

Plant BIOSECURITY

COLLABORATIVE RESEARCH INITIATIVES



CRC **PLANT** biosecurity

FOREWORD

Productive collaboration is fundamental to the success of a cooperative research centre. This publication, *Plant Biosecurity: collaborative research initiatives*, has been developed to provide readers with insight into the innovative and productive collaborative research we have undertaken in the past five years.

By reading the articles in the ensuing pages you will discover a myriad of projects, people and organisations which constitute the Cooperative Research Centre for National Plant Biosecurity. We are fortunate to have Australia's experts from traditional plant science disciplines (entomologists, pathologists, virologists and bacteriologists) as well as economists, statisticians, mathematical modellers, social scientists and agricultural engineers working on our projects. Most project teams consist of employees with contrasting skills from a number of different organisations based around the country. Faced with geographical challenges and the complexity of biosecurity research, they are collaborating effectively to achieve successful outcomes.

Many of the following articles also unfold our comprehensive array of PhD projects. We have a large number of postgraduate students whose research projects are addressing issues offshore (the best place to handle a biosecurity challenge) and within Australia where incursions occur or can be foreshadowed. In the latter case 'prevention is much better than cure'. We are committed to increasing Australia's plant biosecurity capability through our PhD program and other various training and capacity building activities, including primary school education.

Our research collaboration includes the whole spectrum of the plant biosecurity community. From government to research institutions, scientists to farmers and industry to the consumer. Each link in this chain plays an important role in maintaining Australia's privileged biosecurity status. The last link, the consumer, is particularly important because enhancing community awareness of biosecurity is one of the best means of early detection of emerging biosecurity threats.

Many of our projects would not generate the required research data without the support of Australia's farming communities. Farmers and growers, the backbone of our extensive plant export industries, recognise the importance of scientific research to sustain their market access. Property owners across the country have provided not only facilities, but also information about their production systems for our experimental work. We thank them for their cooperation, without which we could not achieve our mission of delivering plant biosecurity technologies which will reduce risk to, and ensure the sustainability of, Australia's plant industries.

This publication also provides an opportunity for you to learn more about our collaboration with organisations across the globe which improves their biosecurity effort and helps us to better understand the risks associated with plant pests and diseases not present in Australia, and to devise strategies for keeping them out.

In an environment of increasing biosecurity risk one thing is certain, success of all of our research projects has only been achieved through cooperation and collaboration. We congratulate all of the people involved in the featured projects and the many others who support our various activities. We hope you enjoy reading *Plant Biosecurity: collaborative research initiatives*.



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The **Cooperative Research Centre for National Plant Biosecurity** started operating in November 2005 in recognition of the need to strengthen the plant biosecurity scientific capacity of Australia. Our corporate office is located in Canberra, where we centrally coordinate plant biosecurity research across the country. We have an extensive collaborative network of researchers and educators from 24 participating organisations representing industry, universities, state and Australian government.

A key strength of our CRC is the involvement of our participants who are, in many cases, end-users of research results. This ensures maximum benefit and impact in the delivery of project outputs, development of new products and services and capture of intellectual property. Through international collaboration, we can also facilitate broad acceptance and adoption of our new technologies, enhancing Australia's trading position.

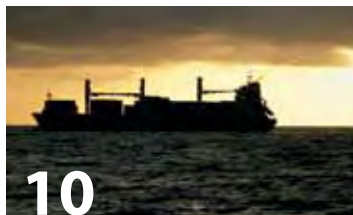
We aim to provide leadership in the development, execution and delivery of plant biosecurity research to:

- safeguard Australia's plant industries
- ensure food security for Australian consumers, and
- improve market access for agricultural exporters.

Our vision ... is to be a world leader in the generation, development and delivery of plant biosecurity science and education.

Our mission ... is to foster scientific collaboration and engage stakeholders to deliver plant biosecurity technologies that will reduce risk to, and ensure sustainability of, Australia's plant industries.

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Bigger menaces, smarter solutions

Biosecurity helps us control what we let into Australia – and what we keep out. Protecting billions of dollars worth of exports, our domestic food security, native landscapes and tens of thousands of jobs requires not only vigilant quarantine, but also razor-sharp science.

By Julian Cribb

The killer exploded out of Africa the year of the millennium. Stealthily, borne by the wind and on people's clothing, it spread from country to country – Uganda, Kenya, Ethiopia, Sudan, leapt the Red Sea into Yemen, then hurdled the Gulf into Iraq and possibly Afghanistan. Today it is poised on the edge of one of the world's greatest 'bread baskets', the Indo-Gangetic region of India and Pakistan. And from there it is barely a monsoon's breath away from Australia.

The 'killer' is Ug99, a stem rust lethal to wheat plants with the potential to wreak havoc on the world's bread supply: 85 per cent of the wheat varieties grown globally are susceptible to it, making it potentially the biggest bread threat in history. While human diseases such as SARS and bird flu dominate the headlines, the public is far less aware that equally large threats exist in the realm of plant biosecurity – that is, the protection of the world's crops and plants from the horde of insect pests, moulds and diseases that can ravage the food supply and, each year, devour almost half of it.

Like human disease pandemics, plant biosecurity is emerging as a critical global issue for the 21st century. Harmful plant pests and diseases occur in almost every country, every year. These impact on food security, trade and, ultimately, the viability of farming industries and communities. Australia alone discovers an average of 30 to 40 incursions of new plant pests or diseases every year.

The 2008 review of Australia's quarantine and biosecurity arrangements (the Beale Review) attributes the rise in threat level mainly to the forces of globalisation: the massive increase in trade, travel and tourism that is creating an expanded highway system for these unwanted hitchhikers. Also driving risk is the settlement of new regions (where unknown pests and diseases may occur), the surging global trade in genetic materials, the opening of fresh opportunities for pests under climate change and the risk of 'agro-bioterrorism' – the deliberate targeting of the food supply by terrorists.

All this comes at a time when the world's scientific resources for combating the upsurge in pests and diseases are weakening. Cutbacks in agricultural science worldwide and the retirement of a generation of expert plant and pest scientists have exposed a grave weakness in the world's scientific and technical defences against pests such as Ug99, the Beale Review has warned.

Today, Australia enjoys relative freedom from many of the plant pests and diseases that ravage agriculture overseas thanks to sharp quarantine backed by a strong

scientific effort. Nevertheless, our \$10 billion of food exports are at constant risk, as is the nation's food security. The absence of many overseas pests and diseases gives us a vital competitive edge in world trade only so long as we keep them out: the price of national food security is eternal vigilance, argues Dr Simon McKirdy, CEO of the CRC for National Plant Biosecurity.

A lapse could prove costly. The Productivity Commission, for example, estimated a foot and mouth disease outbreak in Australia could cost the nation \$8 to \$13 billion a year, along with many jobs. Since plant industries underpin the livestock sector, the costs of a serious plant pest incursion may be equally massive.

To deal with the twin challenges of rising threats and limited resources, the 1996 Nairn Review of Australian quarantine urged a far greater national emphasis on using scientific approaches to combat the menace. This resulted in the Australian Biosecurity System, which aims to: prevent new pests entering the country and becoming established; ensure we are prepared and can respond quickly to an incursion; and meet our international trade and treaty obligations with regard to biosecurity.

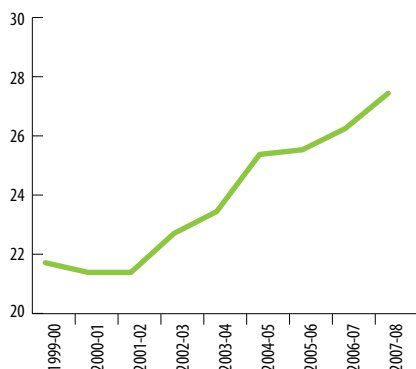
The CRC for National Plant Biosecurity was set up in 2005 to bring together the leading scientific agencies, government bodies and industries to deliver the core science that underpins Plant Health Australia Ltd and the Australian Biosecurity System. Specifically, it serves two goals of the

Biosecurity: the protection of the economy, environment and human health from the negative impacts associated with entry, establishment or spread of exotic pests and diseases.

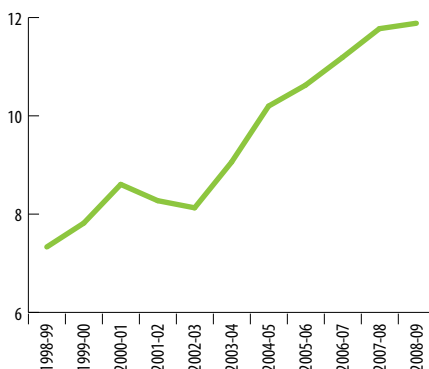
– BEALE REVIEW

Rising global trade and travel increases biosecurity risk

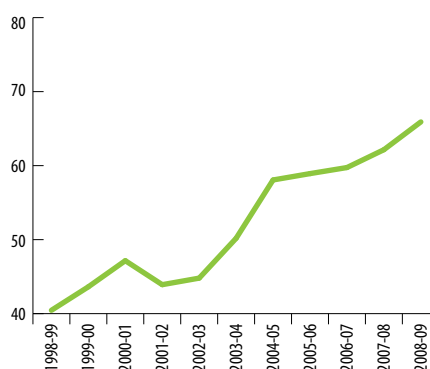
Port calls by international freight vessels (thousands)



International air passenger arrivals in Australia (millions)



International aircraft arrivals in Australia (thousands)



Source: Bureau of Infrastructure, Transport and Regional Economics



The spread of plant pests is a constant threat to the production of global food supplies.

Australian Government's National Research Priority 'Safeguarding Australia': to protect the nation from invasive diseases and pests, and from terrorism and crime.

Dr McKirdy explains that the CRC's research activities span the full range of biosecurity issues, from identifying and combating threats outside Australia, to border and post-border protection, right down to identifying and controlling pests at farm, food-chain and export level. It carries out scientific research in five main areas:

- preparedness and prevention, which aims to head off biosecurity problems before they even start
- diagnostic research, which improves our ability to identify possible risks to plant health as early as possible
- surveillance research, which develops new ways to detect possible invaders before they become a problem

- impact management research, which identifies better ways to nip emerging problems in the bud, and
 - post-harvest integrity research, which explores ways to improve the quality, health and safety of the grain and food-supply chain and provide clean, safe, quality products to our export customers.
- These are supported by two further programs of investment:

- education and training, building Australia's scientific and research capacity, and
- delivery and adoption, to put the latest research findings and scientific advances in the hands of those who can best make use of them.

"A really important advance has been our Plant Biosecurity Toolbox – a knowledge system containing images and diagnostic information that enables almost anyone to rapidly identify a possible plant pest

or disease," Dr McKirdy says. The toolbox is available throughout Australia via the internet as part of the Pests and Diseases Image Library (PaDIL).

"Scientifically, it is fairly straightforward. The point is that it's universally accessible to anyone – expert, researcher, farm adviser, pest control officer, farmer or bushwalker – at whatever level of detail they require. If they see something unusual attacking a plant or crop, they can check it out online and report it. Having many more 'spotters' on the lookout for invaders is a major help in mounting an early response to invading organisms."

The CRC's approach begins with an offshore focus, to identify the various crop and plant pests causing problems around the world and, from among them, to pinpoint those that pose the greatest risk to Australian crops and plants. Under development is a system that gives different

weights to various plant pests and diseases worldwide and then uses a sophisticated analytical tool to identify those that present the greatest risk to Australia based on all the possible pathways they could use to invade. This helps biosecurity planners develop military-style strategies for heading off likely incursions – preferably long before they make it to Australia's shores.

Among potential invaders, two notable targets for preventative action by the CRC are Khapra beetle, a devastating worldwide pest of stored grain that has not yet established in Australia, and citrus greening, a bacterial disease spread by a psyllid insect, which has also not reached our shores, Dr McKirdy says.

"Both of these could ravage our industries and exports, so we have major research programs designed to keep them out, or detect and deal with them swiftly if they do show up here."

One of the CRC's most spectacular projects involves constructing a microscope network with a global reach to detect pests. This began

by networking a number of microscopes around Australia, via webcams and the internet, so researchers could study particular pests and consult with experts online, in real time – even though thousands of kilometres apart. The network has since expanded to include microscopes in New Zealand, East Timor, Thailand, Malaysia, Indonesia and Laos and is shortly due to link up with microscopes in Canada and the United States. New software developments should lead to automatic identification of insect pests. "It's remarkable: it gives us the power to study any pest offshore or onshore and consult the world's top expert on it, in real time. It's a major achievement by Australia to get this to work on such a scale," Dr McKirdy says.

At a quite different level, the CRC is working to further educate farmers and rural communities in Indonesia to spot plant pests and diseases, both to protect their own crops and to provide us with early warning of their proximity to Australia – a good example of how vital international cooperation

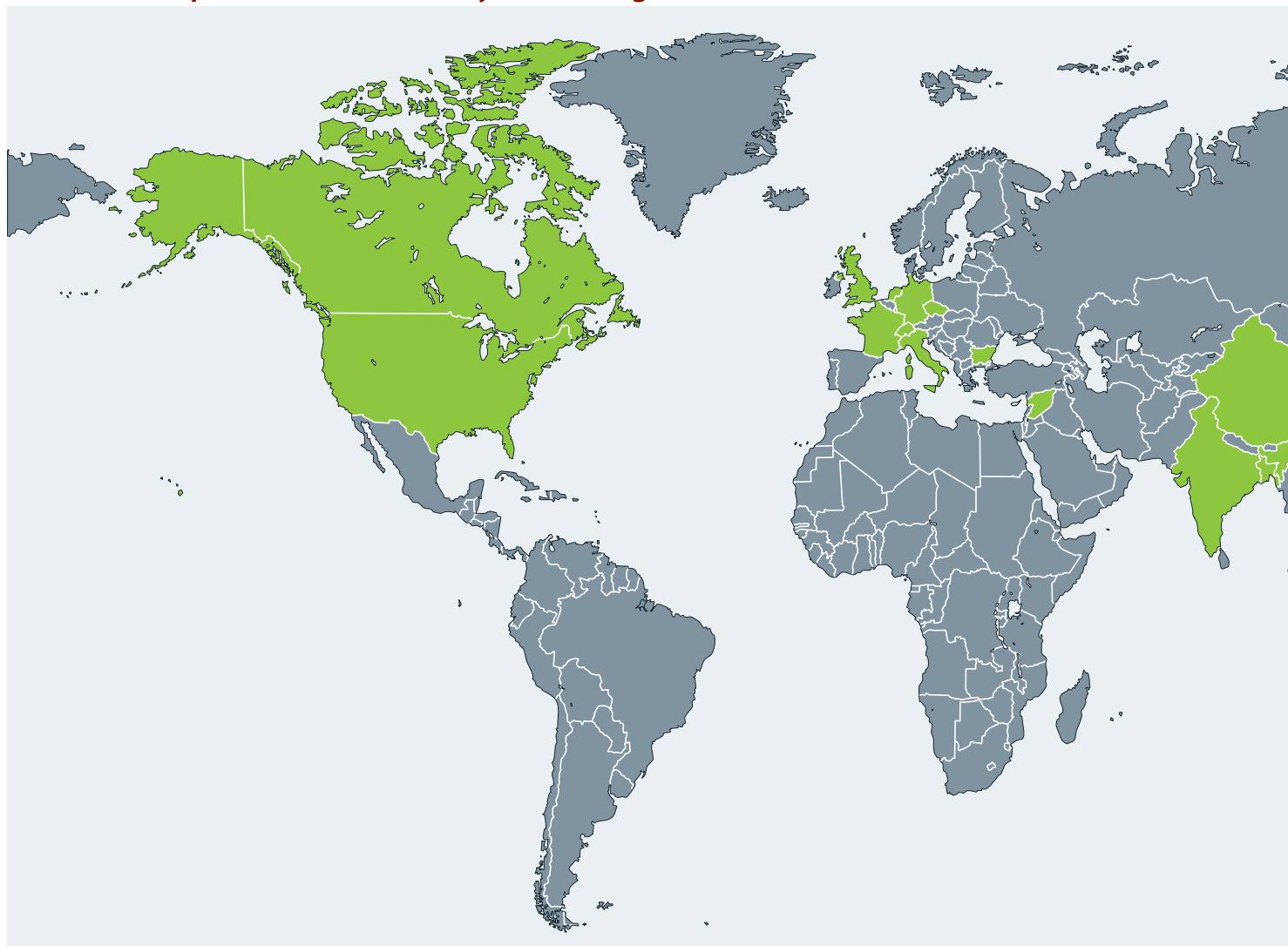
is to biosecurity. Similarly, Indigenous communities in northern Australia are rediscovering a role not unlike their World War II 'coast watcher' responsibilities – as biosecurity defence officers. By undertaking surveillance patrols across remote parts of the country, which the CRC is helping them to recognise, they are helping to keep all Australians and our farming industries safe.

Another project is delineating the likely risks to Australia's largest grain crop, wheat, imposed by the effects of climate change on various diseases that attack grain yields. As rainfall, temperature, moisture and vegetation patterns alter with the climate,

Plant biosecurity: a set of measures designed to protect crops, pastures or native species from harmful plant pests and diseases at national, regional and farm levels.

– EMERGENCY PLANT PEST RESPONSE DEED,
PLANT HEALTH AUSTRALIA

International partners in biosecurity research (green)



pathogens such as stripe rust, crown rot and barley yellow dwarf virus, and the insects that help to spread them, alter their ranges and invade new areas.

Part of adapting to climate change involves anticipating and, if possible, preventing them from gaining a foothold.

As far as Ug99 is concerned, Dr Rohan Rainbow of the Grains Research and Development Corporation says Australia is somewhat better placed than North America or other wheat-growing regions: only half our wheat varieties are susceptible, whereas nearly all North American varieties are. Having a rust research program that has run for 75 years without a break is a major asset, he says. Australia is now supplying resistance genes to countries around the world to help them protect their vulnerable wheat crops. At the same time, the CRC is investigating how rust fungus actually travels around the world, exploiting transport and traveller movements. This illustrates how good biosecurity science here can make a major

contribution to global food security, as well as protecting our own industry.

Other priorities for prevention by the CRC and its partners include the wheat disease Karnal bunt, for which the CRC is developing a sensitive diagnostic test for early detection, and the Russian wheat aphid, for which strategic pre-breeding of resistant wheat strains has already begun. "The experts think it is probable the aphid will get here eventually, so we want to be sure we have resistant wheat varieties on the shelf, ready to go," Dr Rainbow says. "The CRC is a major help to industry in identifying the risks we face, in sharpening our preparedness and in working out how to plug any gaps we find."

In another project, a CRC team is developing biomarkers that identify plant diseases based on their ability to cause damage. This is a far more effective way of judging which disease variants pose the greatest risk and avoids overreacting to harmless strains. At the same time, it is working on novel detection technologies such as hyperspectral imaging, which can scan entire crops for the 'invisible' signatures of attacking plant pathogens.

In its impact-management research, the CRC seeks to reduce the level of crop losses inflicted by attacking insects and diseases. Approaches include the development of new techniques to eliminate an emerging pest early in its attack without having to destroy the whole crop, and tactics that will raise Australia's preparedness status against possible future invaders such as rice blast or Russian wheat aphid.

A central role for the CRC is to uphold Australia's outstanding reputation for delivering clean, safe, high-quality food exports to global customers. Professor John Lovett, CRC Chairman and former Managing Director of the Grains Research and Development Corporation, says "there's basically a zero-tolerance attitude to grain pests by many importers worldwide nowadays – one weevil can spoil your entire day".

"Australia markets a very clean product, but in recent years we have been struggling to maintain the scientific effort necessary to keep that quality in the face of developing insect resistance to the main fumigants. Through the CRC we have managed to pull together the best researchers and experts in Australia for a national focus on better fumigants and ways to improve all our stored grain handling processes." Among the more promising, he says, is the use of the inert gas nitrogen, for which the pests have no evolutionary answer.

Professor Lovett recalls a memorable

quote from the global summit 'Food security in a climate of change', held in London in October 2009. Reflecting on the increased demand for food by 2050, delegates were told that the challenge is to 'Grow more: lose less', emphasising that biosecurity is critical not only while crops are in the ground but also after they have been harvested.

The value of having a CRC to spearhead national plant biosecurity research lies not only in being able to draw together the best scientists and resources, but also the close partnerships with industry and government, which translate the science into real action on the ground, Dr McKirdy says. "We think the cooperative model has worked really well in achieving rapid uptake of new scientific findings at all levels, right down to grassroots farmers."

International collaboration

Another key role for the CRC is ensuring that Australia can uphold its international plant biosecurity obligations. These are derived from the World Trade Organization's *Agreement on the Application of Sanitary and Phytosanitary Measures* and other WTO agreements, as well as guidelines and standards under the *International Plant Protection Convention*. All are science-based and require a high level of scientific expertise to comply with – and to enhance.

Of concern, Dr McKirdy says, is whether Australia is training sufficient plant biosecurity researchers with the skills needed to keep our borders and industries safe in the future. "We have a new generation in training right now – 38 PhDs within the CRC alone, which is more than we expected. It is clear quite a few young scientists are challenged by the ideal of helping to keep Australia's borders safe from plant invaders. But there has been a general decline in enrolments in agricultural and related sciences at universities Australia-wide, in response to cutbacks in state and federal agencies over the years – and it is far from clear if we will have all the necessary skills and dedicated scientists available in future.

"Overall, our biosecurity capacity is diminishing, as older scientists retire and are not replaced by young ones coming through – despite all we can do within the CRC. This is something we need to take serious note of, as a nation. We are getting better at detecting invaders and potential invaders, but we are also discovering there are more and more chinks in our armour, any one of which could cost us dearly."

An example is the recent discovery in Australia of a disease called myrtle rust,



which attacks native shrubs. It is a close relative of guava rust which, if it established here, could spell death to many Australian eucalypts. The thought of an Australian landscape devoid of eucalypts, simply because we had ignored the possibility, is chilling. "This risk is large," Dr McKirdy says, "and so are the resources we would have to throw at it to combat an established invasion. Much better we do the science that will help us to avoid invasion in the first place, or to detect and stamp it out before it can get a hold. Let's hope we can successfully eradicate myrtle rust and limit the impact on our valuable environment."

His views are echoed in the Beale

Review, which advocated a substantial overhaul of Australia's frontline biosecurity defences. It concluded that Australia operates a good biosecurity system, indeed one that is often the envy of other countries, given its comprehensiveness, transparency and scientific rigour. "However, the system is far from perfect. It has been subject to strenuous criticism, at home and abroad, for carelessness, opaqueness, excessive time delays, perceptions of political interference, poor communication with stakeholders, for being too restrictive and for being too liberal." Above all, the Beale Review found that Australia needed to substantially lift its funding of biosecurity and biosecurity

science by around \$260 million a year if the country's defences were to remain strong.

