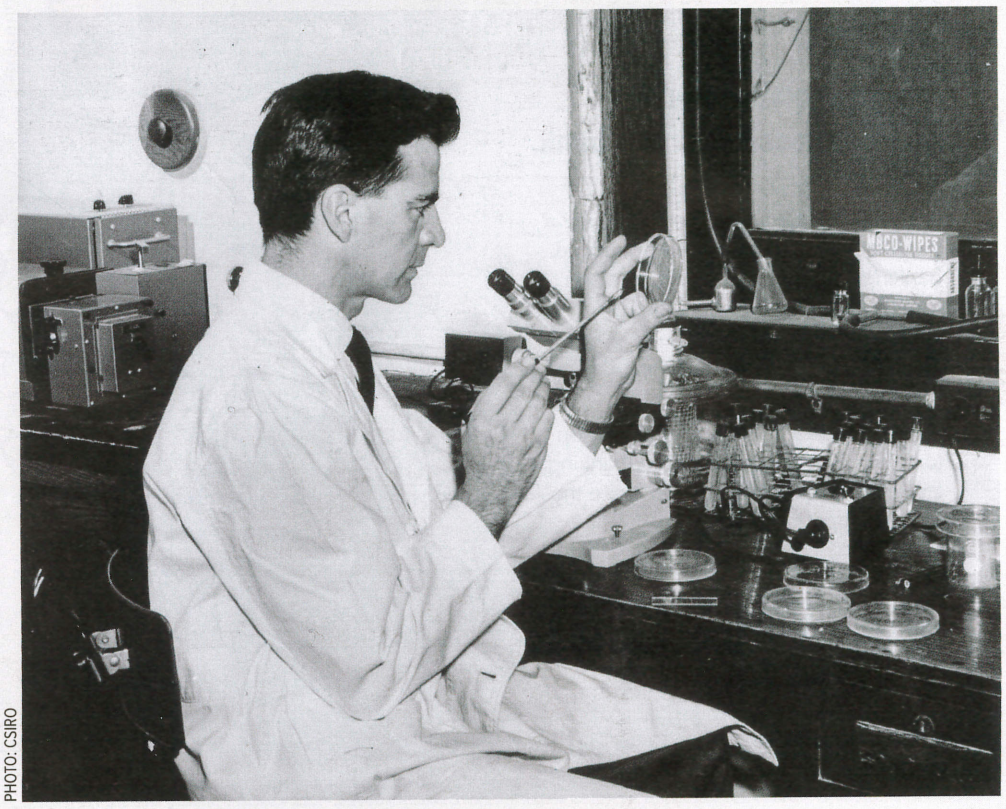

PHOTO: CSIRO

It was a significant milestone, but still proved to be a long way shy of what was needed and what would become PreDicta® B.

"The DNA extraction system had to be reliable to monitor changes in population density and distribution of soil-borne pathogens caused by different cropping practices and environments across Australian grain-producing regions. It also had to match the sensitivity of the bioassay methods, so the benchmark we set was to reliably detect one CCN cyst in 500 grams of soil."

During this period, support from the South Australian Grain Industry Trust (SAGIT) was critical. This underpinned an enormous effort to overcome the sizeable technical challenges associated with the diversity and chemically hostile nature of Australian soils.

It took several years to perfect, and the method is still confidential intellectual property. "The great advantage of this system is that it extracts DNA from all organisms in the soil sample," Dr McKay says.

PHOTO: BRAD COLLIS

Alan McKay viewing a DNA-testing PCR instrument at work.

HOSTILE RECEPTION

"If you think about the diversity of soil types across Australia, there are some pretty hostile soils, particularly at depth, so our system has had to be both simple and robust. There are numerous chemical compounds in soils that can inhibit DNA extraction and all of these had to be overcome. The first DNA extraction system was too slow and each DNA test (for different organisms) had its own customised protocol and that wasn't a sustainable proposition."

In 1999 the SARDI group formed an alliance with Dr John Curran of CSIRO Entomology. Dr Curran's group was more advanced in developing DNA tests, so the SARDI team focused on DNA extraction. The research alliance paid off and over the next 12 months the DNA assays were all redeveloped from scratch and, importantly, the rate of development of new tests began to increase.