The big costs, at a time of fiscal rigour, may have proved a show-stopper: so far the findings of the Beale Review mostly still await action. While the global biosecurity threats mount, question marks gather around our capacity to deal with fresh invasions. "As with human disease, you can never become complacent about plant biosecurity," Dr McKirdy says. "Humanity has created ideal conditions for the accelerated spread of plant pests and diseases around the planet – we absolutely have to get smarter at controlling and containing them." ■

Conference promotes global exchange of ideas



Early in 2010, Australia hosted the world's first international conference devoted to agricultural and environmental biosecurity as a global issue – an event that recognised the growing importance of biosecurity and also Australia's leading role in research, development and science-based risk management in this area. More than 450 delegates from 24 countries attended.

The CRC for National Plant Biosecurity, the Australian Biosecurity CRC for Emerging Infectious Disease and the Invasive Animals CRC combined to host the event with Horticulture Australia Ltd, the Grains Research and Development Corporation and the Australian Centre of Excellence for Risk Analysis as major sponsors.

In opening the conference, CSIRO Chief Executive Dr Megan Clark emphasised the importance of collaborative efforts in a world where the more frequent movement of people, plants and livestock, and climate change, are increasing biosecurity threats.

"Business as usual will not prepare Australia for the future," Dr Clark said. "We need to keep making the point that this challenging issue can be addressed if all sides are willing to engage openly in dialogue, learn from our experiences and work together to prepare Australia and the globe for the future. Now more than ever we need to look at partnerships to address these challenges."

During the conference Dr Tony Gregson, Chairman of Plant Health Australia, launched the *National Plant Health Status Report*, which summarises the research and biosecurity systems that help protect Australia's agricultural and forestry industries from exotic pests. The report also identifies nearly 300 high-priority plant pests that are exotic or of significant quarantine concern to Australia.

Promoting international partnerships and collaboration was a key element of the conference, with participants across the agricultural and environmental sectors sharing and exchanging the latest biosecurity research into drivers, threats and impacts, knowledge and systems. With many new biosecurity technologies being used in areas such as surveillance and diagnostics, the conference also encouraged participants to consider how these ideas and research could be applied in their own environmental, plant or livestock context.

– Catherine Norwood



A trade stand during the international biosecurity conference provided the opportunity to promote the CRC for National Plant Biosecurity's research.

PHOTO: DI HARRIS PHOTOGRAPHICS

CRC for National Plant Biosecurity

program areas



Preparedness and Prevention Research

Preparation, including a better understanding of risks, can help us to prevent problems before they occur. This research program provides information on the risk of entry, establishment and likely spread of harmful plant pests. The emphasis is on developing risk-weighted, science-based decision-making systems and filling critical gaps in the knowledge of the ecology and epidemiology of high-priority harmful plant pests.



Diagnostics Research

The ability to identify a problem is an essential part of Australia's biosecurity capacity, as rapid identification and a quick response can reduce the impact of the incursion and the cost of eradication. The CRC aims to develop world-class diagnostic skills for the early identification of harmful plant pest incursions, using accurate, reliable and cost-effective technology to provide training and access to relevant diagnostic data and expertise.



Surveillance Research

Effective surveillance is essential in monitoring for potential new plant pest threats and for proving area freedom to maintain access to markets. This research program aims to develop cost-effective national surveillance systems using scientifically sound sampling tools and survey methodologies. This will enhance Australia's biosecurity practitioners' ability to capture a wide range of plant health information and to quickly identify incursions and prove area freedom.



Impact Management Research

Some exotic plant pests have the potential to cause more damage to Australian crops and the environment than others. This program aims to minimise the social and economic impact of potential incursions from harmful pests by prioritising risks and developing risk mitigation and more effective control or eradication strategies in response to an incursion.



Post-Harvest Integrity Research

The Australian grains industry is a major partner in the CRC and this program aims to help protect the value and integrity of the post-harvest grains sector in both domestic and international markets. It is focusing particularly on pests of stored grain in order to maintain Australia's competitive advantage as an exporter of pest-free grain through the development of innovative and cost-effective techniques to identify and prevent infestations of existing and new pest and disease threats.



Education and Training

Building capacity to address future biosecurity risks and responses is an integral part of the CRC's mandate. This program enhances the quality and quantity of plant biosecurity education and training available to the research and scientific community within Australia, and in collaboration with international trading partners. It also provides primary and secondary education programs and some industry-specific vocational programs.



Delivery and Adoption

The aim of all the CRC's research programs is to address industry needs. This program focuses on increasing awareness of biosecurity issues within government, industry, education and research communities, facilitating the commercialisation of program outputs, such as new information, technology and processes, and maximising adoption of research outcomes to improve Australia's biosecurity.

Will climate change favour pathogens?

A CRC project is using models to predict how Australia's most damaging wheat pathogens will fare



By Rebecca Thyer

The feeding frenzy of a cereal aphid attack damages plants in more ways than one.

Not only do aphids consume the stems, leaves and heads of cereals, but as they chew on their host plants their tiny 'teeth' or stylets can transmit yellow dwarf viruses into the plant tissue, spreading one of Australia's most damaging group of cereal viruses.

Yellow dwarf viruses are among the country's most serious wheat pathogens and biosecurity experts and managers, researchers and wheat growers understandably want to know how they can reduce their impact.

Yet, in a changing climate, this goal can be somewhat of a moving target. Climate change predictions indicate that carbon dioxide (CO₂) levels and temperature will increase and rainfall will decrease in most wheat-growing areas, affecting plant growth.

Dr Jo Luck, from the Victorian Department of Primary Industries (DPI), admits that the variables involved make it difficult to assess what these changes will mean for plant pests and diseases. But it is a task she is determined to address via a CRC for National Plant Biosecurity climate change project.

Dr Luck and her team are concentrating on what climate change may mean for three of Australia's most damaging wheat pathogens – stripe rust, crown rot and one of the yellow dwarf viruses, barley yellow dwarf virus, and its aphid vector, the bird cherry-oat aphid.

The challenge, she says, is to understand how each component of the system will affect each other and then to synchronise them. "First, we need to work out how plants may change in the future and then how the pest or pathogen affecting it may respond to the changes in the plant."

For example, barley yellow dwarf virus is spread by the bird cherry-oat aphid. Yet, under a different climate scenario aphid populations may change and they may, for example feed and transmit the virus at a greater rate than under existing conditions.

To be able to answer what may happen under a changing climate and provide scope to biosecurity managers, wheat breeders and the industry, Dr Luck's team includes

mathematical modellers, spatial scientists, entomologists and pathologists. The team is working on virtual models that complement real-time experiments.

"In the field and in glasshouses we can look at increased CO₂ levels and increased temperatures. In virtual models, we can obviously look at everything and the two work well together."

The work is already revealing some interesting facts, especially about 'doom and gloom' scenarios. "Most people assume pests and diseases are all going to increase with a change in climate but that is not the case," Dr Luck says. "Climate change brings good and bad news in terms of diseases, pests and biosecurity."

For the team, the next step is to pull its crop, aphid and virus 'sub models' together into an integrated model and assess how climate change affects the overall system.

Dr Luck says the work will help industry address future climate change issues, such as the potential for pests and diseases to shift geographically – important for disease monitoring and surveillance.

This information will help to develop management plans for biosecurity workers, agronomists and growers and create future plant breeding objectives. For example, Dr Luck says there is no complete resistance to crown rot. "So, if what we're seeing is the case, then more resources should be allocated to resistance breeding for this pathogen."

PHOTO: VIC DPI



Researchers monitor plant growth and disease development in a heightened CO₂ environment established at Hamilton.

As an example, after three years of testing stripe rust development in elevated CO₂ field plots the work is showing that pathogen levels may not rise. "With temperature increases and reduced water levels, it could become less of a problem for growers."

Similar work on crown rot tells a different story. "It is already becoming increasingly important in drier years, which will only increase with climate change. Findings from our CO₂ studies suggest it could become even more of a problem, so that is one to watch."

The research is at an earlier stage with the barley yellow dwarf virus. "This is more challenging work because we need to work with the crop, the aphid vector, the virus and the interactions between them."

On a larger scale, the results could influence future biosecurity policy and management.

The importance of Dr Luck's work has also been internationally recognised, resulting in an Asia-Pacific Network for Global Climate Change Research project. That project will address the climate change impact on the disease, potato late blight. Potatoes are an important staple crop in many countries in the region and Australia is, so far, one of the few countries that does not have the new, aggressive strains of the pathogen. There is concern that if these strains reach Australia they could devastate the country's \$400 million-a-year industry. ■

Heavenly eye to keep watch on crop invaders

Locust plagues will help to test satellite-based predictive modelling of exotic pest incursions



By Kenn Pearce

Australia's plant industries have taken a heaven-sent opportunity to innovatively respond to the earthly problem of pest insects.

A researcher working with the CRC for National Plant Biosecurity is using the global mapping expertise of the National Aeronautics and Space Administration (NASA) in the United States to gain an upper hand over voracious harmful plant pests.

John Weiss, from the Victorian Department of Primary Industries, is midway through a PhD research project at the University of Melbourne assessing whether NASA's Terrestrial Observation Predictive Systems (TOPS) can be applied to the management of exotic pest incursions.

Pest insects use various methods to identify suitable hosts for their banquet: some use smell, others are attracted by taste, while others mainly use visual cues. NASA's TOPS technology 'sees' a wheat field or paddock the way an insect might see it – on the wing.

"The satellites fly overhead and have a variety of sensors that see particular wavelengths; they see what the insects are seeing," Mr Weiss says. "Obviously you can't predict what will happen in a week's time just from a satellite image but by incorporating it with a model of the insect pest's preference, and what will be the host plant's suitability, you may be able to improve predictions on



where insects may move to.

"To test the predictive ability we're looking at historical invasions and doing retrospective and trace-back analysis.

"We're working with NASA because they've built a vegetation growth (Gross Primary Production) model of photosynthetic activity that uses climatic data to predict, up to a week in advance, what the crop's conditions or suitability is going to be."

Mr Weiss' research also looks at natural insect dispersal. It will have predictive ability for wind speed and direction, with estimates and probabilities for where the insect pests are going to land during the following week, with data readouts from space providing information about crop location, growth rate and colour hues.

"It's now at the stage of testing with Australian plague locusts to see if we can predict where the adult insect will go in a swarming event," Mr Weiss says.

Locusts were chosen because the Australian Plague Locust Commission

(APLC) has good data sets about incursions and movements of the pest – crucial for modellers. Fortuitously for researchers, although not for farmers, a potential locust plague in spring 2010 – following widespread swarms in south-eastern Australian in autumn 2010 – will provide a test for the robustness of the system.

He says one dilemma for researchers is the desire for superfine spatial and temporal resolution, and for daily updates; but those factors are generally mutually exclusive.

"If you want a satellite to go overhead every day it's essentially got to take bigger-picture views," Mr Weiss says. "Some have resolution of about five metres but only acquire an image of an area every fortnight or month. The one we're using (MODIS) is of a scale of one pixel per square kilometre resolution but we have daily acquisition."

Finer resolution data can be affected for weeks when a fortnightly satellite encounters cloud cover. But daily, lower-resolution fly-overs mean cloud cover one day might be followed by clear sky the next.

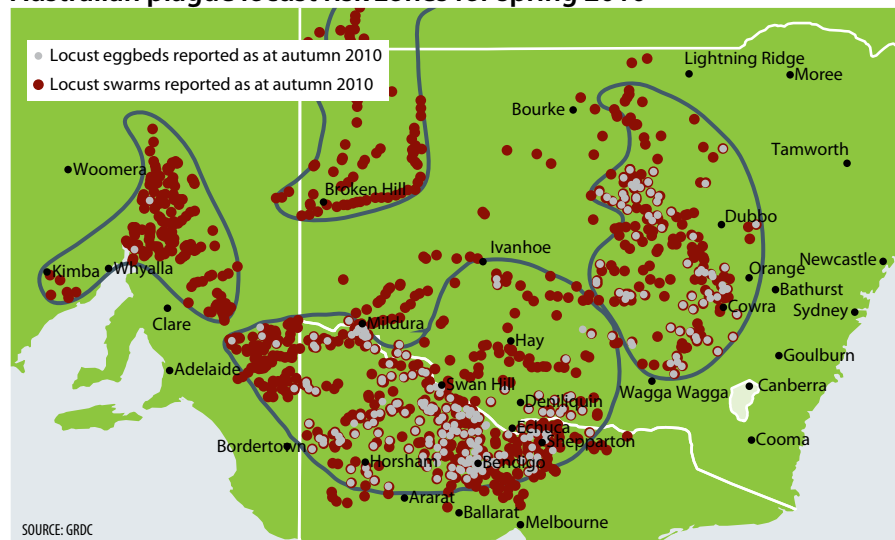
"We're trying to build some qualitative processes to see if we're getting better predictions on where insects land," he says. "In some ways it doesn't matter if TOPS gives us only five per cent better predictive ability; if it gives an extra five per cent we should use it."

"At international biosecurity meetings we all talk about how to model pest dispersal, how you look at natural dispersal versus human-assisted, how to do forward predictions. It's a universal problem that most people dealing with biosecurity and pest incursions are facing."

He believes the project will deliver scientific capacity by providing a potential new national biosecurity tool. "One of the advantages of the CRC is it has this national approach and breaks down the barriers between states or organisations."

In Australia there are collaboration with the APLC and CSIRO and overseas with the US Department of Agriculture and US Geological Survey. CRC CEO Dr Simon McKirdy is one of Mr Weiss' PhD supervisors and has also spent time at the NASA research centre, building relationships as part of a long-term biosecurity partnership between the US and Australia. ■

Australian plague locust risk zones for spring 2010



Charting pest rankings: a concert of challenges

The universal principle of 'like attracts like' applies to plant pests as much as to people



By Catherine Norwood

Like rockstars, roadies, fans and promoters congregating at a concert to feast on the music and money, groups of plant pests with shared appetites can also be found gathered at common venues.

CSIRO entomologist and CRC for National Plant Biosecurity researcher Dr Dean Paini calls these pest groupings 'assemblages'. He uses the concept of self-organising maps to identify pest groupings that affect Australian agriculture and the environment. By identifying those pests currently absent from these groupings in Australia, but present in similar groupings overseas, he determines the pests that are most likely to establish here.

"If 10 species have established in a particular region, and nine of the same species have established in another region with a similar environment, then the likelihood of the tenth species establishing in the second region is very high," he says, "like the ticket scalpers finally arriving on the concert stadium steps."

His research has matched assemblages of 844 plant pathogens and insect pests across 459 different geographic zones around the world. Most regions represent either a country or, for larger nations such as Australia, the United States, China and Canada, the various states or provinces that make up that country. Every species was given a risk rating, on a scale of zero to one, according to how likely it was to establish in Australia based on the mapping of pest assemblages.

"All around the world biosecurity departments are looking for this kind of information to help them prioritise risk and allocate resources where they will be most effective," he says. "This is an objective, analytical tool that can be used to support expert knowledge."

Dr Paini says identifying whether a pest is likely to establish in Australia is only part of the risk-assessment equation. Other elements include the likelihood of a pest actually arriving and the potential cost to industry and to the Australian environment of an exotic pest incursion.

Not everything that makes its way to Australia will find favourable conditions and

PHOTO: ISTOCKPHOTO



Under the title 'Six degrees of preparation', new research will study how 'indirect' international shipping could bring pests to Australia.

not every exotic pest will have a significant impact on industry, even if it does establish itself here.

Among the highest-ranked pests in Dr Paini's mapping analysis were the yellow stem borer and the purple stem borer, both pests of rice crops, with risk indexes (out of one) of 0.7924 and 0.7722 respectively. However, the comparatively small size of the Australian rice industry suggests that even should these pests establish here, the impact on the domestic economy would be relatively small.

The same cannot be said of the oriental fruit fly (*Bactrocera dorsalis*). Although it ranked 31st in Dr Paini's research, with a risk index of 0.5008, it has the capacity to attack a wide range of fruit and horticultural crops. Complementary CRC risk-related research led by CSIRO economist Dr David Cook has identified potential costs of almost \$652 million to the apple and pear industries alone, over 30 years, associated with an incursion of this pest.

Dr Cook's research team has developed an enhanced risk-analysis model to assess the potential cost impacts of incursions and to help industry groups prioritise risks and areas for biosecurity investment.

Apple and Pear Australia Ltd (APAL) was among a number of horticultural groups that took part in Dr Cook's research.

APAL general manager Tony Russell says the industry has had a long interest in biosecurity issues, crystallised by the potential of New Zealand apple imports bringing fire blight into Australia. Dr Cook modelled the impact for a range of pests nominated by APAL and fire blight was the pest with the highest impact, with an outbreak estimated to cost \$846 million over 30 years.

"The modelling has provided us with an economic assessment, which helps to start the process of prioritising pests and making decisions about investment required," Mr Russell says.

"The next phase will be the ability to model the spread of an incursion under

different circumstances. To do that we realise we need better information about where our commercial orchards are located – so that we can better plan and respond to an incursion.”

Other horticultural groups that took part in the project, as members of Horticulture Australia Ltd, included the potato and vegetable industries, along with the Rural Industries Research and Development Corporation.

Dr Cook says an important part of the modelling project was actually gathering detailed information about the pests involved, which was collated into threat data sheets. Based at the Department of Agriculture and Food, Western Australia, Dr Abu-Baker Siddique spent three years collating detailed information about the 69 nominated exotic pests affecting 13 different horticultural industries involved in the project.

The data sheets include valuable specialist knowledge gathered from around the world, such as information on the different control and treatment regimes. They have provided essential information to the modellers developing the risk-management tool.

The modelling produces statistical information about the likely economic impact of the nominated pests over a 30-year period. It takes into account control costs, loss of production and lost markets.

When combined with a deliberative multi-criteria evaluation (DMCE) framework, it includes an allowance for environmental and social costs of pests. For instance, where a pest has the potential to affect native flora and fauna, the implications of this damage can be compared with the agricultural damage to get a much clearer picture of the threat posed to Australia.

The tools developed by Dr Cook’s team allow industry and government participants to determine the trade-offs they may be willing to make in terms of allocating resources and potential impacts of an incursion. They also provide information that can be used to inform biosecurity policy decisions and establish cost-sharing arrangements for control measures.

“We found, when we began using the tools with stakeholders, that they were extremely interested in the assumptions and calculations used in putting the modelling together,” Dr Cook says. “But the model we had developed was extremely complex and it wasn’t easy to ‘crack it open’ to demonstrate the variables and how they influenced the outputs of the model.”

That’s one of the reasons for his next project – ‘Communicating uncertainty in biosecurity adaptation’. Dr Cook says it’s about developing a simpler form of the model that can be more easily understood and manipulated by non-modellers.

“I call it the ‘war games’ version. We’re planning to develop it as PC-based software that, with some training, industry groups will be able to use to try out different incursion and impact scenarios to help with their decision-making. It will have a map-based interface, which should be easier to use than the statistical outputs of the original model.”

Dr Paini is also moving on to a new risk-analysis project to identify the potential pathways for incursions into Australia – the ‘likelihood of arrival’ part of the risk equation. He is specifically looking at indirect shipping connections that could bring high-priority pests to Australia.

The project, ‘Six degrees of preparation’, is founded on network theory, well known in public health circles for studying the transfer of disease, such as the pandemics of SARS in 2002 and H1N1 influenza in 2009.

“You have to be able to identify what may be only a single link between two otherwise unconnected communities,” Dr Paini says. “For instance, goods coming from the United Kingdom could be transferred to containers from Asian countries that have previously carried grain infested with a pest that’s not commonly found in the UK, and that is not found in Australia. Those goods are then delivered to Australia and before long a bug crawls out of the woodwork and we have an exotic incursion on our hands.” ■

Search for fire blight’s genetic weakness



To the average person, mapping the genome of *Erwinia amylovora*, the bacteria that can lead to fire blight in pome fruit, sounds incredibly complex. But La Trobe University PhD candidate Rachel Powney, who has mapped four strains of the bacteria in the past two years, says it is not as difficult as it sounds.

She was given a head start by German researchers who had already mapped one strain of the bacteria and many related species before she began, providing a guide for her own work.

By mapping as many species of *E. amylovora* as possible, Ms Powney is hoping to identify the DNA sub-groups that cause one strain of the bacteria to develop as fire blight while others don’t. Australia is so far free of the disease, which is estimated to cost the fruit industry in the United States more than \$100 million a year in crop losses and disease control.

With support from Horticulture Australia, Ms Powney’s CRC for National Plant Biosecurity PhD project will address unreliability in current testing techniques for the bacteria. Polymerase chain reaction (PCR) tests are the current internationally accepted diagnostic standard, but they do not meet the reliability required in Australian standards.

PCR tests often fail to detect all strains of the *E. amylovora*

bacteria or they give a positive result for closely related species, including those that do not lead to fire blight. Ms Powney’s research indicates that genetic identification will provide a solid basis for more accurate testing.

“When talking about such a destructive disease, correct identification is very important for Australia’s biosecurity,” she says. Greater knowledge of the genetics of *E. amylovora* is paramount not only for developing reliable diagnostics but also to “understand the enemy’s biology and identify its weak points”.

Based at the Victorian Department of Primary Industries Knoxfield horticulture research facility, Ms Powney expects to complete her thesis in 2010.

– Kellie Penfold

PHOTO: JO ANN ASSELIN



PhD candidate Rachel Powney travelled to Cornell University in the United States to see the impact of fire blight on fruit trees first-hand.

Protecting the environment is part of doing business on Barrow Island for Chevron Corporation

By Tim Treadgold

It was back in 1910 that the WA Government recognised the unique flora and fauna habitat that exists on the island, located some 50 kilometres off Australia's north-west coast.

Keeping it a special place, and as free from introduced plants and animals as possible, has led to the formation of a unique project – commissioned through the CRC for National Plant Biosecurity – involving Queensland University of Technology, the Department of Agriculture and Food, WA, and one of the world's biggest oil and gas companies, Chevron Corporation.

Of the various organisations involved, the key player is Chevron because it is the operator of the giant Gorgon liquefied natural gas (LNG) project, which will use a corner of Barrow Island for its gas-processing facilities.

Before Chevron could receive government approval to use the island for LNG production it had to demonstrate that it could:

- protect rare and endangered species unique to the island
- prevent the incursion of species not native to Barrow Island, and
- convince government that it could detect such species with a minimum 80 per cent certainty to ensure control and eradication can be considered.

To clear the hurdles set by government, Chevron turned to the CRC and other government agencies to design a quarantine management system (QMS) to prevent the introduction of non-native species, and detect and eradicate 'invaders' where necessary.

This combination of agencies dedicated to protecting the environment, and an oil and gas producer dedicated to extracting resources might initially seem an odd marriage. Historically, however, there is nothing new about the island serving a dual role as a nature reserve and oilfield.

Pimpla ruficornis
♂, lateral view

0.30 mm

Chevron


Barrow Island Quarantine

Passenger Declaration Card

(For those returning from overseas)

SURNAME/FAMILY NAME _____

GIVEN NAMES _____

DATE ____ / ____ / ____
 ____ / ____ / ____

Please answer every question below and sign.

Are you carrying any:

Fresh fruit or vegetables?	Yes <input type="checkbox"/> No <input type="checkbox"/>
Fresh or dried plants or plant material? - Seeds or nuts?	Yes <input type="checkbox"/> No <input type="checkbox"/>
Fresh, unprocessed meat?	Yes <input type="checkbox"/> No <input type="checkbox"/>
Other food products?	Yes <input type="checkbox"/> No <input type="checkbox"/>
Animals or insects?	Yes <input type="checkbox"/> No <input type="checkbox"/>
Items made from or containing wood?	Yes <input type="checkbox"/> No <input type="checkbox"/>
Soil?	Yes <input type="checkbox"/> No <input type="checkbox"/>

You may be carrying quarantine risk material. Have you:

Thoroughly cleaned all your footwear?	Yes <input type="checkbox"/> No <input type="checkbox"/>
Checked your cuffs, pockets and clothing?	Yes <input type="checkbox"/> No <input type="checkbox"/>
Ensured your bags are clean (inside and out)?	Yes <input type="checkbox"/> No <input type="checkbox"/>
Checked any Velcro fasteners are clean?	Yes <input type="checkbox"/> No <input type="checkbox"/>

 Passenger signature

Garçon Project

(Top) The big-headed fly is one of 181 fly species so far identified as being present on Barrow Island. (Bottom) All visitors to Barrow Island must complete a quarantine risk assessment.

In fact, since oil was discovered in 1964 one of the world's more interesting symbiotic government/corporate relationships has developed, with a consortium of oil companies emerging as frontline defenders of the environment.

By keeping Barrow Island a 'closed shop', Chevron and its partners ensured that the island remained a safe habitat for the 24 insect and animal species that occur nowhere else in the world. It also formed a useful role as an occasional 'set' in the production of Australia's original (and best) television wildlife series, Harry Butler's *In the Wild*.

Operating a relatively small oilfield is one thing, but developing a \$43 billion LNG project is quite another and Chevron quickly

recognised that in order to win government approval, especially from environmental protection agencies, a significantly enhanced level of quarantine was required.

Dr Peter Whittle, principal research fellow at Queensland University of Technology, says the QMS created for Barrow Island is a world-first.

"Our job was to design surveillance systems that would not only detect invader species, but do so with a statistical strength that was provable," Dr Whittle explains. Apart from baseline studies of flora and fauna already on Barrow Island, identifying the possible points of entry of unwanted species was an early step in designing the QMS. That meant looking closely at the construction site itself and modes of transport to the island.

One obvious outcome is that anyone visiting the island starts each trip by completing (and signing) a passenger declaration card that is almost identical to that filled in by international passengers entering Australia.

The card has 12 questions such as 'Are you carrying fresh fruit or vegetables?' and 'Have you thoroughly cleaned your footwear, checked your cuffs and any Velcro fasteners?' Prime QMS targets at this level of personal inspection are plant seeds and small insects such as ants. For machinery being transported to the island it is even more rigorous, with items wrapped in heavy-duty plastic and fumigated – even 35-tonne trucks.

All cargo bound for Barrow Island is subjected to rigorous inspection. Every employee and contractor is inducted into the island's QMS and provided with a spiral-bound, 250-page booklet to enable them to identify insects, spiders and snails common to the island (left alone) and the most likely suspects among the potential foreign invaders (doomed for eradication).

Chevron's Gorgon Quarantine Manager, Johann van der Merwe, says the system goes far beyond anything seen before in Australia, and perhaps anywhere in the world.

"Quite simply, we recognised that what we had been doing in the early years of oil production at Barrow Island would not fit the Gorgon development," Mr van der Merwe says. "Everybody at Chevron knew it couldn't be an expansion of the existing procedure."



Equipment is plastic wrapped and fumigated to remove any pests before being installed at Barrow Island.

PHOTO: CHEVRON CORPORATION

12 different pathways by which things can get to Barrow Island. They include people and luggage, sand and aggregate, plant and mobile equipment and marine vessels.

"We then looked at each item of risk and said if we do nothing the value (risk) will be a score of 10. So what can we do to reduce the risk and drive values down. With ships, we could clean them and spray them, and then ask the independent panel what it thought. If that wasn't enough, we could take further steps, with each one lowering the level of risk to an acceptable level."

On a day-to-day basis most cargo heading for Barrow Island is in specially built containers that have no air vents to prevent insect entry, but do have fumigation nozzles. If a cargo has to be on a pallet, only plastic pallets are used to prevent wood-borne insects. If a wood pallet has to be used for load bearing, the pallet has to meet a permanent immunisation standard.

Once a cargo is containerised it is gassed with an insecticide similar to household spray such as Mortein, or in combination with another 'knockdown' gas. If that's not enough, the temperature in a container left in the sun can easily exceed 60°C, the point at which no insect can survive. The result is no known insects surviving.

In a more extreme example of protecting the island, Chevron recently wrapped nine 35-tonne dump trucks in a white plastic wrap. Not only did the wrap permit fumigation but a side benefit was that the trucks arrived clean.

One of the more curious aspects is that designing a perfect quarantine system requires baseline studies, and that means knowing exactly what lives on Barrow Island – which is a question with an answer that keeps on growing.

"When we started the invertebrate count we thought we would get to 1,200," Mr van der Merwe says. "The baseline at the moment is more than 2,100. More importantly, only 268 had binomial names, the rest known by higher orders of naming and referencing only. We wrote many papers describing species new to science."

A sample of what's alive at Barrow Island can be found at the Pests and Diseases Image Library (www.padiil.gov.au), under the Barrow Island drop-down menu of a seemingly endless variety of ants, bees, beetles and insects.

Chevron's aim today is to apply its QMS to protect what should be on Barrow Island, keep out what should not be there, and eradicate any invaders. ■

So the company devised a quarantine plan that went well beyond best practice.

"We knew we had to form partnerships that involve all parties, including the CRC for National Plant Biosecurity, government agencies and universities around Australia," Mr van der Merwe says.

"For us, this is far more than just getting a licence to operate. It's all about environmental stewardship and preventing biodiversity loss, and that means we needed to know as much as possible about the animals and plants on the island."

This led to a key role for the CRC, designing surveillance systems for invertebrates on the island, including traps, cameras, surveys and incidental sightings. Another step involving the CRC members (including Dr Peter Whittle, Dr Frith Jarrad, Dr Susan Barrett and Professor Kerrie Mengersen) was to create a statistically measurable surveillance scheme.

"Each component of the surveillance system has its own range of creatures that it can identify and its own degree of effectiveness, with the overall effect combined into a single statistic," Dr Whittle says.

Looking back, the starting point for the systems that have developed on Barrow Island is the original negative reaction from the government. But as well as saying 'no' to Chevron, the government made a positive

contribution by asking for a set of standards of acceptable risk which might earn it a 'yes'.

For Mr van der Merwe and his team, this was a significant challenge. "We have no record in the world where standards of acceptable risk have been set for biodiversity."

Years of negotiation followed before the government acknowledged that the QMS devised by Chevron and its partners was 'likely to be the world's best' and that it was 'not possible for them to recommend any additional controls'.

What Chevron and its partners devised is an engineering methodology reshaped into a biological tool and overseen by layers of independent advisory panels.

"The process is unique," Mr van der Merwe says. "I know of no risk assessment in the biological field that has gone down this route. We have not just assessed risk ourselves, we have asked the wider community and independent experts what risks they see, and then we ask them to assess our response."

Some of those people chosen to assess risks were not friendly to the Gorgon project, which meant the risks nominated were often at the extreme end of the spectrum.

What evolved was a risk matrix that identified risk and then methodically worked through ways to lower that risk to a standard acceptable to all stakeholders. "We identified

Surveillance systems you can count on

Statistical modelling provides greater confidence in pest surveillance systems



By Catherine Norwood

“You cannot prove an area is free of a particular pest”, says Queensland researcher Mark Stanaway, “you can only estimate the probability. The more information you bring into your surveillance systems, the more accurate your estimate.”

Mr Stanaway and Western Australian researcher Nichole Hammond are both working on CRC for National Plant Biosecurity PhD projects designed to analyse the effectiveness of current surveillance systems and identify areas for improvement.

Mr Stanaway’s project is based on surveillance data and modelling of the spread of spiralling whitefly, a sap-sucking bug from South America first identified in Cairns in 1998. Ms Hammond has focused on an assessment of surveillance systems

in WA for the fungal disease Karnal bunt, which affects cereals.

Both are using hierarchical Bayesian models to estimate the uncertainty attached to each level of decision-making in the surveillance process and ranking the importance of each decision, to arrive at a ‘confidence rating’ for the effectiveness of their respective surveillance systems.

For his project, Mr Stanaway has collated more than 11 years of surveillance data to track the spread of the exotic spiralling whitefly (*Aleurodicus dispersus*) through northern Queensland. His central challenge is to calculate the likelihood that spiralling whitefly is in a particular area, based on the available surveillance strategies and current knowledge about the pest, by quantifying the uncertainty in the surveillance system.

Since arriving more than a decade ago, spiralling whitefly has established itself in

tropical coastal Queensland, from Torres Strait to Gladstone, and has also been found in the Darwin region. Restrictions have been placed on the transport of nursery stock from Queensland because the insect attacks crops and ornamental plants.

Mr Stanaway’s modelling includes all known information about the insect, such as growth and reproductive rates and its spread to date. It also includes uncertainty calculations about the pest’s ecology and the sensitivity of monitoring techniques. It effectively provides a statistical fact check for predictive modelling about how the insect may spread, which can highlight problems in surveillance systems and in the modelling.

He uses a scoring system that identifies how good the ‘presence’ and ‘absence’ data collected by the surveillance program is – how likely a ‘false negative’ might be and whether the insect is actually present,

Pest outbreak scenarios test responses



SARS caught the world napping and the rapid spread of the human and economic misery it created brought home sharply the wisdom of the motto: be prepared.

The outbreak sent the scientific community into overdrive, trying to predict the path and the momentum of the disease. If there was a silver lining to this and subsequent flu pandemics, it was the demonstration of pooled scientific technologies and disciplines generating effective disease control strategies.

Now these lessons are being applied to plant biosecurity with the development of pre-emptive surveillance systems to simulate the real-time rates of pest and disease spread.

Leading a CRC for National Plant Biosecurity research project to develop this ‘what if’ scenario-planning tool is Professor Mark Reynolds, at the University of Western Australia’s (UWA) School of Computer Science and Software Engineering. Also working on the project are Dr Juan José García Adeva (UWA) and Dr Maria José de Sousa Majer, from the Department of Agriculture and Food, Western Australia (DAFWA).

The aim is to develop a geographically based automated simulation system for surveillance and response applications to predict the spread of emergent plant diseases and pests.

Researchers currently provide biosecurity managers with biological parameters for harmful plant pests including host range, mode of spread and potential distribution based on climatic factors. But being able to predict the emergence of disease and pest

infestations helps biosecurity teams implement more rapid, more effective responses.

“An automatic simulation system requires information about initial (perhaps hypothetical) infestations, host and food locations, weather, and barriers in the landscape,” Professor Reynolds says.

“It then uses understanding of the pest life cycles, triggers for spread and pattern of spread, to predict the spatial-temporal pattern of infestation evolution.

“We cooperate closely with DAFWA entomologists and those in other CRC teams, including the team of Dr Michael Renton at UWA, who is designing a system for collating data about a wide range of pests – the type of data that enable us to model the pests’ life cycle and behavioural characteristics.”

This information is then used to manage response strategies and carry out hypothetical reasoning to determine the range of risks facing the managers of a potential outbreak – and the best distribution of resources to contain it.

While the initial focus has been on pest infestation, Professor Reynolds says the platform could be adapted to the management of plant diseases.

“So far we have made most progress [in simulations] with fruit flies and similar air-borne pests, although we have started to expand it to incorporate a diverse range of pests from grain beetles to blights.

“The way that outbreaks develop, and the environmental factors which affect them, varies greatly from pest to pest, so our simulator incorporates a modular design. This will allow us to quickly modify the system to simulate the behaviour of any particular new pest.”

– Barry Pestana

despite the fact that it hasn't been found.

His analysis has already identified the most effective host plants to monitor for the whitefly and a method of assessing how effective inspectors are.

He says his analysis will be used to estimate whether spiralling whitefly has spread as far south as it is likely to, based on 'absence' data from southern-most monitoring points for several years, and the fact that once the pest spreads to a new location numbers build up to the point where it is easily identified in surveillance. This finding may allow restrictions on the movement of nursery plants to be reviewed.

He says the statistical surveillance information is being used in conjunction with a geographic information system (GIS) database and predictive modelling to develop a risk map for the spread of the insect. "The risk map is a much more powerful tool than the surveillance data because it provides a visual representation

of changing insect or disease presence over time; it gives a sense of movement or change, which can be otherwise difficult to assess."

In WA, Nichole Hammond has considered both active and passive surveillance systems for the exotic fungal pathogen *Tilletia indica*, which causes Karnal bunt of wheat, rated an 'extreme' threat to the grains industry if it ever gets through Australia's quarantine defence.

The Department of Agriculture and Food, WA (DAFWA) has run an active surveillance for the past 10 years, collecting grain samples from the bulk grain handler the CBH Group. Each round of DAFWA surveillance tested approximately 200 grain samples and no Karnal bunt has been detected.

Passive surveillance systems involve the constant scanning of crops by farmers, farm workers and agronomists for anything 'out of the ordinary'. Ms Hammond says passive surveillance has historically been used to support claims of freedom from plant pests,

but its effectiveness has not previously been formally evaluated.

Her research analysed the community detection and reporting processes – the likelihood that growers would detect a possible infection and report it, based on existing surveillance practices for reporting pests and diseases.

She found that the active and passive surveillance systems provided greater than 90 per cent confidence that WA is free of Karnal bunt. This was based on 10 years' worth of harvest data and the level of infection expected, if the disease was present in WA.

Ms Hammond's analysis identified how sensitive each stage of the surveillance and identification process was. It highlighted where these could be improved, such as providing more information to growers and agronomists about signs and symptoms of exotic grains pests to improve confidence in on-farm detection. ■

Target on deadly passengers



The biosecurity arsenal of Australian airports could soon be expanded with a new CRC for National Plant Biosecurity and Grains Research and Development Corporation (GRDC) study to determine whether minute plant pathogens are being brought into the country.

Project leader Ms Dominie Wright, from the Department of Agriculture and Food, Western Australia, says it is possible that deadly pathogens, invisible to the naked eye, could be hitching a ride on passengers' clothes, shoes or hair. The results of her research will determine whether a CSI-like forensic test could be developed to detect the tiny hitchhikers.

Ms Wright says researchers, growers and travellers often visit farms or related enterprises while on holidays or working overseas. "These individuals represent an undefined risk for inadvertently introducing exotic plant pathogens into Australia," she says

The GRDC is assisting with the project, asking its scholarship-holders to collect samples from either washed or unwashed clothing after returning from overseas.

Ms Wright is analysing the material collected and returned in sealed evidence bags to determine whether spore shape has an impact and whether particular fabrics are more conducive to transportation. Additionally, she will investigate whether a simple wash is enough to remove the suspect spores.

Air-borne pathogens such as cereal rusts will be first in the firing line as researchers identify whether they are being introduced. Some strains of cereal rust are a major international threat, with the potential to wipe out entire wheat crops. Australian wheat varieties have in-built resistance to many rusts but overseas strains of the disease, such as the stem rust Ug99, have already caused crop destruction in regions where they have taken hold.

"It could be as simple as making sure people wash their clothes

PHOTO: DAFWA



Dominie Wright examines samples taken from the clothing of travellers for evidence of plant pathogens.

after they have been out in the field and before they get on a plane to come home," she says. "But we won't know until we finish the work."

The findings will be used to underpin communication campaigns targeted at travellers visiting agricultural regions. Ms Wright says there is a need for the Australian community to be better educated about the potential biosecurity risks when travelling interstate or overseas – and that prevention is the best defence. Educating the community on the most appropriate material to wear when visiting high plant-biosecurity-risk regions will also be an important output of the research.

– Melissa Marino

The inspiring adventures of a plant investigator

An innovative education program aims to inspire the next generation of Australian scientists



By Kellie Penfold

Sam has a sick plant and his sister Lily is trying to help him find out why. Sam, Lily and the clever scientist Dr Wheaten are the main characters in the book *Plant Pest Investigation with Lily and Sam*, which the CRC for National Plant Biosecurity has developed as the introduction to an educational resource that provides a science program for primary and early secondary classrooms.

It is a simple story, pitched at five to eight-year-olds who follow Sam and Lily on a journey to investigate the cause of their sick plant. Along the way they learn about the world of plant pests, biosecurity and science.

CRC education program leader Dr Kirsty Bayliss, based at Murdoch University in Western Australia, says the strategy – developed to address Australian Government reports on declining science participation and an anticipated future shortage of skilled scientists – is important in ensuring future biosecurity.

“We hope the schools education program creates enthusiasm for science that will stay with students until their adult life. Ultimately, we hope it results in young people taking up a career in plant biosecurity science,” Dr Bayliss says.

When he launched the *Plant Pest Investigation with Lily and Sam* big book in 2008, Australia’s former Chief Scientist, Dr Jim Peacock, applauded the CRC for fostering interest in science and educating children about plant biosecurity.

“Resources such as this book provide the younger generation with awareness of important issues and are also fundamental to capturing enthusiasm for science at an early age,” Dr Peacock said.

While Lily and Sam and a package of activities based on the book are pitched at lower primary students, for upper primary and lower secondary school students the CRC has produced another science unit called *Plant Pest Investigators*. This unit illustrates how important science is to everyday life through learning about plants, invertebrates and diseases, which is backed up with presentations, activities and online

PHOTO: NIMIC PRODUCTIONS



The ABC’s Surfing Scientist, Ruben Meerman explains the importance of plant biosecurity at the launch of *Plant Pest Investigation with Lily and Sam*.

resources to help teachers implement the program in the classroom.

“The units are very hands-on and we have had reports of classrooms being filled with very interesting things, such as breeding and monitoring fruit flies and children collecting plant samples to look at pest signs and symptoms. Teachers are really building on the units we have developed and even though it’s designed to be a package delivered over one term, it’s been carried throughout the year,” Dr Bayliss says.

“Many of the units link back into current CRC research and students are encouraged to visit websites and follow projects.”

The strategy and resources were also introduced to science teachers at the 2009 annual conference for science teachers, CONASTA, where the CRC had an exhibition booth and conducted workshops.

“Many were impressed by the beautiful illustrations in the book and its ability to break down a complex message to create awareness of plant biosecurity in a younger generation,” Dr Bayliss says.

The teachers became students when

Dr Bayliss and former CRC education officer Melanie Hay presented a workshop on ‘Plant Biosecurity in the Classroom’. After an introduction by Dr Bayliss, Ms Hay engaged the attendees in activities designed for their students including classifying invertebrates, building model pests, instigating surveillance projects and looking at ways to engage students.

Teachers who have trialled the program in the classroom provided positive feedback. Terri, a teacher at Tuart Hill Public School, says it integrated perfectly with environmental studies lessons. “Activities were easy to follow and students were enthusiastic about activities. The PowerPoint presentations were particularly useful and we purchased microscopes, which added a new dimension to our research activities,” she says.

Lynda, a teacher at Western Australia’s Baldivis Primary School, found the program comprehensive and easy to use. “Use of the big book and PowerPoint presentations was a great introduction, which helped motivate students for the topic,” she says. ■

Shared expertise protects food security

Mutual benefits are expected to emerge from a Papua New Guinea biosecurity delegation to Australia



By Catherine Norwood

Sharing Australian biosecurity expertise with Papua New Guinea (PNG) is expected to help in the detection and management of harmful plant pests and diseases of concern to both countries, such as the new, highly virulent strains of potato late blight that wiped out crops in PNG in 2003.

Potatoes are an important cash crop for many smallholders in PNG, particularly in the Western Highlands, and biosecurity is essential to protecting local food security, more so than protecting trade. While original strains of potato late blight were responsible for the Great Irish Potato Famine in the mid-1800s, new strains of the blight pathogen (*Phytophthora infestans*) have emerged in the past 20 to 30 years. These are much more aggressive and are a high priority threat for the Australian potato industry.

In PNG, the Australian Centre for International Agricultural Research's (ACIAR) program to help manage the disease highlighted gaps in the general pathology and training of local biosecurity staff. To address this issue, the Crawford Fund



Dr Brendan Rodoni (right) explains the finer points of virus diagnostics to PNG delegates.

sponsored a two-week training program in 2008, supported by the CRC for National Plant Biosecurity, which has resulted in the further exchange of information and development of biosecurity networks between the two countries.

The program, based at the Victorian Department of Primary Industries facilities in Knoxfield, brought together six delegates from a range of PNG government and industry organisations with specialists from Plant Health Australia, the Australian Quarantine and Inspection Service (AQIS) and Victorian Department of Primary

Industries, including potato blight expert Dr Dolf de Boer.

National Biosecurity Extension Coordinator for Plant Health Australia Jo Slattery helped to organise the training program that was designed to improve delegates' understanding of how the Australian biosecurity system works and to gain specific skills in plant pest detection and diagnosis.

"The participants were able to visit Melbourne Airport and docks to see first-hand the front-line activities of Australian Customs and AQIS services," Ms Slattery says. "Each delegate also spent a day with an expert of their choice, relevant to their roles in PNG, such as virus diagnostics, pest insect identification and management, and pathogen-tested propagation and multiplications schemes."

Ms Slattery says the PNG delegates identified a number of improvements that could be made to their own biosecurity systems and handling of pest incursions. They were also keen to see further training and networking between Australian experts and PNG biosecurity managers and policy makers to facilitate this. ■

Masterclass builds biosecurity networks



An international plant biosecurity masterclass held in Kuala Lumpur, Malaysia, in 2008 has led to new opportunities for cooperative projects among members of the Association of Southeast Asian Nations (ASEAN).

The masterclass was an initiative of the Crawford Fund, which partnered with the CRC for National Plant Biosecurity, CABI agricultural publishing service (south-east Asia) and the Department of Agriculture and Agro-based Industry, Malaysia, to host the event.

Participants from 10 countries took part in the two-week event, which was designed to establish networks and share skills among scientists working in biosecurity. It was targeted particularly at those who interact with policy makers and regulators in their home countries and, potentially, abroad.

Research leader for the CRC for National Plant Biosecurity Dr David Eagling was among the presenters at the masterclass. He says the event was an opportunity to build partnerships with neighbouring countries.

"Biosecurity is a global issue," he says. "These events provide the

CRC with the opportunity to assist our ASEAN partners to develop skills and networks that can help protect market security – theirs and ours."

The masterclass included an intensive program of presentations, practical work, discussion groups and field visits, with the presentations and discussion groups the most highly valued, although feedback on all elements of the event was positive.

Presenters were drawn principally from CABI and the CRC, with Australian presenters discussing the 'big picture' issues of global biosecurity and the use of technology in biosecurity, including a demonstration of remote microscopy.