CSIRO also had a prior arrangement with French company Rhone-Poulenc to commercialise the technology when it was robust enough. The commercialisation rights were exercised and in 2000 C-Qentec Diagnostics was formed to launch PreDicta® B. The 'B' is short for broadacre.

"In the first year we only had tests for two organisms and processed 1100 samples. Today, with support from most of the RDCs, covering grains, horticulture, vegetables, viticulture, pastures, aquaculture and recently sugar, we have over 150 tests, most of which were developed by Diana Hartley (CSIRO), and regularly process over 30,000 samples a year."

Twenty years on, SARDI is again investing in the technology, this time to double the capacity. The rate of test development is also increasing and new projects are expected to result in test development time being reduced to about three months. Working out the relationship between the DNA result and actual level of disease risk is the main bottleneck.

Dr McKay says that while an increasing number of consultants and growers are using PreDicta® B, their main clients are other researchers, particularly those running trials that are testing different disease treatment effects.

The soil sampling strategy is also critical and is the greatest source of error. Over time, the in-paddock soil sampling strategy has also changed, particularly as crown rot has become a more prominent disease.

"Initially we sought to piggyback on soil nutrition sampling," Dr McKay says. "But these samples are taken from between rows and stubble is avoided; this reduces the potential to pick up crown rot and other stubble-borne pathogens.

PADDOCK PRACTICE

"We now strongly promote that PreDicta® B soil samples need to be collected from the rows of the last crop and that small pieces of stubble from the base of the old plants must be added to the sample. This, of course, is diametrically opposite to what you would do for a soil nutrition sample, so it means an agronomist needs to collect two separate soil samples if wanting both PreDicta® B and soil nutrition data."

Sampling is the greatest source of error, and research undertaken by Dr John Heap and others has shown that it is important to sample within a production zone (an area with the same growing characteristics, such as soil type, organic content, slope and subsoil constraints).

This can be tricky, but important: "Neighbouring production zones can have quite different disease risk profiles, so it is best to keep soil cores from each zone separate," Dr McKay says.

"Our recommendation is to identify the zone in the paddock that is the most important and where you don't want any surprises. Alternatively, if you are confident about the good zones, but want to find out if there is a disease presence responsible for the poorer zones, then you might target these areas for testing."

Dr McKay describes PreDicta® B as a glorified counting system. "If the paddock is sampled properly, it can tell growers the levels of each organism tested. For some tests that have been used by disease management projects, we have a pretty good idea of what the numbers mean for disease risk and appropriate management options. For the newer tests, the results can be used to benchmark the levels in individual paddocks against rest of industry."

FUTURE DIRECTIONS

As we approach 20 years since SARDI committed to establishing PreDicta® B, it is tempting to speculate on what the next 20 years will bring.

"When the current upgrade is completed by the end of the year, we will have the capacity to monitor changes in soil-borne diseases across a wide range of industries. Research is also progressing to broaden the application of the technology to monitor spore and insect traps for area-wide surveillance to support market access, as well as warn growers of impending risks.

"In the medium term we will need a new DNA technology to cope with the growing number of tests that will need to be run per sample. It is easy to get seduced by the emerging technologies, but finding the right one for this work will be a challenge.

"In future, the greatest benefits will probably come from integrating PreDicta® B data with emerging 'big data' technologies. The impacts from this could be large on both growers and researchers. I expect potential problems and solutions that are currently difficult to observe at an individual farm level, will be identified. These can then be studied in more detail to provide growers with targeted solutions, hopefully before significant losses occur."

The GRDC has been a major investor in PreDicta® B and currently funds project DAS00137 to further develop PreDicta® B for the grains industry. The GRDC is also negotiating with SARDI to fund several bilateral projects to speed up test development and broaden application of the technology to grains researchers and growers nationally. □

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Many of the original staff still work in the group, including Dr Herdina, Ina Dumitrescu, Russell Burns, Danuta Pounsett and Irena Dadej. Diana Hartley still provides advice on test design. Daniele Giblot-Ducray now leads the DNA laboratories. Several new staff are being appointed to enhance the test development capability. Dr Kathy Ophel-Keller is research chief of SARDI Sustainable Systems and maintains a strong interest in the group.