Dr Eagling says the masterclass content has proved highly relevant, with ongoing contact between participants resulting in deployment of some of the CRC technologies discussed during the class such as remote microscopy. "It is very pleasing to see Australian research advances such as remote microscopy being taken up by neighbouring countries," he says.

"These sorts of outcomes highlight the value of training activities, such as the masterclass, and the opportunity they present to make a difference to a country's capacity to undertake plant pest diagnosis and the chance to help reduce the risk of pest incursion to Australia."

– Catherine Norwood

Generational change to protect Australian grain

A determined effort to improve fumigant options is designed to protect Australia's grain trade

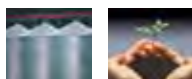


PHOTO: MURDOCH UNIVERSITY

Murdoch University's Professor Giles Hardy (left) and Associate Professor YongLin Ren in the new laboratory established for stored grains research.

By Rebecca Thyer

In a bid to help grain growers and bulk handlers prepare for a future without the same access to cheap fumigants – such as phosphine – stored grains expert Associate Professor YongLin Ren has moved across the country, from Canberra to Perth, taking on the role of principal scientist with the Department of Agriculture and Food, Western Australia's (DAFWA) stored grains research team.

Under a three-year appointment in which Associate Professor Ren has been seconded to Murdoch University through a CRC for National Plant Biosecurity initiative, he will help train the next generation of biosecurity scientists while working on CRC

stored grains projects, such as phosphine alternatives and storage integrity.

For Associate Professor Ren the work is vital for protecting Australia's export grains industry. "If just a small part of the grain kernel is damaged by insects the whole commercial value of the grain is lost, and Australia's reputation is damaged," he says.

With more growers now storing grain on-farm following wheat market deregulation, the need for on-farm stored grain research is essential, he says. However, bulk handlers and exporters also need similar research into fumigation alternatives and improved storage options.

To address these issues, Associate Professor Ren, who has almost 30-years'

research and industrial experience in grain storage, has overseen the CRC project to establish a stored grains research laboratory at Murdoch University.

He says that although phosphine will continue to be an important industry-wide fumigant, alternatives are needed. "Phosphine is popular and cheap, but there are issues with occupational health and safety (OH&S), residues and insect resistance. Increasingly, Australia's international grain markets are also demanding alternatives. "Although international maximum residue limits (MRL) allow for 0.1 milligrams of phosphine per kilogram of grain, some individual buyers, as well as many countries are reducing this," Associate Professor Ren says.

"Spain has reduced the limit to 0.01mg/kg and China and Japan to 0.05mg/kg. It is now very easy to monitor for very low levels of phosphine residue and Australia needs to be wary of this." He says all phosphine users should also be aware of the scheduled Toxicological and Residue Re-Evaluation Review in 2014.

For Australia, these changes could become even more important as phosphine resistance increases. That is because as resistance builds, so too do the chances of increased residues and greater OH&S risks as more phosphine is needed to eradicate harder-to-kill pests.

One alternative the team is researching is nitrogen, an inert gas that comprises 78 per cent of the atmosphere. Using pressure swinging adsorption (PSA) technology, the atmosphere within stored grain facilities can be modified to remove oxygen, leaving nitrogen to effectively fumigate a grain load.

As a fumigant, nitrogen offers many benefits, Associate Professor Ren says. It is safe to use, environmentally friendly, and electricity is the only significant operating cost. It also produces no residues, so the grain can be traded at any time, unlike other fumigants that have withholding periods.

Nitrogen is already in use at bulk-handler and on-farm-storage sites. For example, GrainCorp's Newcastle terminal uses nitrogen to treat export-ready grains. The site has access to cheap nitrogen, a byproduct of air-purification work, so running costs are kept low. WA's Lake Grace Farming Group has recently purchased a

Fumigating with nitrogen

A nitrogen-based, controlled atmosphere technology system for protecting and disinfesting grain has several advantages over other fumigants:

- It constitutes 78 per cent of the air, and air is a rich, free source of nitrogen.
- It is not toxic.
- Its use would greatly reduce occupational health and safety (OH&S) and environmental risks.
- It provides an 'organic' and residue-free grain.
- It has no known resistance problems.
- It does not react with construction materials.
- There is no need for ventilation before grain can be marketed.
- There is no need for product registration.

PSA unit (producing 30 cubic metres of 99.5-per-cent pure nitrogen per hour), a diesel generator and an air compressor to fumigate farmers' silos with nitrogen, with the equipment moved from farm to farm on a trailer.

Although both systems are working well, Associate Professor Ren says further research is needed to find out: how insects respond to low oxygen and high nitrogen concentrations; nitrogen's relationship to phosphine resistance; nitrogen's effect on grain quality; and if improvements can be made to existing PSA technology. A new and cheaper nitrogen generator that can be adapted to on-farm grain and bulk-

handler storage is also being developed, with a focus on maintaining the quality and commercial value of oilseeds, pulses and malting barley.

With research, he says, nitrogen could become a viable phosphine alternative. However, in recognition of phosphine's importance to the grains industry, other CRC projects are concentrating on maintaining the viability of phosphine.

The Capacity Building and Stored Grain project is exploring how to improve phosphine use on-farm. Associate Professor Ren says many farm silos are not well sealed (making phosphine application ineffective); do not have recirculation systems (causing uneven phosphine application); and application – via aluminium phosphide tablets usually inserted at a silo's pinnacle – is an OH&S risk.

The project is working on simple ways to address these issues, such as ground level applications of phosphine and a thermosiphon to enhance the distribution of phosphine through the silo. A similar project that aims to improve grains storage structural integrity also includes bulk handlers.

Underlying all the CRC work that Associate Professor Ren oversees is the training of the next generation of researchers and collaborating with major trade partners, such as the United States, China, India, Korea and countries in Europe, on grains storage, alternative fumigants, phosphine resistance and Khapra beetle treatment. "Any grain treatment technologies developed in Australia need to be accepted by grain-importing countries," he says. ■

Biosecurity surveillance to benefit trade



The grains industry will be able to trace costs associated with transporting grain and the impact of biosecurity threats, such as phosphine resistance, through a new surveillance system created by CRC for National Plant Biosecurity PhD candidate Hoda Abougamos.

Based on a year's worth of data from 2008-09, which tracked the costs of grain travelling from some 14,000 farms through more than 100 receival sites to Kwinana, Western Australia's largest grain export port, the system employs mathematical modelling to plot the most efficient path to market. Operating as a piece of computer software, the model could allow industry to clearly track or predict costs at each stage of the grain's journey, identify points of risk and reduce export losses resulting from potential pest attack.

Phosphine resistance is a major cause of insect infestation in stored grain, which can emerge in the transportation process or at storage sites and can lead to the rejection of entire shiploads of export grain.

Mrs Abougamos says the system will help identify at which point it is most economically advantageous to take action in the face of phosphine resistance. Her research could also save industry the cost of rejected grain by identifying the vulnerable points of the journey, highlighting the need for prevention or treatment.

"The system is like a map so you can trace the grain as it goes through storage nodes and you can see if biosecurity measures should be concentrated at a certain point," she says. "If infestation happens in one node we can determine how much it will cost to control at that point and how much it will cost if it goes to another node."

The CRC's scholarship project, being conducted at the University of Western Australia, is due for completion at the end of 2011. The project is co-supervised by the university and WA bulk grain handler the CBH Group.

Mrs Abougamos says her ultimate aim is to provide CBH and the grains industry with the means to trade most efficiently. "The main aim is to prevent grain from being rejected for export," she says.

– Kellie Penfold

Double gene drives borer's phosphine resistance

Genetic research may provide some answers to the resistance issues threatening grain markets



By Catherine Norwood

The capacity for stored grain insects to evolve their way around the limited number of environmentally safe fumigants available is a constant challenge for the Australian grains industry. Insect infestations also have the potential to jeopardise annual grain exports worth in excess of \$7 billion.

Dr Pat Collins, based at AgriScience Queensland, a division of the Department of Employment, Economic Development and Innovation (DEEDI), is leader of the CRC for National Plant Biosecurity's Post-Harvest Integrity Research Program. He says growing resistance to the fumigant phosphine puts Australian grain traders in a precarious position.

"The Australian industry has a zero tolerance for live insects in its export grain shipments, which has given us an advantage in highly competitive international grain markets," he says. "But rising levels of insect resistance to phosphine have the potential to jeopardise these markets."

The CRC has a number of projects focusing on phosphine resistance, including identifying how resistance develops, developing new fumigation protocols and tests for resistance, improved fumigation practices and alternative fumigation products.

The resistance issue stems from the worldwide popularity of phosphine. Phosphine is cheap and effective, easy to apply and is environmentally benign. It can be used for multiple commodities and is effective against a wide range of insects. There is also no readily available, comparable alternative.

In Australia, more than 80 per cent of all stored grain is treated with phosphine, which means it is the chemical most in contact with insects, creating the greatest chance for insects to develop resistance. This begins when some insects start to survive treatment, often because of inadequate procedures by users.

Dr Collins says phosphine resistance is a greater issue for Australia because grain is harvested and stored during the hottest part of the year, when insects are most active. Other grain-growing countries such as the

United States and Canada do not have this problem to the same extent because their grain goes into storage in cooler weather.

Managing phosphine resistance has also been made more complicated by the deregulation of the grains industry in Australia. There are new players entering the grain marketing and export business and there has been a substantial rise in on-farm grain storage. It means more people need to be informed about the issue and trained in fumigation procedures.

Resistance model

One of the greatest threats to emerge is the development of resistance to phosphine in the lesser grain borer (*Rhyzopertha dominica*), one of the most common pests of stored grain. Low levels of resistance were first found in Australia in the early 1990s, and in 1997 highly resistant borers were discovered in storages in Queensland.

A senior lecturer at the School of Mathematical Sciences at Queensland University of Technology, Dr Glenn Fulford, has been modelling the development of resistance in populations of lesser grain borers as part of a CRC project.

Molecular analysis has found that two genes on separate chromosomes control phosphine resistance in lesser grain borers, each of which produces a relatively weak resistance.

Most creatures have two copies of each gene, one inherited from their mother and the other from their father. Hybrids that carry a single copy of a resistance gene and a single copy of the susceptible gene are known as heterozygotes and they are generally susceptible or very weakly resistant. But if an insect carries both resistance genes, they are called homozygous. When the resistance genes are homozygous they produce a higher level of resistance. However, strong resistance is only found when an insect carries both copies of both resistance genes.

When resistance genes first appear in a population they are rare. Resistant individuals carry only one copy, but may have enough resistance to survive low doses of phosphine. These heterozygotes become relatively more common and breed with

others, producing homozygous-resistant insects. Further fumigations continue to purify the insect population, producing almost entirely resistant strains.

Dr Fulford says where insects have one gene they will be 2.5 to 30 times more resistant to phosphine than insects with no resistance genes, depending on which of the two chromosomes the gene occurs.

However, insects homozygous for both resistance genes are at least 250 times, and possibly upwards of 600 times, more resistant than insects with no resistance genes. The double-gene nature of resistance in borers has delayed its development and given the industry some breathing space to develop counter-measures. But it also significantly increases the level of resistance where it occurs.

In eastern Australia almost every strain of lesser grain borer tested has at least one of the resistance genes. Insects that inherit both genes from both parents are still relatively rare, occurring in only about five per cent of insect samples.

Dr Fulford says CRC PhD candidate Jason Thorne is building on the current resistance modelling for the lesser grain borer by adding life cycle factors to genetic influences.

"We already know that lesser grain borers are less susceptible to phosphine during the egg and pupal stages and this greatly affects the success of fumigations. Other research the CRC is doing will help identify exactly how much more resistant they are during this stage, so that we can refine the modelling and help develop more effective fumigation strategies."

Varying levels of phosphine resistance have been identified in eight other insect species collected from farms and central grain storages around Australia, with the highest levels of resistance emerging in the flat grain beetle (*Cryptolestes ferrugineus*) in recent years.

Other species identified with varying levels of resistance include two additional *Cryptolestes* species, rice weevils (*Sitophilus oryzae*), granary weevil (*Sitophilus granarius*), rust-red flour beetle (*Tribolium castaneum*), saw-toothed grain beetle (*Oryzaephilus surinamensis*) and the psocid *Liposcelis bostrychophila*.

Current techniques to test for

resistance are either relatively blunt or time-consuming. They rely on identifying survivors at prescribed fumigation doses and breeding further generations of insects from survivors to confirm initial diagnoses, which can take six weeks or more.

Genetic markers

Dr David Schlipalius says this technique does not effectively identify strains with low numbers of resistant insects. Based at DEEDI AgriScience Queensland, Dr Schlipalius is working to identify changes in the genes of the lesser grain borer and in the rust-red flour beetle.

"We will then be able to use markers

that will allow us to quickly identify resistant insects and the level of resistance," he says.

An earlier CRC project had hoped to use the proteins encoded by genes as a shortcut for identifying resistance. But there was no distinctive difference between the proteins from resistant and non-resistant insects, which sent researchers back to the DNA itself to identify changes in the sequence of genes.

Research partners in the US were able to provide a full sequence for the rust-red flour beetle genome, which has helped identify a number of candidate resistance genes, with one in particular a prime candidate for a likely molecular marker for resistance.

Dr Schlipalius says this has indicated where researchers should look within the genetic sequencing of the lesser grain borer to find the corresponding gene.

He says developing a genetic marker will allow researchers to identify more subtle differences in the level of resistance in insect populations and to track how it develops in populations over time – before and after fumigation and even over several years.

"This will help us to identify the types of storages or practices that tend to select for resistance, so that we can change those practices and prevent a build-up of resistance in the field. At the moment we can only track resistance when fumigation fails." ■

PHOTOS: CHRIS FREEBAIRN, DEEDI



Phosphine resistance is most widespread in lesser grain borer populations (left), but the highest level of resistance has emerged in the flat grain beetle (right).

Population dynamics added to resistance equation



When a farmer augers grain into a silo, little thought is given to the mathematical modelling behind efforts to control pests likely to eat this stored bounty. But equations could prove to be a vital link in work to halt the spread of pesticide resistance in Australia's stored grain insect pests.

Dr Mingren Shi is a computational mathematician who is working on his PhD by undertaking research for the CRC for National Plant Biosecurity on the issue of stored grain pests. Dr Shi is creating individual-based mathematical models of the evolution of resistance to pesticides in the lesser grain borer (*Rhyzopertha dominica*).

As a result, he says, industry will have greater ability to predict the growth of populations, the evolution of resistance and the spread of individuals and resistance genes under different management scenarios.

This, in turn, will help identify effective long-term strategies for monitoring and managing resistance.

"A wide range of management tools is particularly critical when the pest management strategy is reliant on a single pesticide (phosphine), as is the case for the lesser grain borer," Dr Shi says.

By incorporating processes such as organism life cycle and biology, the effects of environment, genetics, effects of management, population and spatial spread within and between storage facilities, mathematical models can predict population dynamics, the evolution of resistance and the spread of individual borers and resistance genes under different management scenarios. Two models (a one-resistance-gene and a two-resistance-gene model) have been simulated and tested. Based on the two models, Dr Shi has begun to develop a spatially explicit 3D model to address the question 'How is the evolution of resistance affected by the factors regarding spatial distribution within silos?'

– Kellie Penfold

Borers take cover in native vegetation

Researchers discover stored grain insects thriving far from their preferred food source



By Catherine Norwood

The lesser grain borer, a prime pest of stored grain, is just as likely to be found in native vegetation and farm paddocks as it is in grain silos, a new study into the insect's behaviour has found.

The unexpected finding poses real challenges for efforts to control the insect, says Queensland's Dr Greg Daglish, who is leader of a project researching the ecology of the lesser grain borer and rust-red flour beetle.

Dr Daglish, principal entomologist with Queensland's Department of Employment, Economic Development and Innovation (DEEDI), says the ecological research is the first to establish in any detail where these grain pests are located outside of grain silos.

The lesser grain borer (*Rhyzopertha dominica*) and rust-red flour beetle (*Tribolium castaneum*) were selected for the study because bulk grain handlers identified these species as the most problematic in terms of developing resistance to fumigants.

"With increasing concern about resistance in recent years, we realised we needed to understand the ecology of these insects if we are to manage them better," Dr Daglish says.

His project is part of the CRC for National Plant Biosecurity's Post-Harvest Integrity Research Program for the grains industry, which focuses on insect resistance to phosphine.

Initial trapping of lesser grain borers and rust-red flour beetles began in September 2008 and concluded in December 2009. Traps were set in southern Queensland between Condamine and Moonie. In southern New South Wales, Dr Mark Stevens, principal research scientist at Yanco Agricultural Institute, has collaborated on the project, setting traps in the Leeton district.

Traps were placed adjacent to farm silos, in cereal paddocks at least one kilometre from grain silos, or in native vegetation. There were two additional sites at regional grain depots.

Pheromone traps were set for both insects at all sites. The traps were put out for one week every four to six weeks, and the insects caught were sorted and frozen

for further analysis. More than 40,000 lesser grain borers and 5,000 rust-red flour beetles were trapped during the study, with numbers in individual traps varying greatly. The greatest number of lesser grain borers caught in a single trap was 1,419 in NSW, and Queensland recorded the highest number of rust-red flour beetles in a single trap (387).

Dr Daglish says trapping shows that these beetles can be found across the rural landscape. In particular, the lesser grain borer's distribution is more widespread than he initially believed. "The borers have been consistently caught in traps that are significant distances from grain storages and, in some cases, in greater numbers compared to traps adjacent to silos."

On one occasion, 887 lesser grain borers were caught in a single trap in a Queensland national park, at least five kilometres from

the nearest farm. Rust-red flour beetles were concentrated around silos, but were still caught in traps away from farm storages.

Dr Daglish says it is not yet clear whether the lesser grain borer is surviving on unknown host plants or whether they are travelling long distances to the vegetation sites. "Findings that demonstrate this species is reproducing and developing on native host plants would pose a whole new set of challenges."

A more likely explanation is that adult beetles are flying great distances from infested grain and being caught in the traps.

Further analysis of the insects preserved from traps may help to answer the question of travel. Population genetics will identify how closely related insects found around silos are to those found in paddocks and further afield. A close genetic relationship in all populations could indicate insects are travelling much longer distances than previously thought; a more distant relationship could indicate that separate populations are surviving on alternative host plants. Results available so far show a high degree of relatedness between rust-red flour beetles trapped in the Queensland study area.

There have been differences in the trapping results between the two states, with NSW traps catching more insects when they are around, but for shorter periods. In the two months following a heatwave in southern NSW in January 2009, when temperatures reached 40°C or higher for more than a week, the number of insects of both species trapped declined dramatically. In Queensland, warmer-than-normal temperatures in late winter coincided with a major increase in insects trapped, compared with previous months.

These findings suggest weather conditions may have a significant bearing on insect populations and further research will investigate these links.

Other aspects of the project include identifying the levels of phosphine resistance in different insect populations, although early results suggest little difference between silo- and paddock-collected lesser grain borers. ■

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PHOTO: I&I NSW

Dr Andrew Ridley (left) and Dr Greg Daglish, from Queensland's DEEDI at Indooroopilly, setting up a pheromone trap adjacent to silos on a farm near Moonie, Queensland.

Five-hour test guides grain fumigation protocols

Rapid identification of phosphine resistance is helping grain handlers to fumigate more effectively



By Catherine Norwood

There is nothing subtle about the rapid test developed to determine whether flat grain beetles (*Cryptolestes ferrugineus*) found in stored grain are resistant to the fumigant phosphine.

It's a simple 'knock-down' assay that exposes insects to a high dose of phosphine and produces results within five hours of insects being delivered to laboratories for testing.

The development of phosphine resistance in stored grain insects is a high priority for the grains industry because of the potential for insects to damage international trade. The test, developed through a CRC for National Plant Biosecurity project, is helping grain handlers to better manage phosphine resistance by quickly confirming resistance and allowing them to alter their fumigation and grain management practices in response.

Project leader Dr Manoj Nayak, from AgriScience Queensland, a division of the Department of Economic Development and Innovation (DEEDI), says that so far the knock-down assay has only been developed for flat grain beetles. However, the testing method will be evaluated in the near future for other key pest species including the rice weevil (*Sitophilus oryzae*) and rust-red flour beetle (*Tribolium castaneum*).

The flat grain beetle test applies phosphine at a rate of two milligrams per litre or 1,440 parts per million (ppm). This kills non-resistant insects in only 30 minutes; insects with moderate resistance survive 30 minutes, but fail to survive 24 hours.

"Any insects that survive five hours are suspected of 'strong resistance' and grain storage managers are advised immediately so that they can take remedial actions," Dr Nayak says. "We observe these insects over a 48 to 72 hour period at the same dose and if they survive it confirms the 'strong resistance' diagnosis and we inform the storage manager."

In the past three years, 74 samples of flat grain beetle with strong resistance have been identified through this test, and have survived a more comprehensive test of seven days at 720 ppm, validating the resistance findings.

PHOTO: DEEDI



(From left) Dr Manoj Nayak, Lawrence Smith and Linda Bond organise a field experiment on strongly phosphine-resistant pests in a bunker storage of sorghum at Mallu, Queensland.

Grain Protection Manager for GrainCorp Robin Reid says the knock-down test usually gives them results within 24 hours, from the time live insects are found in storages, to the notification of results from the testing laboratories.

"It has been an important tool for us in managing phosphine resistance and identifying whether we need to change to another product altogether for a particular batch of grain. There is no use re-applying a treatment that is not going to work, especially when it ties up the grain for four weeks during treatment with potentially no benefit," he says.

GrainCorp checks all storages monthly for signs of insects or other problems. Mr Reid says if insects are found after fumigation, or if they appear earlier than expected – within three months of a fumigation – samples are immediately sent to government laboratories for testing.

"In the past, if insects survived fumigation, operators might think it was because a silo or bunker had not been sealed properly, or perhaps there was a wet patch or a hot spot in the grain that the fumigant had not been able to penetrate.

"Knowing there are resistant insects makes a difference to our response and to the treatments we use. It is an issue we need to keep in front of, to protect the reputation of the Australian grains industry and the value of our product."

In conjunction with the rapid resistance testing, Dr Nayak has been developing new fumigation protocols and an eradication

plan that can be used once resistance is confirmed. Protocols to control all life stages of the strongly resistant lesser grain borer (*Rhyzopertha dominica*) require phosphine at a rate of 720 ppm for five days. For flat grain beetles, new protocols require phosphine at 720 ppm over 24 days, or 360 ppm over 30 days, to effectively kill all insects.

GrainCorp and Dr Nayak have worked together to develop an eradication plan for the flat grain beetle where strongly resistant populations have been identified. It includes the use of registered contact grain protectants and other fumigants at ports, use of registered contact grain protectants at country storages, an intensive hygiene program at infested storage sites and continuous monitoring of insect populations for resistance.

Dr Nayak says 82 different strains of the original flat grain beetle species *C. ferrugineus* have been diagnosed with strong resistance. Insects have been collected from 71 central grain storages and two farm storages during the past three years.

Of the three different *Cryptolestes* species found in Australian grain storages, only *C. ferrugineus* strains have shown strong resistance to phosphine. The other two *Cryptolestes* species have shown only low-level resistance to date.

"At this stage we suggest the industry should focus on managing strongly resistant *C. ferrugineus* as the current registered rates of phosphine will control the other two species," Dr Nayak says. ■

Fumigant trial to push phosphine longevity

Identifying gas distribution patterns in storages will improve application effectiveness



PHOTO: VITERRA

A wheat storage bunker is fitted with real-time monitoring equipment to identify how phosphine is distributed through the grain.

By Rebecca Thyer

In a 12,000-tonne, steel-sided, tarpaulin-covered wheat storage bunker at Roseworthy in South Australia, perhaps the country's biggest ever fumigant monitoring trial is taking place.

The 120-metre-long, eight-metre high and 30-metre wide bulkhead has 120 monitoring lines running across its floor and snaking down from the top. Combined with five monitoring boxes, each with 20 monitoring points, the trial is collecting, in real time, information on the Australian grains industry's dominant fumigant – phosphine – and its distribution through a grain stack.

The trial is part of CRC for National Plant Biosecurity research being led by Viterra's grain hygiene manager Greg Hopkins, with other bulk grain handling companies GrainCorp and the CBH Group also collaborating on the project.

Mr Hopkins says the project's main aim is to experiment with different storage facilities and phosphine types to determine gas movement. "Essentially, we want to know how it moves across large storage facilities, what its efficacy is in these conditions and what impacts weather conditions have," he says.

"With limited monitoring, but industry experience, I've seen wind movement outside impact phosphine gas inside storage bunkers. So the hope is that by doing in-field work we will be able to validate mathematical models being developed by others involved in the project."

Phosphine is used by bulk storage operators and grain growers to fumigate grains to meet domestic and international demand for insect-free supplies. Used for decades, it is still the Australian industry's number-one choice for the job because of its effectiveness and environmental suitability. However, with its efficacy being compromised by an increasing number of phosphine-resistant stored grain pests, the industry needs to ensure its long-term use through a better understanding of how it works.

"Phosphine is very important to the industry and this project will give us a good understanding of how it moves around storage facilities, the amount needed to fumigate effectively, and when it should be applied," Mr Hopkins says.

Using actual data, the project team aims to build and validate a model that would allow industry to better predict phosphine movements and efficacy through grain bulks, minimising areas of lower

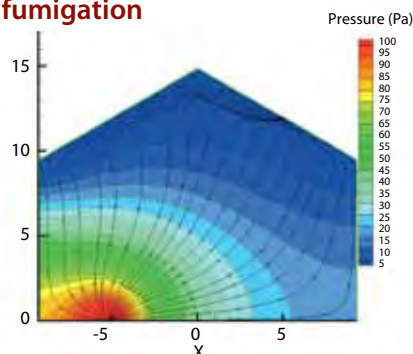
concentrations and whether top-ups may be needed given other parameters such as temperature or wind speed and direction.

The first trial at Roseworthy began in February 2010 using a solid form of phosphine – aluminium phosphide tablets – used by the majority of the industry. Other trials will collect data on other phosphine technologies, called Eco2fume and Vapor3phos, in future scheduled trials at CBH and GrainCorp sites over the next 12 months.

Mr Hopkins says it is too early to make any assumptions from the Roseworthy trial. "But phosphine doesn't seem to move as effectively as I thought it would longitudinally down the bunker," he says.

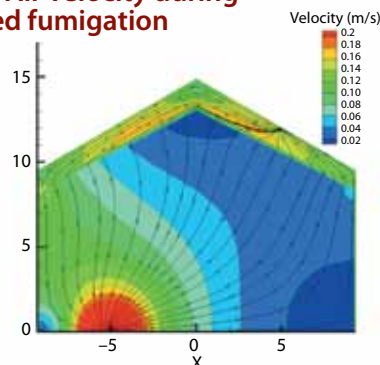
Although he leaves the science and modelling to the researchers, Mr Hopkins says he is happy to manage the project and work with researchers from CSIRO, the University of Western Australia, Queensland University of Technology and other industry partners. "The great benefit of being part of a CRC project is this whole-of-industry collaboration. If we didn't work together we wouldn't be able to do this sort of work on our own. Yet ensuring that we can continue to use phosphine effectively into the future is so important to Viterra and the whole Australian grains industry."

Figure 1 Air pressure during fan-forced fumigation



A 2-D cross-section view of air being 'fan-forced' through wheat held in a 1,500-tonne silo, from an off-centre aeration trench. Static pressure (Pa) contours of the flowfield are indicated in different colours with the arrows indicating the airflow paths.

Figure 2 Air velocity during fan-forced fumigation



Colours indicating air velocity in metres per second (m/s). The airflow shadow (dark blue) in the lower right hand corner indicates that it takes a long time for fumigant to reach this part of the grain stack.

Win-win from fan-forced fumigation

CSIRO senior engineering scientist James Darby has been leading a team of researchers as part of a CRC for National Plant Biosecurity project to model fan-forced fumigation, and to develop and field-test a mobile fumigation unit. The University of Western Australia is an important member of this team, providing computational fluid dynamics computing expertise, led by Dr Ming Zhao.

Mr Darby says mathematical modelling has been used to identify the three-dimensional flow of gaseous grain fumigants in fan-assisted applications (figures 1 and 2). The model takes into account the size of the grain stack, absorption of the fumigant by grain, dispersal and flow fields through the storage, insect death and the size of any

reticulation system provided.

The modelling produced 'believable results' and from there the project joined with New South Wales silo manufacturers Modern Engineering and Construction (MEC) to design a new mobile application system that could be attached as required to silos.

The new design includes a 'fail-safe' gas-reticulation feature, and it was field-tested during the 2009-10 grain harvest on a 1,500-tonne farm storage near Corowa in southern NSW. In addition to accelerating the fumigant through the grain stack, the fan-forced system allows the fumigant to be purged more quickly and can be used to cool the grain, reducing the risk of further insect infestation. Insect growth is suppressed once temperatures fall below 20°C.

Mr Darby says the development of the

mobile fumigation unit, which can be used with cheaper forms of metal phosphide preparations for faster, more effective results in killing stored grain insects, is a major outcome of the project. It provides a tangible benefit of the research that will be available to industry commercially. A key part of this is the capacity to reliably disinfest grain, even if resistant species are present.

In addition to the farm storage trials, a further trial is proposed in conjunction with one of the bulk-handling companies to target squat silos with an 8,000-tonne capacity.

Mr Darby says while the use of fans to drive fumigants through grain stacks may improve the effectiveness of applications, an essential requirement remains the provision of gas-tight storages. ■

Designs for better fumigation



Although there have been some advances in sealing techniques for storage facilities in recent years, the development of more easily sealed, alternative structural designs has been limited.

Well-sealed storages are essential in the effective use of phosphine, says Associate Professor YongLin Ren, from the Department of Agriculture and Food, Western Australia, and Murdoch University. He leads a project reviewing the structural integrity of grain storages for the CRC for National Plant Biosecurity.

Well-sealed storages allow efficient concentrations of gas to be maintained in the silo so that the gas has enough time to penetrate through the bulk grain and kill target pests, reducing the likelihood of insect resistance to phosphine developing.

The CRC project will review storage design and develop alternative sealing and fumigant application technologies and techniques for bulk handlers and growers.

"We often look at either on-farm storage or bulk storage, but this project will address both, from silo bags to bunkers, with bulk handlers and manufacturers involved, and is focusing on grain storage issues nation-wide," Associate Professor Ren says.

Associate Professor Ren says the failure of existing technology to maintain sealing standards has led to increases in maintenance costs and capital expenditure and to the deterioration of storages, which then fail to effectively manage biosecurity risks associated with stored grain pests. This creates a vicious cycle: "It then makes maintenance of existing storage networks cost prohibitive and results in the inevitable, further deterioration of the sealed storage network," he says.

Any new technologies and techniques developed through the project will need to cater for phosphine and any alternative fumigants likely to come on to the market in the future.

With his team, Associate Professor Ren is working to improve bunkers' gas holding or seal so that more effective phosphine fumigations are possible, helping to reduce resistant pest problems.

The project also aims to:

- develop design specifications for low-cost structures for bulk handlers and growers that incorporate improved engineering for fumigant distribution and gas tightness, with estimates of structural costs
- develop flexible storage technologies to minimise potential biosecurity threats from multi-commodity systems, and
- better manage potential threats to human and animal health in the supply chain.

– Rebecca Thyer

Phosphine still a killer in cooled grain

Grain cooled through aeration in on-farm storage can be effectively fumigated with phosphine



By Rebecca Thyer

Warm winter days can provide highly fertile conditions for insect pests, especially in southern Queensland and northern New South Wales where toasty temperatures provide an ideal breeding ground for stored grain pests.

With more growers storing grain on-farm to take advantage of a deregulated grain market, stored grain insects have become a greater problem in recent years. Increased on-farm storage is contributing to, and suffering from, growing insect resistance to the industry's main fumigant, phosphine.

Most grain in Australia is harvested in the warmer months in conditions that can lead to heat-damaged grain and mould; conditions that are favourable to insect pests. To combat this many growers aerate stored grain, to reduce grain temperatures to 20°C or less.

Although lower temperatures maintain grain quality and reduce insect population growth, industry researchers have questioned whether phosphine is as effective at actually killing insects in grain stored at a cooler temperature.

Now, Queensland Department of Employment, Economic Development and Innovation entomologist Dr Greg Daglish and research development agronomist Philip Burrill have determined that phosphine can still be effective at lower temperatures, within certain parameters.

Over a number of years and using both laboratory trials and trials with on-farm silos, they found that phosphine is generally less effective at lower temperatures; but in the right conditions, that is, in a properly sealed, gas-tight silo and with adequate exposure periods, phosphine fumigation of cool grain to control resistant insect populations is possible.

"The benefits of cooling and phosphine fumigation are that cooling preserves grain quality and reduces insect population growth, and phosphine kills insects and has a residue-free status in all major markets," Dr Daglish says. "The work has proved that cool grain fumigation is an option for growers and meets market needs."

However, changing temperatures do affect some phosphine-resistant strains of

key pests. Dr Daglish says that although the most resistant Australian strains of two pests are known to respond similarly to phosphine, the project showed that one species became much harder to control in cool grain.

It is an important finding for an industry reliant on phosphine to treat stored grain for pests, and where these pests are becoming more resistant to it.

Mr Burrill says the majority of tools growers had for dealing with grain pests 10 to 15 years ago are falling over. "The only thing we can say to growers is that having a well-constructed, aerated and sealable silo gives you the option to fumigate with

Sorption is the major cause of phosphine loss in well-sealed silos. However, in cooled wheat or sorghum, the sorption rate is lower at lower temperatures, meaning that higher concentrations will be achieved for longer.

Another discovery, that older grain tends to be less sorptive, means that delaying fumigation could also help keep phosphine concentrations higher for longer.

Although results for silo trials varied, three general observations were made about phosphine concentrations: lower concentrations tended to be measured deeper in the grain mass; lower

PHOTO: BRAD COLLIS



'Cool grains': DEEDI's Philip Burrill is part of the research demonstrating that phosphine can be effective at lower temperatures.

phosphine, which when done correctly is still effective against all our pests."

The 'cool grains' work, which started as a Grains Research and Development Corporation project, has also highlighted some vital information about phosphine and its interaction with stored sorghum.

For example, an early finding relates to sorghum's sorption rate (how quickly it absorbs phosphine), which influences fumigation performance. Generally sorghum is more sorptive than wheat.

"It means that when it comes to phosphine fumigation in sorghum, there is less margin for error than for, say, stored wheat, so fumigations need to be spot on," Dr Daglish says.

concentrations tended to be measured on silos' northern sides; and concentrations measured higher in the grain mass tended to peak earlier.

Dr Daglish says aluminium phosphide tablets must not be mixed with the grain itself, so tablets are often placed on trays in the silo headspace. When phosphine gas is liberated from one point it takes time to diffuse throughout the whole grain bulk, so gas concentrations are not always distributed uniformly in all parts of the storage.

"It is essential that sufficient time is allowed to ensure maximum gas distribution, to achieve the best results from phosphine fumigation," he says. ■

New grain sampling strategies to deliver savings

Looking in the right place at the right time will better target pest infestations in grain



By Kenn Pearce

Australia's multi-billion-dollar grains industry is poised for superior ways of combating incursions of harmful plant pests and diseases, once a study into a 'better way' of detecting insects and fungi in bulk storages bears fruit.

The presence of these pests affects grain quality, but could potentially have devastating economic consequences for one of Australia's most valuable exports. Early detection of insects and fungi is crucial in demonstrating that grain consignments are pest-free.

Leading the CRC for National Plant Biosecurity project on stored grains sampling strategies, Queensland University of Technology's (QUT) Dr Grant Hamilton says that from the viewpoint of an insect or fungus, "grain is a wonderful place to hang out and breed".

His three-year project follows CRC research led by Dr Sharyn Taylor at Plant Health Australia, which documented the types and effectiveness of surveillance strategies used across the grains industry.

Dr Taylor identified the need to implement specific surveillance strategies for different priority pests, rather than trying to detect everything with the same sampling technique.

"Being able to detect different grain pests obviously depends on a range of factors that include the timing of surveillance, the pest's detectability in the plant host or commodity, the resources available for surveillance and the sensitivity of sampling," Dr Taylor says.

Some strategies rely on increasing general awareness of the symptoms of something like a fungal infection, for instance, so that anyone along the production and supply chain looking at grain is also keeping an eye out for the infection, which could occur anywhere within storage grain.

When it comes to surveillance for stored grain insects, surveillance and sampling strategies need to consider preferred locations within storages where insect populations cluster.

"Finding the most effective sampling

PHOTO: CHRIS NEWMAN



Targeted grain sampling strategies will be more effective in identifying pests.

strategies for different pests is essential, particularly in export grain, where there is a nil tolerance for live insects. A few live insects leaving Australia can turn into millions by the time the shipment arrives at its destination, and result in the shipment being rejected," she says.

Dr Taylor's work was the precursor to the sampling strategies project Dr Hamilton is working on with Dr David Elmoultie, a CRC research fellow at QUT who joined the project in May 2009.

Dr Hamilton says contemporary grain-handling methods are based on an incorrect notion that insects are evenly spread throughout a grain bulk. His project incorporates "realism and thinking" about what insects do and is developing sampling strategies accounting for that.

"If you're not sure about the pest load within the grain, taking just a few kilos of grain per truck really is not good enough; depending on conditions, you can be under-sampling," Dr Hamilton says.

"We're getting to the stage of showing that traditional grain-sampling systems perhaps leave a little to be desired and are developing a sampling method that can substantially improve on the old method of doing things.

"In some cases [grain handlers] may have to sample more intensively. In the end it's

going to be better: they will find insects that are there that they couldn't find previously."

Dr Hamilton says it is no longer acceptable to "throw some chemicals" at insects discovered in grain. Industry must find smarter solutions. "If you don't know what's going on in the bulk grain you don't have an idea about treatments you should be making," he says.

The on-ground work – talking to bulk grain handlers, government agricultural agencies and farmers – has been an important step in better understanding the pest and surveillance problems.

The South Australian Research and Development Institute's Dr Andreas Kiermeier has collaborated on the project, incorporating statistical modelling into software that has been developed to inform and guide sampling staff. He found it challenging to think about grain being moved through the supply chain and distilling the process down to commonalities.

"The model allows grain handlers to try out sampling strategies at various points in the supply chain and estimates how successful they are at detecting different levels of harmful plant pests and diseases," Dr Kiermeier says.

Dr Hamilton says the sampling intensity information was still to be added to the software before distribution of the program was finalised with the CRC.

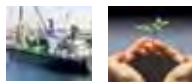
"Sampling is really a core issue in this industry. To make the changes we're talking about, while not huge, as in construction of new buildings, they will fundamentally change the way that industry works."

It is possible sampling strategies could change from region to region. "We go from places like Emerald down to South Australia, across to Western Australia ... there is not only the complexity of environment – heat and humidity – but different supply chains. We're confident our strategy can deal with these complexities and give really good on-ground outcomes to grain handlers."

Benefits flowing from his team's program will favour the end-user, particularly larger bulk handlers, but will also be applicable to farmers. "In certain circumstances what we're suggesting is actually going to save them time and money," Dr Hamilton says. ■

Russian aphid ancestry offers clues to its control

Understanding Russian wheat aphid's genetic diversity will help Australia develop response strategies



By Catherine Norwood

Invasive swarms of Russian wheat aphid have caused major crop losses and devastated cereal-growing regions around the world during the past 30 years, but at home in western China, among other places, the aphid is of relatively little concern to growers and Australian researchers are trying to identify why.

One research project is investigating the level of genetic diversity in the Russian wheat aphid species, and whether genetic changes have occurred that distinguish non-destructive biotypes from their more voracious and invasive familiars.

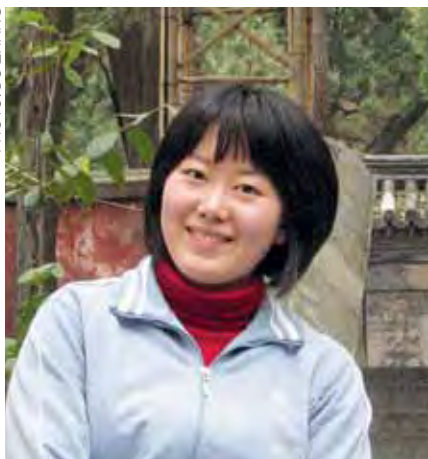
The CRC for National Plant Biosecurity has a number of research projects underway, led by CSIRO entomologist Dr Owain Edwards, to better understand the aphid and to protect the Australian grains industry from potential incursions, as Australia is the only major wheat-growing region in the world still free of this pest.

Working on the genetic diversity project for the CRC is PhD candidate Bo Zhang,

who is based at the Chinese Academy of Sciences, in China.

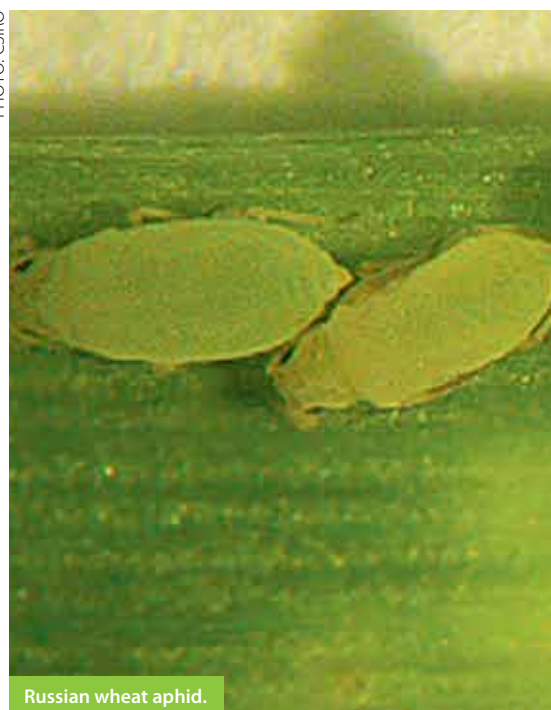
Dr Edwards says Ms Zhang's project will address questions about whether the aphids have evolved distinct, more damaging and invasive strains, or indicate whether perhaps there is something in the nature of cultivated wheats and barleys that encourages this behaviour in the insect.

PHOTO: BO ZHANG



PhD candidate Bo Zhang is investigating the genetic diversity of Russian wheat aphid.

PHOTO: CSIRO



Russian wheat aphid.

Ms Zhang has already collected genetic evidence confirming that, although Russian wheat aphid was not formally reported as a pest in crops in western China until the 1970s, it is indeed native to the Xinjiang region, which is adjacent to regions of the former USSR that have always been considered part of the aphid's native range.

Following an international workshop on Russian wheat aphid research held in Singapore in April 2010, scientists attending the conference have agreed to provide Ms Zhang with samples of aphids collected in their countries for comparison, giving her access to the genetics of more than a dozen international collections.

International direction on Russian wheat aphid research



An international workshop held in Singapore in April 2010 was the first time scientists from all six continents affected by Russian wheat aphid had gathered together to coordinate an international research effort against the devastating pest.

Evaluating current global research efforts and developing cooperative control strategies were key discussion points for the 27 scientists from 11 countries.

The workshop was sponsored by the CRC for National Plant Biosecurity and the Grains Research and Development Corporation, while Murdoch University and CSIRO organised the scientific program.

It identified several areas for future research to help safeguard crops in Australia and around the world. This includes the need to better understand the ecology and genetics of the Russian wheat aphid in its native range, where it is generally not considered to be a pest.

Murdoch University's Associate Professor Mehmet Cakir and CSIRO entomologist Dr Owain Edwards have been researching cereal strains resistant to Russian wheat aphid in collaboration with researchers from the United States, France, Turkey, Syria, Iran, South Africa, Kenya, Ethiopia and Argentina.

Presentations to the workshop indicated that once Russian wheat aphid populations have established in a new country, they appeared to occur in larger numbers, with more damaging consequences near mountains. The workshop identified the need for further research into the role of topography and climate to help develop control strategies in affected regions.

Scientists also agreed on the importance of integrated pest management (IPM) in controlling the pest and will focus on identifying and promoting the effects of natural control agents, such as predators and parasites. Feedback from participants indicated that the workshop had provided an important opportunity to review the progress of research and identify gains research findings into IPM strategies.

– Catherine Norwood



"We know when and where the insects have spread across the world, but we don't know if each new population has been established from the same genetic base, or if there are different strains that have colonised different areas, and which may perhaps respond differently to Australian conditions, should there ever be an incursion here," Dr Edwards says.

It is research Ms Zhang could not do in Australia: so destructive is the pest that none of the aphids can be brought into the country, even for research purposes.

Instead, Australian scientists are relying on their international partners to help them study the insect and prepare for possible incursion, and are using the pea aphid as a surrogate for local research.

Dr Edwards is working with an international consortium that is sequencing the genes of aphids in a bid to eventually breed wheat varieties that have long-term resistance to the pest.

He says since the 1970s the aphid has spread rapidly through the major wheat-growing regions of the world, from eastern Asia, the Middle East and the Balkan states.

"It causes major crop destruction for the first five to seven years after it appears in a new region, until the ecosystem establishes a balance, with occasional outbreaks causing ongoing problems.

"It is still a major pest in the United States and South Africa. These countries spent 10 years developing and rolling out resistant wheat varieties, but all of the original resistant wheats relied on a single resistance gene. Within six or seven years the

aphids evolved to overcome that resistance," Dr Edwards says.

His research involves genomic sequencing of the salivary glands of the aphids, in an attempt to map how they evolve to secrete new forms of proteins – proteins that wheat and barley no longer recognise as precursors to attack. Dr Edwards says the changes in aphid saliva indicates a rate of evolution 50 to 100 times faster than that of other insects.

When aphids attack a resistant plant, the plant detects particular proteins in the aphid saliva and cuts off the flow of sap to that part of the plant. Finding no sap, the aphids leave the plant and fly on to neighbouring plants.

Tasting is the primary mechanism aphids have for identifying their food. If after several attempts to feed they are unsuccessful, they

can launch themselves into the air currents and can travel hundreds of kilometres in search of a new food source.

In non-resistant plants, the wheat does not recognise the salivary protein and allows Russian wheat aphids to feed without triggering any defence mechanisms. This results in white streaks on the wheat leaves – symptoms similar to drought stress. The leaves also curl, trapping the heads of the plants and making it impossible to harvest the grain.

"Contact insecticides are relatively ineffective because the aphids are inside the curled leaves, where the chemicals can't reach them," Dr Edwards says. "It only takes relatively small numbers of these aphids to cause significant damage."

None of the wheat or barley varieties currently released in Australia have Russian wheat aphid resistance, and rolling out resistant varieties from the US or South Africa would be relatively ineffective if aphids arriving in Australia are those that have already evolved to overcome plant resistance.

"Identifying the mechanisms aphids use to generate new proteins and developing resistance to those proteins at a more fundamental level is the strategy we are pursuing. Once we understand the underlying mechanism, the appropriate resistance can be developed for a range of different aphid species and crops," Dr Edwards says.

The research is being conducted in collaboration with colleagues at Kansas State University in the US and the Chinese Academy of Sciences. ■

PHOTO: CSIRO



Dr Owain Edwards is leading research to prepare Australia for the possibility of a Russian wheat aphid incursion.

Biosensors develop a nose for grain pests

Mimicking an insect's sense of smell, computers are helping to find insects in stored grain



By Kenn Pearce

Detecting insects inside large grain storages has been compared to finding the proverbial needle in a haystack. Now, a groundbreaking CRC for National Plant Biosecurity collaboration between CSIRO's Food Futures Flagship and the South Australian Research and Development Institute may make finding those 'needles' easier.

The collaborators are working to develop biological recognition elements using insect pheromone receptors. Results from that research should help develop a biosensor that can tell grain storage owners when and where grain insects are resident.

This is a spin-off application of a CSIRO project to develop a 'Cybernose[®]', with applications ranging from measurement of wine aroma and monitoring for food safety to detecting explosives. There are strong synergies between the CRC collaboration and CSIRO's broader research in this area.

CSIRO's Dr Alisha Anderson says detecting infestations in large grain storages is problematic because insects often congregate in 'hot spots' and it is impossible to visually sample all the grain to detect insects.

The grains industry repeatedly applies phosphine in a costly bid to combat insect pests. However, this accelerates the evolution of resistant insects and there are concerns regarding toxicity and unwanted environmental consequences.

"With a biosensor you can essentially suck air off the grain and sample the whole silo at once," Dr Anderson says. "If it's an aerated silo, you might have a biosensor incorporated in the aeration system, so you're continually sampling and checking for insects."

She says the 'end game' would be to develop a portable version of the Cybernose[®] device that would be used to probe grain and indicate the presence of pests in silos.

The CRC research team is developing knowledge of olfactory (scent organ) receptors of grain pests, in particular rust-red flour beetle (*Tribolium castaneum*).

"We have copied the genes from the insect and expressed them in our system,"

she says. "They're exactly the same sensing elements the insect uses but we hook them up to a computer rather than an insect's brain to read the signals."

Research of this type would have been impossible without the 2008 publication of the sequenced genome for the rust-red flour beetle by the Tribolium Genome Sequencing Consortium. "It's certainly right at the edge of the scientific capability at the moment," Dr Anderson says.

This project only began in September 2009, but research outcomes are focused on finding receptors in the beetle that respond to known volatiles.

"One of the postdoctoral students working on this project, Dr Bradley Stevenson, is using an olfactometer to test what the insects are attracted to," she says. "Air carrying different chemicals is blown down two arms of a Y-shaped tube. Insects are introduced at the bottom of the Y and make their way up the tube. When they make a choice at the junction it tells us a lot about the volatiles they're attracted to."

"We're also using electrophysiology, measuring the change in electrical signal in the insect's antennae when it's exposed to different odours. This tells us if an insect has the ability to detect an odour. If so, we can be sure it has receptors for that odour."

It is well known that insects have a very sensitive system for detecting sex

pheromones. Finding mates is important, so they have receptors dedicated to that purpose. If researchers use those same receptors to detect the sex pheromones it will tell if the pest is present.

Dr Anderson sees several practical ways in which their research might be useful to industry. "As well as using a biosensor to continually sample air coming out of a silo, a second option would be to sample air from the end of the auger or spear used to collect grain for sampling," she says. "When the biosensor detects that there might be insects, you could take grain samples in that area, rather than doing it blind."

The project will also build scientific capacity with two postdoctoral fellows working on the project. Both will gain considerable experience in working on grains industry problems by the end of the three-year project.

"There's also a lot of off-the-shelf technology, such as electronic noses," she says. "Finding insects in grain is like trying to find a needle in a haystack. The problem is electronic noses are not yet selective enough to find that needle. But they can compare haystacks."

Current research to identify and isolate the biological receptors will end in 2012. "The first Cybernose[®] will be targeted at explosives. It will probably be a minimum of six or seven years before we'll have a working prototype in the grains industry." ■

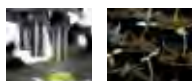
PHOTO: PAUL JONES



Biosensors are being developed to help with the difficult task of finding insects in grain storages.

Rapid test helps protect against Karnal bunt threat

An Australian test could become the new international standard for identifying Karnal bunt in wheat



By Nicole Baxter

Scientists have developed a rapid diagnostic test to help better protect the nation's grain growers from a devastating exotic pest incursion and preserve market access for Australian wheat.

In an international collaboration, researchers have developed a new test procedure to detect one of the top biosecurity threats to Australian grain: the exotic pest *Tilletia indica*, a fungus that causes Karnal bunt in wheat.

Australia is currently free of Karnal bunt, but if spores enter Australia the pathogen could spread quickly through the movement of infected seed, farm equipment and, over short distances, by the wind. Spores can remain viable for several years, germinating when weather conditions are favourable, infesting wheat flowers and reproducing on the kernel embryos.

Research leader Dr Mui-Keng Tan, of Industry & Investment New South Wales, says diseased grain smells like rotting fish and products made from severely infected grain are unpalatable. She points to a Monash University study that estimates a Karnal bunt incursion could cost Australia up to \$1 billion annually through decreased grain quality and the loss of export markets.

The existing test involves classifying spores based on their structure, followed by labour-intensive spore germination and a molecular test to confirm their identity. The disadvantages of this procedure are:

- germinating the spores takes at least two weeks
- not all spores germinate, which adds another 14 days to the test, and
- the similar species *T. walker* is difficult to distinguish under the microscope when there are fewer than 10 spores.

Dr Tan says the existing test is too slow to address the concerns of Australia's grain trading partners. "Also, in a suspected incursion, a large-scale screening program for Australian wheat could be costly."

By contrast, the new test prescribes how to access genetic material from a single spore for direct, sensitive and definitive molecular identification of the fungal pathogen in just two to three days.

PHOTO: PADIL



Australia's need for a rapid, accurate and internationally accepted test became apparent in early 2004 when Pakistani authorities claimed to have found Karnal bunt in an Australian wheat shipment. In response, several overseas markets stopped buying Australian wheat until the shipment was confirmed Karnal-bunt-free. "During that time, more than 50 ships carrying Australian wheat – worth more than \$400 million – were en route to markets," Dr Tan says.

To respond more efficiently to trading partner concerns, the CRC for National Plant Biosecurity project supported Dr Tan in 2006 to develop a new test that could distinguish *T. indica* from *T. walker*.

The first challenge was to successfully extract genetic material from autoclaved spores, something never before attempted. After Dr Tan looked at the killed spores under the microscope, most had broken walls and did not contain the genetic material she needed. "But we were fortunate to have some spores with intact walls," she says. "These were not viable, but gave us the DNA we needed."

A second challenge was to develop a robust test procedure that would withstand the rigours of international scrutiny. Geography also added to the project's difficulty – Dr Tan and collaborator Ms Dominie Wright, of the Department of Agriculture and Food, Western Australia, were located on opposite sides of the continent.

Nonetheless, the pair used every opportunity to discuss and plan for a national and international analysis to assess the repeatability and sensitivity of the protocol in the hands of molecular biologists from laboratories in Western Australia, Victoria, New South Wales, South Australia, Italy, China and the United Kingdom, which plan to use the test for quarantine diagnosis.

When participating collaborators sent back their tests, Dr Tan was glad to see positive results from those who had been trained to perform the diagnosis.

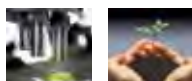
A seed technologist with Science and Advice for Scottish Agriculture, Marian McNeil, was a member of the international testing group, and says the Australian test method to distinguish between the different *Tilletia* species shows promise.

The chair of Australia's Sub-Committee of Plant Health Diagnostic Standards (SPHDS), Jane Moran, says the test will be most useful in surveillance programs to demonstrate Australia's freedom from Karnal bunt, but she hopes Australia will be fortunate enough never to require the use of the protocol during an incursion.

The new test protocol is currently with SPHDS awaiting national endorsement. With further refinement, it is hoped the new test will become the internationally endorsed test procedure for detecting Karnal bunt in wheat. ■

DNA tool breaks this hitchhiker's disguise

A new testing protocol has given researchers confidence that Australia can keep out Khapra beetle



By Kellie Penfold

Khapra beetle (*Trogoderma granarium*) is one of the five highest-priority threats to the Australian grains industry. Internationally, it is considered one of the most damaging pests of stored products and is of quarantine significance. Economic analysis has estimated that the establishment of Khapra beetle in Australia could cost the grains industry at least \$500 million annually in trade restrictions.

Identifying a Khapra beetle in a sample of grain can be difficult, requiring extensive training and experience. This is because the beetle will often be a little worse for wear when discovered. The grain acts like sandpaper and sloughs off the beetle's hairy cover and most of the identifying features. This assumes that the beetle makes it at all – Khapra beetle larvae usually eat their dead adults for dinner.

It can also be difficult to identify the Khapra beetle from other, less harmful *Trogoderma* species and related Dermestid insects. There are more than 100 described and possibly 50 undescribed species in the *Trogoderma* group, which includes the Khapra beetle. There are 52 described and relatively harmless *Trogoderma* species endemic to Australia.

For the past three years, senior entomologists at the Department of Agriculture and Food, Western Australia, (DAFWA) have been working on the CRC for National Plant Biosecurity *Trogoderma* diagnostic project. There are three elements to the project: developing protocols for DNA identification of different *Trogoderma* species, establishing a national *Trogoderma* laboratory and creating an international *Trogoderma* reference collection.

The overall aim of the project is to protect Australia's valuable grain export market by identifying unwanted *Trogoderma* visitors as quickly as possible to facilitate eradication and to distinguish them from similar native, but non-destructive, pests found in grain leaving our shores.

Working on the *Trogoderma* diagnostics project, DAFWA's Mike Grimm initiated the development of a new set of protocols for molecular laboratory testing in 2007. These

PHOTO: AMANDA SMITH



Mark Castalanelli's CRC scholarship has paved the way to a career in Khapra beetle research.

protocols are establishing real-time PCR (DNA) procedures that will allow species confirmation in a matter of hours.

Mr Grimm says Khapra beetles are a major threat because they are able to survive in many products and situations and will eat anything they can get their teeth into – grains, spices, herbs, dried fruit, meat, wool and many other products coming from overseas – so they can easily 'hitch a ride'.

"Their capability to stay inactive in larval stage for years means even empty containers may have a healthy population of

Khapra beetles capable of infesting anything loaded in it," he says.

While there has only been one Khapra beetle incident in Australia (found by owners in furniture imported in a shipping container and successfully eradicated), Mr Grimm says the risk is great.

"What we are talking about is protecting the hard work by Australian farmers in producing a valuable commodity for this country," he says. "Misidentification of exotic pest species poses just as much of a threat to our industry through the imposition of

trade barriers as having Khapra beetle in this country.”

Mark Castalanelli has been working on the new DNA protocols for the CRC as part of his PhD project, through Curtin University’s Western Australia Biomedical Research Institute.

The extraction technique he has developed in Australia allows DNA to be taken without physical damage to the specimen. In the past, it had to be crushed and ground to extract DNA. By keeping the specimen intact, visual morphological identification can take place.

“We can’t have DNA identification without morphological identification,” Mr Castalanelli says. “One backs up the other and are equally important when deciding if an intercepted beetle sample is Khapra beetle or not.

“Once we are able to positively identify the Khapra beetle we need to test as many *Trogoderma*, *Trogoderma*-related and other stored grain pest species as possible to exclude the false positive results,” he says.

There is still a lot of work to be done since the protocol needs to be verified by independent laboratories; it is already being evaluated by the United States Department of Agriculture. Following its successful acceptance, the protocol may be included in the international Khapra beetle identification protocol.

In conjunction with this work, the CRC has established a national *Trogoderma* laboratory and international reference collection, which Mr Grimm says is the first facility of its kind for this pest in the world. “Other countries have offered their support in bringing it together,” he says.

Trogoderma laboratory project manager

Dr Oonagh Byrne, a molecular entomologist with DAFWA, says the new diagnostics laboratory will be world leading through its use of a suite of tools, including DNA screening with non-destructive DNA extraction methods and imaging using photomontage and the web-based Pests and Diseases Image Library (PaDIL), and Lucid keys (a computer-based identification system).

“The diagnostic equipment purchased to date – the real-time PCR machine and the automated liquid handling system – are widely used in other DNA diagnostics, which is beneficial for other species and many people know how to use them,” she says.

But the equipment is the easy part. Also required are the new testing protocols, accrediting the laboratory and building the collection of species characteristics – through DNA profiling, the morphological validation and development of Lucid keys and an image library of species from all over the world. It is a multi-faceted approach that will take time to complete.

As part of this project, diagnostic entomologist and *Trogoderma* specialist Andy Szto is building the international reference collection of *Trogoderma* specimens by visiting collections around the world. Having travelled in Europe and the United States on his mission, this year he will visit France and Russia to view significant collections and he hopes to visit Asia and the Indian subcontinent next year.

After examining thousands of specimens, Mr Szto has morphologically identified more than 100 *Trogoderma* species, which will back up the DNA profiles.

Locally, the first national *Trogoderma* trapping program is complete with pests

trapped at 64 grain storage sites around Australia. The samples will be the basis of taxonomic studies and the development of DNA markers of native and non-native storage pest Dermestids, including a closely related but less serious pest, the Warehouse beetle. The trapping program will also establish the biogeographic diversity of the different species. A second trapping program will begin later in 2010.

Dr Byrne says many of the specimens held in collections are old. “While useful for morphological validation and development of Lucid keys, they are often unstable for DNA analysis – meaning DNA profiling is more likely from freshly sourced specimens from the trapping program and from sources such as the Australian Quarantine and Inspection Service.” ■

PHOTO: PADIL



Khapra beetle has reached Australian shores but been quickly eradicated. A new testing protocol aims to ensure our doors will never be open to this unwanted visitor.

Online access to grain-handling training



Deregulation of the grains industry means more people are involved in storing and handling grain, so Charles Sturt University (CSU) has revised its grain-handling course to meet the need for more training.

The CRC for National Plant Biosecurity project is reviewing the CSU course, which has been streamlined and formatted for online delivery. It also meets National Training Quality Framework requirements and participants will receive a nationally recognised Certificate of Attainment.

Dr Paul Weston and Associate Professor John Kent at CSU have jointly been responsible for the revisions. Associate Professor Kent says the new course expands on the previous course the university offered and now includes biosecurity issues and how these influence international trade and quarantine regulations. There are also units on

identifying stored grain insects, what to do when an infestation occurs and the correct procedures for fumigating silos.

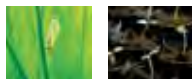
Associate Professor Kent says previous participants were generally silo operators and mid-level managers at bulk grain companies, but he expects a broader range of people to take advantage of the revised course. He says flexible, online delivery will make the course more accessible, although there will still be a three-day workshop at the CSU Wagga Wagga campus for hands-on insect identification and silo fumigation practice.

Associate Professor Kent says participants should expect to spend a few hours a week for two months completing the online work, with a combination of workplace and online assessments in addition to the workshop. For more information about the course contact Dr Paul Weston (02 6933 2420, pweston@csu.edu.au).

– Catherine Norwood

Software evolves for real-time surveillance

Paper trails make way for the digital age with PDAs, GPS software and smart phone data collection



By Catherine Norwood

It is no longer enough to check surveillance traps for the presence or absence of insects as proof of area-freedom from a particular pest or disease. Western Australian researcher Rob Emery says international trading partners increasingly require more detailed information from biosecurity programs: not just 'absence' data, but information about the data itself.

"That's why we've come up with personal digital assistants (PDAs) as a way of capturing this additional information in a way that is much more efficient than filling out forms," says Mr Emery, who led stage one of the PDA-assisted surveillance project for the CRC for National Plant Biosecurity. Associate Professor Giles Hardy from Murdoch University is leading stage two of the project.

Software has already been developed for a number of different surveillance activities, with the initial challenge being to develop a program to help with the basic trap checking.

The PDAs can automatically collect detailed GPS date and time information about traps, while operators enter data about the contents of the traps. Photographs can be attached to the data for each location to provide visual clues, including photos of samples found at those locations. Samples can be collected from traps and the PDA used to generate barcoded labels, printed on a hand-held printer in the field, to provide a detailed, sealed chain of evidence.

Mr Emery says when operators return from the field, the information can be downloaded into a central database and all information for individual sites collated in one place. This can then be updated or instructions added for operators and reloaded on to the PDAs.

The latest incarnations of the program and the smart phone technology actually allows operators to send their surveillance data wirelessly via the internet to a central server, providing near-real-time monitoring.

Programming has most recently been expanded to allow the grains industry to improve phosphine treatments of stored

PHOTO: SIMON EYRES



Nicolas Garel (left) and Rob Emery with PDAs that bulk grain handler the CBH Group is trialling to improve protection of the nation's vast grains storage system, generate cost savings and enable greater market access.

Samples can be collected from traps and the PDA used to generate barcoded labels, printed on a hand-held printer in the field, to provide a detailed, sealed chain of evidence.

grain through more extensive monitoring and onsite evaluation of fumigation effectiveness.

"A map of phosphine levels across grains bulk storage could be created that shows potential inconsistencies and leaks, which the grain handler can then act upon before they become a problem, generating considerable cost savings," Mr Emery says. Western Australian bulk grain handler the CBH Group is currently field-testing the PDAs and related software and will provide feedback to help refine the programming.

"This is a living project and the system is constantly being reviewed and improved and different applications are being considered as they become available," Mr Emery says. "This technology is very exciting and could potentially be used in a

range of different industry applications."

The PDAs have already been successfully trialled in urban surveillance programs in New South Wales. Forest Science Centre entomologist Dr Deborah Kent is a convert to the technology and has been using a PDA with Mr Emery's software for almost two years, initially for a survey of exotic Sycamore lace bug (*Corythucha ciliata*) in London plane trees in and around Sydney.

Dr Kent says hers is a 'dumb' unit – it is only a PDA, rather than one of the latest smart phones being used with the software. However, it does have TomTom GPS software that automatically records the exact latitude and longitude of the trees she surveys while she enters surveillance information. "You do have to stand still for a few minutes so that you get an accurate location reading, but that is generally only as long as it takes for me to enter my survey information," she says.

Dr Kent verifies the location information using Google Maps and with photos she takes of the trees she visits. "Given that we're talking about one tree in many and six decimal places in recording locations, a transcription error in handwritten notes could be the difference between a site in Canberra or Goulburn, not just the wrong tree," she says.

"This system eliminates those kind of errors and I estimate it saves me about 20 per cent of the time it would otherwise take to record everything on paper and then transcribe it, and would save even more with repeat visits to the same site. When I get back to the office with the PDA, all the site information is already there."

Dr Kent says information can be downloaded into geographic information system (GIS) software that allows her to visually track the presence of the lace bugs and identify possible host sites nearby, and to share the information easily with anyone who has access to Google Maps.

She says in the case of an emergency outbreak, when large numbers of new or casual staff are employed, having survey or trapping sites already recorded using the PDA system would make it possible for people who had never been to those sites to accurately locate them. With GPS programming, the PDAs can even provide directions to get from one site to another.

Sensor technology monitors fumigation from a distance

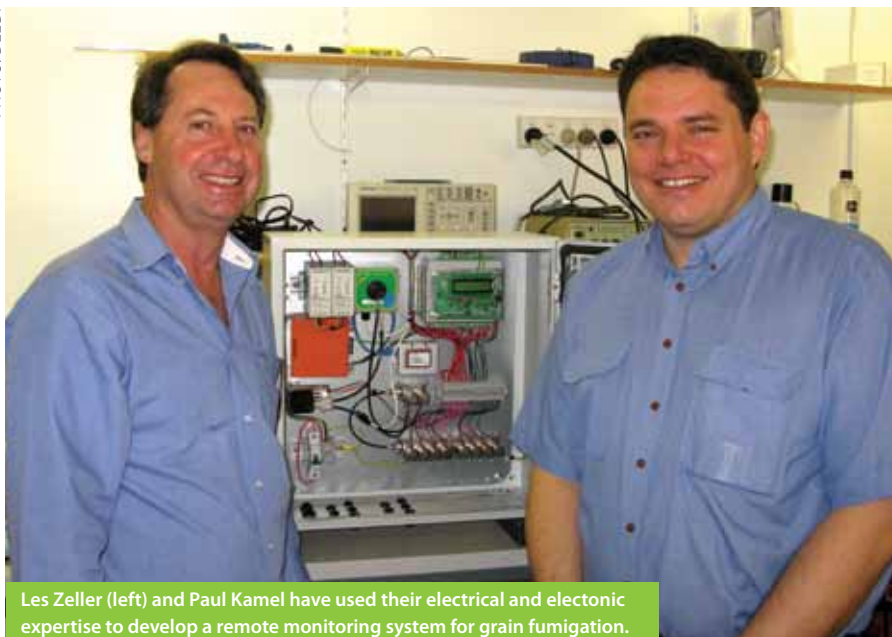
When electrical engineer Les Zeller was called on to help with the CRC's smart phone project, he was initially asked about linking PDA or smart phone devices to sensors that would provide real-time data on stored grain fumigations. The original concept was data transfer from sensors set up in silos to devices via Bluetooth wireless technology.

But Mr Zeller, who works with the Queensland Department of Employment, Economic Development and Innovation, says the problem is that you need to be on-site to do that. He thought a system that allowed operators to check fumigation concentrations via the internet – without needing to leave the office – would be better, and he has come up with a system to do just that.

He has developed the sensor technology to fit into fumigation systems, linking it with a commercially available internet monitoring system that is also used by graziers to monitor water levels at stock watering points. Trials to test the system have been under way at a series of five connected silos at Tammin in Western Australia.

During winter and spring the system has been tested with clean air flowing through the fumigation system. Grain will begin arriving in December 2010, when testing with phosphine can begin. Mr Zeller and his new team member Paul Kamel have

PHOTO: DEEDI



Les Zeller (left) and Paul Kamel have used their electrical and electronic expertise to develop a remote monitoring system for grain fumigation.

developed a system that can be set to check the fumigant concentration through the fumigation system at programmable time intervals from 10 minutes to once a day or once week and to clean the sensor between readings.

Mr Zeller says it is possible for the system to monitor the fumigant concentration levels of up to 12 sample points and also to measure and record temperature and humidity. The Tammin site uses a recirculation system for fumigation of stored grain and a sensor has been installed to monitor the fumigant as it is

being recirculated. Other trials are being established for siroflow grain storages at Macalister in Queensland and Roseworthy in South Australia and a grain storage shed in northern New South Wales.

He says if the trials are successful, the system will be commercialised for more widespread use. Initially designed for phosphine fumigants, Mr Zeller says it can be easily adapted for other gases. It is also possible to access data from hand-held devices such as PDAs, via Bluetooth, as originally envisioned, as well as via the internet. ■

Postgraduate programs meet need for biosecurity expertise



As the volume of trade and travel escalates, increasing the risk of exotic plant diseases and pests arriving in Australia, more professions require an understanding of biosecurity issues.

Agriculture, natural resource management, transport and tourism are all industries at the front-line.

To help meet this growing need, the CRC for National Plant Biosecurity has supported the development of three new postgraduate courses launched in 2010.

A Graduate Certificate, Graduate Diploma and Masters in Plant Biosecurity are initially being offered through Murdoch University in Western Australia, and will soon be available through La Trobe University in Victoria and Queensland University of Technology (QUT).

Units are offered by flexible, online delivery and are taught jointly by a consortium of universities, including Murdoch, La Trobe and QUT, as well as Charles Darwin University and the University of Adelaide.

The program has been developed in consultation with Canada, the United States, New Zealand and the Australian biosecurity profession

to ensure it is recognised both nationally and internationally.

The postgraduate programs are designed to develop participants' ability to analyse, evaluate and synthesise information from scientific and regulatory sources and to apply effective solutions to problems in situations that may be changing rapidly.

The Graduate Certificate is the equivalent of six months of full-time study and includes units on plant pest invertebrates, pathogens and weeds, as well as detection and diagnostic strategies and an overview of the field of plant biosecurity.

The Graduate Diploma articulates from the Graduate Certificate and is a further six months of full-time study, with additional units on the ecological and biological principles of invasions, risk assessment, community engagement and biosecurity policy.

The Masters program is 18 or 24 months of full-time study and, additional to the Graduate Diploma units, includes a six or 12-month research project.

Courses can also be studied part-time and will also be available to international students living overseas. For more information on the postgraduate biosecurity courses see the 'Postgraduate study in plant biosecurity' website (www.plantbiosecurity.edu.au).

– Catherine Norwood

National effort to hold back fruit fly assault

Scientists and industry join forces to counter-attack orchard invaders



Fruit fly are one of the most damaging plant pests in Australia.

PHOTO: I&I NSW

to identify because they are hard to distinguish from each other and from some endemic, 'non-regulated' fruit fly species.

Associate Professor Clarke and his colleague, CRC postdoctoral fellow Dr Mark Schutze, have attracted international attention for their work in this area. Dr Schutze has spent time in Vienna conducting cross-mating and development trials on four different fruit fly species at the entomology section of the United Nations Food and Agriculture Organization International Atomic Energy Agency Seibersdorf laboratories.

Live-insect trials on these species are linked with genetic work being done in New Zealand by Dr Karen Armstrong, of Lincoln University, and Dr Deborah Hailstones at Industry & Investment New South Wales' (I&I NSW) Elizabeth Macarthur Agricultural Institute. This research is being done in conjunction with morphological and morphometric data Dr Schutze has compiled in Brisbane.

"Once together, this project will provide us with our best chance at resolving identification boundaries for the target species," Dr Schutze says.

Other researchers involved in CRC fruit fly research projects include Dr Katina Lindhout and Dr Olivia Reynolds from I&I NSW and Dr Francis de Lima and Shirani Poogada from the Department of Agriculture and Food, Western Australia (DAFWA).

Horticultural Australia Ltd (HAL) is a significant supporter of fruit fly research, as the pest is a major quarantine concern for domestic and international markets. It is contributing to Dr Lindhout's research on developing female lures for improved market access through the citrus, summerfruit, table grape, cherry and apple and pear levy and matched funds from the Australian Government. Contributions to Dr de Lima's research on trapping to better predict and prove fruit fly presence are funded through the citrus, summerfruit and apple and pear levy and matched Australian Government funds.

HAL expects the outcomes of these projects – the development of female fruit fly lures and internationally accepted fruit fly trapping protocols – will strengthen Australia's ability to prove fruit flies are



Dr Mark Schutze has studied the behaviour of different fruit flies at the IAEA Seibersdorf laboratories in Vienna as part of research to improve identification of species.

PHOTO: QUT

By Barry Pestana

Australia's leading scientists are attacking the dreaded fruit fly from all fronts – and, by all accounts, are winning important strategic battles.

But the war is far from over, with Australia's capacity to trade in domestic and international horticulture markets – valued at \$4.8 billion – still challenged by this pest. Fruit flies cost the Australian economy an estimated \$125 million a year.

The CRC for National Plant Biosecurity is investing in fruit fly research to tackle the issue on several fronts.

Australia is up against two key species: Mediterranean fruit fly (*Ceratitis capitata*), found in the southern growing regions of Western Australia, and Queensland fruit fly (*Bactrocera tyroni*) found, naturally, in Queensland, parts of New South Wales, the

Northern Territory and eastern Victoria.

Fruit flies are not impeded by geographic borders and have the potential to infect a range of crops – from mangoes and apples to grapes and tomatoes. Unlike many other plant pests, fruit fly infestation cannot be contained to a single property. Whole regions can be susceptible, affecting not only farmers but also the wider regional communities.

Associate Professor Anthony Clarke, of Queensland University of Technology, is involved in two CRC fruit fly research projects. One of these projects includes the development of new diagnostic methods through a comprehensive study of biological, morphological and molecular issues of the *Bactrocera dorsalis* group of tropical fruit flies. These flies are among the most destructive and invasive horticultural pests and are also among the most difficult

known not to exist, as well as increase the likelihood of gaining new international markets for Australian horticultural produce.

Dr Lindhout and her team at I&I NSW are developing dry lures to attract female Queensland fruit fly as an alternative to the current liquid lures for female flies, which cause trapped flies to decompose quickly into a sticky mess, making identification difficult. As a result, most monitoring programs use dry lures, which attract only male flies. "This has been a problem since it is the female flies that do the most damage," Dr Lindhout says.

Her team is developing both dry lures and semi-solid lures that can attract the female flies, used in combination with a toxin that kills the flies when they enter the trap. In the spring of 2009, testing moved from the laboratory to the field in different climates. DAFWA researchers are also testing the new lures on the Mediterranean fruit fly.

Monitoring fruit flies for regulatory purposes is expensive. Surveillance involves thousands of traps, which are placed at 400-metre intervals in urban areas and one kilometre apart in horticultural areas.

These traps need to be monitored weekly or fortnightly, which makes it a highly intensive and expensive system to maintain.

Dr Francis de Lima is leading a national team to develop more cost-effective ways of trapping to verify area freedom while maintaining or improving trapping efficiency. A dynamic trapping method being developed to target both Queensland and Mediterranean fruit fly strategically moves traps to preferred host plants throughout the year, and has been validated during three years of trials at more than 200 sites in New South Wales, Victoria, South Australia and Western Australia.

Dr de Lima says the new method of trap placement could reduce the costs of trap deployment and reduce eradication costs through the earlier detection and eradication of outbreaks. Growers would also benefit by maintaining market access for longer, and more quickly contained outbreaks would have a lower environmental impact.

He says the research validating the effectiveness of the strategic trap placement will give regulators the confidence to

approve lower-cost systems for areas of low pest prevalence, and to provide evidence of area freedom. This would allow much-needed funds to be directed towards more effective control and eradication programs.

Working with Dr de Lima, DAFWA researcher Shirani Poogada says strategically deployed traps (dynamic traps) in WA have performed better in the capture of Mediterranean fruit fly than traps which remain stationary in a single host using traditional trapping grids. She says the system will allow them to maximise their chances of capturing incursions of flies into the monitored area.

Dr Olivia Reynolds has collaborated on the trapping research in NSW and says selectively placed cure-lure baited Lynfield traps attractive to the Queensland fruit fly appear to be performing better in areas of very low pest prevalence, with mixed results when wild fruit fly populations are high.

"Ultimately, we would like to use fewer traps, while still maintaining the same level of detection that the current trapping grid used in the Fruit Fly Exclusion Zone holds," she says. ■

Control clues sought in fruit fly 'lifestyle'



Two CRC for National Plant Biosecurity studies being conducted at Queensland University of Technology aim to gather as much information as possible about the role of host plants and other resources in the lifestyle of the Queensland fruit fly – and then use that information to control the pest.

Postdoctoral fellow Dr Solomon Balagawi and PhD candidate Sakuntala Muthuthantri are investigating how different host plants and sources of food and shelter influence the species' distribution and numbers. Understanding the distribution and dispersion patterns is expected to help researchers track the origins of the fruit fly infestations, to more effectively target source populations.

Both studies are designed to identify the spatial pattern of fruit flies at various scales, including single host plant level, crop orchard/ farm level and at a larger scale of between districts. Their work is being done in collaboration with the Market Access Focus Group of the Queensland Government's Department of Employment, Economic Development and Innovation (DEEDI).

Ms Muthuthantri says knowing how populations of fruit flies are created, and the role of host plants, can improve the pest management advice to growers. Commercial and domestic plant hosts are being monitored in her research, particularly citrus fruit. She aims to develop predictive fruit fly population models that can be used to support specific area-wide management systems as pre-harvest market access.

Dr Balagawi's project is focusing on the spatial distribution of the Queensland fruit fly and the most effective placement of protein baits

and cure-lure – a male fly attractant used in conjunction with male annihilation technology (MAT).

"This research will significantly improve our knowledge on where to apply these two management tools at both the plant and farm/ orchard level. These will then lead to efficient management of fruit fly in the field," he says.

Studies on the foraging pattern of the Queensland fruit fly for protein on a single plant have used guava and nectarine host plants in the field. These looked specifically at the height of the foliage where flies forage and compared foraging patterns between a fruiting and a non-fruiting host plant.

Dr Balagawi says the results from this suggest higher numbers of Queensland fruit flies forage for protein at heights greater than one metre and that more flies forage for protein on a fruiting host tree than a non-fruiting host plant.

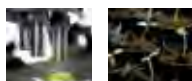
Results from field studies to determine foraging patterns at the farm or orchard level, using strawberry farms and an apple orchard, suggest that:

- Queensland flies tend to be more abundant on the edges of strawberry farms earlier in the season and gradually move into the interior of the farm, thus implying management options intensify on the farm edges.
- Abundance of flies is largely correlated with temperature – that is, as temperatures increase, the fly population in strawberry farms increases.
- The population of Queensland fruit flies was evenly distributed through the apple orchard, implying management efforts need to be evenly distributed throughout the orchard.

– Barry Pestana

Digital diagnostics expand global surveillance

Real-time connections via remote microscopes are improving access to plant pest experts



By Catherine Norwood

Australia's digital pest-detection network is expanding globally, to help identify and combat invasive pests threatening our agricultural industries and markets before they arrive, through the CRC for National Plant Biosecurity's remote microscope network.

Within Australia there are already more than 30 camera-connected microscopes linked to a public, internet-based image library – part of the new digital arsenal being deployed to meet Australia's biosecurity challenges. Connections include remote districts such as Kununurra in Western Australia, which would otherwise have limited access to the expertise required to identify new plant pests and diseases.

Late last year in central Queensland, cotton crops were ravaged by a species of mealy bug thought to be new to Australia.

Almost immediately, remote microscopy was used to send real-time images of the pest via the internet to an expert in California, who was able to identify it as an exotic suspect.

Dr Gary Kong is the project leader for the CRC's digital diagnostic project and principal plant pathologist with the Queensland Department of Employment, Economic Development and Innovation.

"Whenever a suspected incursion occurs, it is vital that a positive identification is made as quickly as possible," Dr Kong says. "Remote microscopy has demonstrated its ability to reach experts wherever they might be and to speed up the identification process. Early detection and confident identification mean that immediate steps can be taken to minimise the risk or impact of incursion."

Internationally, the Australian Department of Agriculture, Fisheries and Forestry is supporting efforts to expand

the remote microscope network, with a particular focus on fruit fly diagnostics as part of the National Fruit Fly Strategy.

Attending a planning meeting of the Association of Southeast Asian Nations (ASEAN) Regional Diagnostic Network in Laos in 2009, Dr Kong was able to provide a real-time demonstration of the network to participants.

With the help of the internet and a microscope connected to a computer in Canberra, scientists at the Laos meeting, 8,000 kilometres away, were able to identify an Oriental fruit fly specimen taken from an Australian research collection. Similar to the Queensland fruit fly, the Oriental fruit fly is a high-risk pest endemic to Asia that has so far been kept from Australian shores – aside from those few specimens, long since dead, kept for scientific reference.

Five ASEAN countries – Laos, Malaysia, Singapore, Thailand and Vietnam – in

Thailand training targets citrus canker, fruit fly and potato viruses



An international training collaboration between Thailand and Australia is helping to improve biosecurity surveillance and diagnostic skills, underpinning more confident trading between the two nations.

Citrus canker is one of several high profile pests and diseases of concern to authorities in both countries. An outbreak in Australia in 2004 took five years to eradicate at a cost of more than \$30 million, with 500,000 citrus trees destroyed.

Citrus canker is known to be present in Thailand, along with a number of exotic fruit flies that Australia does not want to see established here. Thailand is also seeking evidence that the seed potatoes it imports from Australia are free of disease, specifically potato viruses.

As part of Australia's pre-border biosecurity strategy, the CRC for National Plant Biosecurity is training Thailand Department of Agriculture scientists in traditional and molecular diagnostics. This will help to improve identification of these high-profile plant pests and strengthen the Thai diagnostic network.

Project leader for the CRC is Dr Gary Kong, who says diagnostic capability complements border protection, incursion management and surveillance activities. The feedback between these activities is crucial to creating an effective biosecurity system. Implicit in the ability of a country to respond to a potential incursion is a capability to detect and identify exotic pests and pathogens.

The Australian Centre for International Agricultural Research is

co-funding the project, which was initiated in 2008 when four Thai scientists travelled to Australia to spend three months with CRC project teams, learning diagnostic techniques for a range of pests.

Two Thai trainees have also studied taxonomy in Queensland as part of the program and in 2009 Dr Kong led a team of Australian scientists

who spent a week in Bangkok to conduct further training.

They also helped Thai scientists to set up diagnostic laboratories at Bangkok and Chang Rai, including installation of remote microscope equipment, which links them to the microscope network Dr Kong has been establishing in Australia and south-east Asia.

There have been a number of subsequent visits between Australian and Thai researchers for further training, including focusing on molecular techniques for diagnostics and the use of remote microscope technology.

– Catherine Norwood

PHOTO: CRCNPB



Dr Gary Kong (right) helps scientists in Thailand set up a new diagnostic laboratory and protocols.

addition to East Timor, now have remote microscope capability and will form a vital link in Australia's pre-border security, monitoring pest movements throughout south-east Asia.

The CRC's remote microscope project support officer Michael Thompson has recently returned from Thailand, Vietnam and Laos, where he has been installing remote microscope equipment at government entomology research laboratories as well as training staff. The equipment has been supplied by the CRC, the Australian Centre for International Agricultural Research (ACIAR), AusAid and through a partnership that will contribute substantially to Australia's pre-border surveillance.

"We import produce from these countries and if we can identify outbreaks of pests or disease before it leaves these countries we can protect the relevant industries in Australia," Mr Thompson says.

"For instance, all three countries have different fruit fly species from the ones we have. If these species should establish in Australia it would decimate our fruit industries. We need to be able to identify pests like fruit fly, help our neighbours identify fruit fly, and be able to differentiate their species from our own when they reach our shores, and this network improves our ability to do this."

Mr Thompson says the cost of entry-level equipment required to join the network is expected to become more affordable in the near future, with a minimum of an internet connection, a computer and a Dino-Lite digital microscope, with models available for less than AU\$300.

The network is also operating in New Zealand, with extensions to Canada and the United States being negotiated as part of the Quadrilateral Scientific Collaboration in plant biosecurity.

The United States is particularly interested in using the system for pre-border surveillance in the Caribbean in the same way that Australia is helping to establish a network in south-east Asia. Researchers in Florida joined a remote microscope session, with others from the Caribbean island of Aruba, Melbourne and the Australian National Insect Collection (ANIC) in Canberra, using Skype and a Dino-Lite digital microscope to share images of red palm weevil specimens, an exotic pest threat to the United States.

Dr Kong says with these cheap and

PHOTO: CATHERINE NORWOOD



simple technologies, diagnostic events such as these can be organised easily and quickly to share information and images, connecting experts with non-experts in remote and isolated regions.

"The increasing volume of international trade and people movement is placing greater pressure on quarantine systems. At the same time, the pool of expertise to diagnose new incursions is shrinking. Digital tools are helping us to do more with less and increasing access to information that improves our capacity to identify new pests and disease and respond more quickly," he says.

Dr Kong's team has developed system requirements and protocols for the remote microscope network and also developed the CRC's Plant Biosecurity Toolbox, which is hosted in Museum Victoria's Pests and Diseases Image Library (PaDIL).

PaDIL (www.padil.gov.au) contains more than 20,000 detailed images of insects and plant diseases, available publicly through the internet. The website has proven to be a leading international resource for plant and disease identification, with users in more than 190 countries.

In conjunction with PaDIL, the CRC's Plant Biosecurity Toolbox provides details of pests, the symptoms and damage they cause, and links to information about diagnostic tests to confirm the identity of the pest.

An upgrade of the PaDIL website, being launched in 2010, includes a personalised 'dashboard' that will provide an entry point for identification requests and store personal

search history and identification records.

All data relating to identifications will be stored in a new portal known as BowerBird, which will also provide a control centre for all users of the remote microscope network. Dr Kong says that through BowerBird users will be able to request a session with a relevant plant pest specialist and, using a triage process to assess the urgency of each request, sessions will be booked and organised and each session will be given its own unique reference code.

Dr Kong says one of the most important elements of this will be the ability to record all sessions for later reference, to provide a chain of evidence for all identifications and to also add to the body of diagnostic knowledge. Logging all remote microscope events together with diagnostic images is expected to help provide a dynamic picture of pest movement across Australia and even globally.

While the remote microscope network is expected to continue growing in Australia and overseas for the purpose of identifications, it is likely to grow in importance as a tool to deliver real-time training. With remote microscopy, experts can run training workshops from their laboratories for people in remote areas and, with interactive communication, they can demonstrate identification features of specimens and share images. Participants can keep a personal library of relevant images and a record of conversation for future reference. In this way, experts can share their knowledge with others via remote connections. ■

Sipping the wine, saving the vine

A new drastic pruning technique may save vineyards from devastating exotic plant diseases



By Barry Pestana

The ballroom in Singapore's plush Hyatt Hotel is packed with men in black pin-striped suits, Versace casuals and Cartier originals alongside well-groomed, fashionable women with elegant wine glasses in hand, chatting animatedly. They're not drinking just any wine: they're drinking premium Australian wine – the latest releases of an array of whites and reds from one of the country's leading labels.

Australia is the fourth-largest wine exporter in the world, selling \$5.5 billion worth of wine to more than 100 countries. This is a hard-won success story, but it is one that is under constant challenge – not only from the competition of other wine-producing countries but from the potential incursion of exotic plant diseases and pests.

Of these international threats, black rot disease – caused by the fungus *Guignardia bidwelli* – is one of the most destructive. It infects the leaves, stems and petioles of the all-important grapevine and rots the berries.

Traditional responses to outbreaks of exotic diseases in perennial crops involve the destruction of infected plants, or those suspected of being infected, by burning and burying the entire plant – regardless of whether the plants are vines with 100 years of history producing fine shiraz or have not yet borne their first crop.

Research scientist Dr Mark Sosnowski, who is based at the South Australian Research and Development Institute, says current eradication methods are labour-intensive, meaning it takes an inordinate amount of time to re-establish a vineyard to its previous level of economic production and quality. Not to mention the potential destruction of Australia's viticultural heritage.

His research into alternative strategies to eradicate disease, without the heavy economic and social impacts, has led him to a new drastic pruning technique as a possible solution.

He heads a major project for the CRC for National Plant Biosecurity that has established collaborative trials in Victoria and in New York State in the United States to test these techniques.

The Victorian trial is based in the

PHOTO: SARDI



Dr Mark Sosnowski is looking for a strategy to reduce the economic and social impact of eradicating vine diseases.

Sunraysia region, near Mildura, with Dr Bob Emmett of the Victorian Department of Primary Industries as part of Dr Sosnowski's research team. The trial is using black spot, caused by *Elsinoe ampelina* and endemic to Australia, as a disease model for a pruning protocol that is ultimately designed to prepare a response to the more devastating exotic black rot disease.

In a collaboration with Professor Wayne Wilcox and his research team at Cornell University's New York Agricultural Experiment Station, the pruning protocol is being tested on actual black rot infections caused by *G. bidwelli*. The aim is to eradicate infection, while leaving enough residual

plant material to ensure rapid grapevine regeneration.

The Sunraysia trial site was established in 2006 and in 2007 vines were inoculated with black spot, developing symptoms by the following summer. The vines were then cut off at the crown with a chainsaw in July 2008. Material from the vines and debris raked from the beneath the vines was removed to a pit for burning and burial, while the trunks of the vines were drenched with lime sulfur to prevent infection from inoculum caught in the bark of the trunks. Soil between vines was disc cultivated to bury any remaining debris.

But in December 2008 symptoms were found on all control vines and four of the 36

treated vines. Tests showed the re-infection had resulted from infected debris in the soil below the vines.

The protocol was refined in 2009 to include pruning of lower shoots from the vines and the addition of straw mulch on the vineyard floor following pruning, to prevent spores from remaining, inoculum infecting new foliage and to accelerate decomposition of debris.

The refined protocol incorporating the straw mulch was first tested in 2009 as part of the collaboration with Cornell University, and was successful in controlling black rot infection. The trial is continuing in 2010 to further validate results. If validated, it will allow Australian authorities to implement this protocol as an alternative to the whole-of-plant destruction practice, which is the current standard response procedure.

Dr Sosnowski says Australia is fortunate to be free of significant pests that plague agricultural production in many countries. "Fewer pest and disease problems provide an enormous market advantage to Australian producers. But to maintain this edge in the global wine industry, controlling exotic pests and diseases is paramount," Dr Sosnowski says.

PHOTO: SARDI



Drastic pruning is being investigated in a New York trial for the eradication of black rot disease.

"The results from this research have the potential to save the Australian wine industry more than \$18 million in lost production and vineyard re-establishment costs in the event of an exotic disease incursion.

"Ever-increasing globalisation and international trade, while laced with opportunities, also increase the threat of

disease to our wine industry. Which is why it is imperative we remain vigilant.

"We believe further research into drastic pruning as an effective eradication protocol can potentially be used on other high-priority exotic diseases that also threaten high-value grapevines and, therefore, our wine industry." ■

A sharpshooting double-act with grapevines in its sights



The glassy-winged sharpshooter (*Homalodisca vitripennis*) was discovered in California in about 1996 after migrating from the south-east United States. It is a powerful vector for the bacterium *Xylella fastidiosa*, which causes the devastating Pierce's disease in grapes.

The disease kills vines by stopping water being drawn through the plant. There is no cure and, to date, there are no fully resistant grape varieties. The incursion has seriously threatened California's wine industry and economy.

Many of Australia's coastal regions have a similar climate and similar land use to that of California (particularly grapes and citrus, which are also affected), suggesting a similar impact on local industries should the bacterium make its way to Australia.

To better understand the potential risks and potential for spread in Australia, CRC for National Plant Biosecurity PhD candidate Anna Rathe is undertaking study through Charles Sturt University to examine the glassy-winged sharpshooter and *X. fastidiosa*.

She says while many areas here have a climate suited to the establishment of both pests, cold stress may protect some southern areas of Australia. As part of her research, Ms Rathe is spending six months in California studying Australian native plants. While these

trials have not detected the bacterium in Australian natives under natural vector pressure, the pathogen was artificially inoculated into a range of native Australian species and has been recovered from a number of these plants after an over-wintering period. In addition, Ms Rathe says there are early indications that some Australian plants may be able to host the glassy-winged sharpshooter. She has also completed pathogen surveys in Australia, but there is no evidence of *X. fastidiosa* in this country.

PHOTO: PaDIL



The glassy-winged sharpshooter.

She says importation of egg masses on live plants is the most likely way the glassy-winged sharpshooter will enter Australia.

The *X. fastidiosa* bacterium could also enter Australia in living plant tissue as it can be hosted by some plants without causing symptoms.

The aim of her research is to help industry quickly detect the pathogen and its vector insect by identifying the range of potential hosts.

"Known host plants can be targeted for monitoring so that an incursion can be detected early and contained," she says.

– Cassandra Humble

Deluded pests follow scent trail to eradication

Alternative control methods offer hope for easier, more cost effective pest eradication



By Nicole Baxter

An international collaboration of researchers is trialling new biological control and management methods for horticultural insect pests to reduce costs to growers and the environment.

Leading the CRC for National Plant Biosecurity project is Bill Woods, from the Department of Agriculture and Food, Western Australia. He is collaborating with researchers in Australia, New Zealand and the United States to develop effective non-chemical alternatives. Using the light-brown apple moth as a model species, the team is integrating control methods such as the sterile insect technique and mating disruption.

For the light-brown apple moth – a small leafroller moth that attacks grapes, citrus, pears and apples – the sterile insect technique involves exposing males to irradiation so they become infertile. After release, sterile males mate with wild females, which lay unproductive eggs. The research teams in WA and New Zealand have determined the optimal dose to sterilise male moths without preventing them from flying and scientists in the United States, where the light-brown apple moth has recently established, have confirmed the results.

In nature, male moths fly along a pheromone plume (scent trail) to find and mate with virgin female moths. A technique

called mating disruption interferes with this biological imperative. Conventional mating disruption uses hand-placed pheromone ties that confuse male moths, which fly to the ties rather than to females. This inhibits breeding and lowers pest pressure on high-value crops. However, Mr Woods says dispensing ties is time-consuming and expensive. “New techniques to inexpensively apply pheromone over large areas are required.”

In urban areas the research team has tested a new technique – mobile mating disruption – where thousands of sterile medflies to which pheromone has been applied are released; deluded male moths chase the medflies instead of female moths.

The trials were based on earlier work by collaborators Max Suckling, from the New Zealand Institute for Plant and Food Research Ltd at Christchurch, and Eric Jang, of the US Department of Agriculture Agricultural Research Service in Hawaii.

In Perth trials, success was measured by whether the male moths could find a female moth or lure plug in a moth trap that simulated a calling female. In the pheromone-treated plots, no moths were found in traps immediately after release of the pheromone-carrying medfly, but there were good moth catches in untreated plots.

Mr Woods says the technique shows promise as a safe, low-cost way to eradicate light-brown apple moths in urban areas

where placement of pheromone ties is difficult.

In collaboration with the South Australian Research and Development Institute's Greg Baker, another innovative pheromone-application technique is being investigated, appropriately known as ‘SPLAT’ (Specialised Pheromone and Lure Application Technology). The product, dispensed as small blobs into vineyards or orchards, is a wax-based formulation containing a small amount of pheromone that attracts male moths and keeps them away from breeding females.

According to Mr Baker, the SPLAT treatments trialled at Orlando Wines, Langhorne Creek, SA, appear to have successfully disrupted light-brown apple mating. “Our next experiment will help determine the most cost-effective application rate,” he says.

The final phase of the research will integrate several of the novel control technologies to evaluate their ability to eradicate small populations of light-brown apple moth in urban and semi-rural areas of SA and WA.

Mr Woods says, in time, these novel pest-control methods may play an important role in defending high-value produce against attack from other devastating pest threats, such as the European grape vine moth and the false codling moth, should they enter Australia. ■

Accurate moth identification removes quarantine threat



International quarantine inspectors may soon have more tools to distinguish the damaging light-brown apple moth from its harmless relatives, thanks to the efforts of an Australian scientist.

As part of her PhD project, CSIRO's Bobbie Hitchcock is developing methods to rapidly identify different *Epiphyas* species, including the light-brown apple moth (*Epiphyas postvittana*) – a pest of apples, pears, grapes and citrus.

To preserve market access for Australian horticultural exports, Ms Hitchcock is building a ‘decision tree’ to help quarantine officials correctly identify various *Epiphyas* species by looking at important structural features that distinguish the adults of pest species. However, some *Epiphyas* adults and all larvae look similar, so she is also developing a novel genetic reference database for the economically important species.

“Being able to quickly identify a pest threat or respond to trading partner concerns relies on having a large database of genetic variation and this takes time to build,” she says. “This is why our collaboration with other groups through the CRC for National Plant Biosecurity is so important, because anyone with a suspect pest incursion can tap into our gene sequence database to check if the adult or caterpillar of concern is a known pest.”

To build the database, Ms Hitchcock has been collecting moths and larvae from around Australia for the past three years and analysing their DNA with the help of researchers at Industry & Investment New South Wales.

By December 2010, Ms Hitchcock hopes to contribute more DNA sequence variation from Australia to the University of Guelph's Canadian Barcode of Life initiative, so quarantine inspection officers in other countries can check if a species incursion from Australia is a justifiable biosecurity threat.

– Nicole Baxter

Traps capture aphid migration patterns

Understanding flying pest migration patterns will determine the extent of new incursion threats



By Catherine Norwood

South Australian researcher Craig Feutrill has spent three years building a better aphid trap, only to find that the currant lettuce aphids (*Nasonovia ribis-nigri*) he was trying to catch were not so willing to be caught.

However, several other species of aphids and psyllids have been caught, in numbers large enough to allow Mr Feutrill to select another species for the modelling project that inspired the construction of the traps in the first place.

His aim, as part of a CRC for National Plant Biosecurity PhD project, is to identify the factors that cause aphids and other small flying pests to migrate and to model their migration patterns. "If there is an incursion of some exotic flying pest, this should allow us to determine quarantine zones and focus our efforts to more effectively respond," Mr Feutrill says.

His initial choice of the currant lettuce aphid is a case in point. He was working as a horticultural industry development officer in South Australia (SA) when he first saw this aphid during a trip to New Zealand in 2003. An exotic species, it arrived there in 2002 and quickly spread across the entire country, aided by human movement and wind currents.

Although the currant lettuce aphid is essentially a contamination pest – it colonises lettuce hearts and rosettes, rendering them unsaleable – the same cannot be said of Mr Feutrill's chosen replacement species, the bird cherry-oat aphid (*Rhopalosiphum padi*).

This aphid is a high priority cereal pest that can cause serious crop damage through its own voracious appetite, but it also spreads one of the three most damaging grain pathogens, barley yellow dwarf virus.

The traps Mr Feutrill has designed and built are an essential part of his project. He has six automated suction traps placed in strategic agricultural regions – two in northern Tasmania, one each at Waterloo Corner and Piccadilly Valley in SA, one at Cranbourne in Victoria and one at Yanco in New South Wales (NSW). They stand nine metres tall – the height at which migrating

insects can be found – and draw 45 cubic metres of air per minute. The air is funnelled into a fine mesh cone, dropping insects into 70-millilitre jars containing polyethylene glycol (radiator fluid), which preserves the insects, including their DNA.

There are eight jars on an automated turntable, which segregates daily catch samples. Collaborators at each site, such as the Victorian Department of Primary Industries, Industry & Investment NSW (I&I NSW) and Tasmania's Department of Primary Industries, Parks, Water and Environment replace the sample jars weekly, sending them to Mr Feutrill, based at the University of Adelaide, for sorting and analysis.

The data collected from the traps is

to the cherry-oat aphid has not fazed Mr Feutrill, as the aim of the project is to develop a model that can be applied across species, rather than a research project on a specific species.

He says the cherry-oat aphid is better able to handle the higher temperatures of an Australian summer. "Essentially their migration is temperature-driven, and they are easily knocked down by rainfall. They won't fly if there is a lot of water around or a low dewpoint, when humidity is more than 75 per cent, and when winds are more than 10 knots. For these aphids, the condition of their food source – the maturity of cereal crops – also influences their migration patterns."

PHOTO: KATE SCOTT



Craig Feutrill changes the jars used to preserve insects caught in his aphid traps.

analysed and compared to temperature, wind direction and speed, and synoptic weather charts to gain an understanding of migration timing and patterns of dispersal.

The traps were first commissioned between October and December 2008, providing Mr Feutrill with 18 months or more of insect data for his modelling. He is unsure why currant lettuce aphids have escaped the traps when a similar New Zealand design has been successful. "I know these aphids are in the field. It could be that the lower temperatures in New Zealand are more conducive to their migration – air temperatures of 32°C knock them down and above 36°C can kill them outright."

Changing the focus of his modelling

Other aphid species found in high numbers in Mr Feutrill's traps include pea aphids and two types of root aphids. A number of psyllid species have also been caught in traps and the SA Research and Development Institute is using this data to map psyllid species in eastern Australia.

Mr Feutrill says although he has finished collecting data for his PhD, a number of organisations are interested in the continuing use of the traps he has developed, including CSIRO and I&I NSW. Similar traps in New Zealand have actually trapped exotic insects before they were able to establish 'on the ground', providing biosecurity authorities with an early warning of new incursions. ■

Eyes in the north protect culture and country

Indigenous rangers are taking up the challenge of pest surveillance on our northern borders



PHOTO: CHARES DARWIN UNIVERSITY

Laynhapuy rangers are an important part of biosecurity surveillance in northern Australia.

By Catherine Norwood

Wadeye, 400 kilometres south-west of Darwin on the western edge of the Daly River Reserve, is Australia's largest Indigenous town and the home base of the Thamarrurr Indigenous Rangers, who are an important component

of Australia's northern biosecurity surveillance and management.

The rangers monitor 18,000 square kilometres of beaches, mangrove swamps, tropical woodlands and inland river country, although the Thamarrurr region itself takes in only 5,000km² around Wadeye.

Research leader for the CRC for National

Plant Biosecurity Dr David Eagling says engaging with Indigenous communities in the north is an important strategy that benefits both local communities and Australia's biosecurity efforts.

"Australia's northern boundaries are only a short distance from our neighbours in Indonesia and Papua New Guinea, where

Strategies to support local involvement in Papua biosecurity



At the CRC for National Plant Biosecurity, Theo Litaay is working on solutions to the critical issue of food shortages that have at times threatened food security in parts of Indonesia's Papua region.

Mr Litaay realised that the different levels of biosecurity policy – international, national and local – are a significant part of this issue, which launched him into a PhD research project evaluating the concept of biosecurity policy and its interaction with capacity building, food security and local knowledge.

His research is part of the CRC's Australia-Indonesia Biosecurity Community Management Project, which was established in 2007 as a partnership with Indonesia to research how communities in eastern Indonesia and northern Australia might effectively manage plant and food security.

Mr Litaay has identified existing international strategies for community involvement and is examining how policy and legal frameworks can either

facilitate or impede effective national and international biosecurity. His project will examine whether international biosecurity instruments can and should be implemented as part of national policies to assist Indonesia's development and improve food security.

He will also consider how special regional autonomy in Papua and other eastern Indonesian provinces could facilitate the integration of traditional culture into regional, national and international-level policies.

"In looking at how international and national policies flow through to regional and local communities, I am focusing on adoption of local knowledge in biosecurity and food security policies, especially in the Papua region in comparison with the surrounding eastern Indonesian provinces," he says.

"These communities and peoples possess local knowledge that has, in many cases, effectively managed biosecurity in their regions for many years." He says the big question is how governance structures can engage with this knowledge and incorporate it into good biosecurity policy at all levels.

– Catherine Norwood

there are a number of plant pests established that present a risk to us," Dr Eagling says.

"We knew of the work of the rangers and what we wanted was to build on this for the long term. We saw a chance to do something different by building biosecurity as part of sustainable businesses.

"Our aim is help develop sustainable livelihoods managing plant pests and diseases, providing work that offers cultural, economic, social and environmental benefits".

This has provided the basis for engagement with several communities including those in the Thamarrurr region and further east, in the Laynhapuy Homelands in East Arnhem Land.

Through a Caring for Country contract, and other contracts with mining companies and the Australian Quarantine and Inspection Service, 20 Indigenous rangers are being employed to undertake surveillance for plant pests and diseases in the Thamarrurr region, with other duties including monitoring wildlife populations and habitat, documenting and maintaining cultural sites, and passing on cultural knowledge to the next generation.

A similar arrangement is in place with the Laynhapuy Rangers, who manage the Laynhapuy Indigenous Protected Area. Laynhapuy ranger Yinimala Gumara says the land is very important to all Yolngu people, who are the traditional landowners in this region. "Our law, our songlines, our paintings are there. That's why we protect our land," he says.

Both Laynhapuy and Thamarrurr rangers are developing a knowledge of invasive plants and plant pests so they can help make decisions about land management and enterprise development in the future.

Ruth Wallace, from Charles Darwin University, has been largely responsible for driving the CRC's engagement project during the past four years, identifying engagement and training opportunities and negotiating participation through enterprise development that works from Indigenous knowledge and commitment to and strengths in land management.

Ms Wallace says providing enterprise-based contracts for biosecurity services is providing employment for Indigenous people and provides economic, cultural, social and environmental sustainability.

"It is a proactive approach to managing harmful plant pests and diseases. Partnerships that connect biosecurity management with existing economic, cultural and social structures improve the likelihood of long-term success," Ms Wallace says.

The CRC is contributing to the strength-based learning activities Charles Darwin

"We knew of the work of the rangers and what we wanted was to build on this for the long term. We saw a chance to do something different by building biosecurity as part of sustainable businesses."

– DR DAVID EAGLING

University is undertaking as part of the Conservation and Land Management Training Package to encourage connection to country and others' experiences and to meet industry standards. Through these activities, Indigenous rangers from different communities are recording the range of skills and knowledge required to undertake effective plant biosecurity management and are developing teaching resources in Yolngu Mathu, Murrinpatha and English to share that knowledge with other rangers and the community. These will be used to teach young Aboriginal people and other rangers about the role of rangers, identifying plant incursions and the value of being a ranger as a long-term career.

She says it is also important in underpinning the success of a number of new enterprises developing in the Northern Territory and opportunities to provide meaningful work for Indigenous communities. An example of this is the body-care product range being developed by a group of women in the Thamarrurr region. Essential ingredients in their products include sugar-bag honey produced by native bees from native plants and scarlet bloodroot flowers (*Haemodorum coccineum*), which produce a purple or red dye used to colour the body products.

"The biosecurity efforts of rangers play a vital role in supporting this new enterprise and the jobs they provide help to protect the plants they rely on from an exotic pest incursion," Ms Wallace says. ■

Ord aims for a community biosecurity culture



Community members can provide an effective first line of defence in the battle against plant pest incursions, but raising awareness of biosecurity issues is an essential first step in engaging hearts and minds.

In northern Australia, CRC for National Plant Biosecurity researcher Paul Royce has been conducting his PhD from his home base in Kununurra, Western Australia, focusing on community engagement to improve biosecurity awareness in the Ord River Irrigation Area (ORIA).

The ORIA produces a significant proportion of Australia's cucurbits, mangoes, grapefruit and chickpeas and is supported through the OrdGuard program, which is a biosecurity agreement between growers, government, tourism organisations and councils in Western Australia. The program aims to protect the region's comparatively pest-free status by raising biosecurity awareness, policy and practice among all people living in and visiting the Ord River region.

Mr Royce's research into the information networks in the Ord region has found that brochures, road signs and websites are the most common points of information, but that these are static, one-way forms

of communication that do not necessarily engage their audiences.

Within the primary industries sector, information was commonly shared between agricultural agencies and growers, but information provided to other sectors of the community was limited or non-existent.

An analysis of existing social networks in the Ord community identified five groups, in addition to the agricultural industry, with the capacity to play a significant role in biosecurity awareness and action: Indigenous groups, young people, government agencies, tourists and tour operators, and local residents.

"Much of our learning is gained unintentionally, through our everyday involvement in local activities, context and culture," Mr Royce says. "A change in biosecurity attitudes and practice will primarily take place through experiential participation. Biosecurity information should be participatory, reciprocal, transferable and exchangeable."

He says community members are not equally motivated to adopt new knowledge or translate new knowledge into action and OrdGuard's biosecurity strategies need to communicate how biological incursions can affect the individual and the broader community to generate more widespread awareness and action.

– Catherine Norwood

Tracking pathogens through water

Research is examining the potential of irrigation water to spread plant diseases



By Catherine Norwood

Returning to Murdoch University in Perth after three weeks of fieldwork in Western Australia's Ord River Irrigation Area, Rebecca Zappia brings with her more than 500 possible plant pathogen samples isolated from irrigation water in the region.

As part of her PhD project with the CRC for National Plant Biosecurity, Ms Zappia is trying to determine whether irrigation water could contribute to the spread of plant pathogens in the Ord River Irrigation Area – a relatively disease-free and high-value horticultural region.

Her recent sampling expedition, during the 2010 dry season, will provide baseline information about plant pathogens present in irrigation and drainage water within the Ord River Irrigation Area. Previous surveys of crops in the region have identified a number of bacterial and fungal pathogens, but there has been no testing of water as a potential source of infection.

Ms Zappia says there is limited information in Australia about which plant pathogens may be present in irrigation water, or about their viability; for example, some *Phytophthora* species are known to survive in water. She hopes to add to the knowledge of other potential water-borne pathogens.

Her project will help refine the OrdGuard

DIANE WHITE, MURDOCH UNIVERSITY



Rebecca Zappia processes fungi and oomycete cultures found in irrigation water in the Ord River Irrigation Area.

Regional Biosecurity Plan, introduced in 2006 to protect the region's high-value horticultural production. Ms Zappia plans to develop a robust sampling system for ongoing monitoring of plant pathogens

in the supply channels of the Ord River Irrigation Area. The project is also expected to develop procedures that will allow growers to test their own irrigation water for plant pathogen contamination. ■

Evaluating Indonesian citrus greening risk



Only 500 kilometres separates Indonesia from Australia's northern shores and the potential of a citrus greening incursion from our northern neighbour is a serious concern to the Australian horticultural industry.

Indonesian PhD candidate Wayan Mudita is helping to evaluate this risk in his CRC for National Plant Biosecurity project. His research has found that increasing knowledge about the symptoms and risk of citrus greening disease could help reduce the risk of deadly infections spreading as Indonesia expands its citrus plantations.

Citrus greening, or huanglongbing, is transmitted by the Asian citrus psyllid *Diaphorina citri*, a wind-borne insect that prefers a warm and dry climate. It is also spread through the grafting of seedlings from infected mother trees. If seedlings are already infected at planting, the disease will kill the trees before they are able to reach

their fruiting potential, which peaks between eight and 10 years.

Mr Mudita has interviewed growers and community leaders in 18 villages in the highlands of West Timor and other parts of Indonesia, as well as speaking with government officials and members of the nursery industry about how seedlings are produced and distributed, disease control measures and communication strategies.

He says while there is general agreement that pests and diseases are causing citrus decline, communication between governments and communities about the potential risk of citrus greening as either a potential or current threat could be improved.

Mr Mudita says there is an urgent need to provide more information to Indonesia's nursery industry and growers about the disease itself, what the symptoms are and how it is spread.

"Information about the disease vector, the Asian citrus psyllid, is also important because to date the only control measure available for the disease is to control its vector," Mr Mudita says.

– Catherine Norwood

Research to contain banana wilt infection

The race is on to understand and stop the spread of a new strain of the *Fusarium* pathogen



By Catherine Norwood

In less than a decade the *Fusarium* fungal pathogen has devastated the Northern Territory's banana industry, although tough quarantine measures have so far protected the economically critical Queensland industry.

Fusarium wilt is a disease common to many horticultural industries; the form that strikes bananas is commonly known as Panama disease. The first major impact of this disease was observed in Panama (Central America), where between 1890 and the mid-1950s it destroyed 40,000 hectares of banana plantations with the variety 'Gros Michel' banana.

A Northern Territory Primary Industry group researcher and CRC for National Plant Biosecurity PhD candidate, Rachel Meldrum, says relatively little is known about the *Fusarium* strain causing the problem in the Northern Territory. She says tropical race 4 of *Fusarium oxysporum* f. sp. *cubense* (Foc TR4) was detected in the Northern Territory in 1997, although the exact source of the infection remains unknown.

It was identified in a banana farm on the outskirts of Darwin and is genetically similar to the strain that devastated 'Cavendish' plantations developed for export in south-east Asia in the early 1990s. "The industry in here was looking to expand at the time, to fill an off-season niche in production," Ms Meldrum says.

"Production peaked in 2000, with 7,000 tonnes of production valued at \$13 million. But by 2009 the industry was reduced to one-third of its original value, worth about \$4 million, and most of the major growers in the Territory had left the industry," she says.

Ms Meldrum's PhD project aims to create a better understanding of the Foc TR4 pathogen and how it spreads, and to develop more effective control strategies.

She says there are four races of *Fusarium oxysporum* f. sp. *cubense* (Foc) known to exist, and three are pathogenic to bananas. Race 1 causes disease in 'Lady Finger' and 'Gros Michel' varieties and race 2 affects 'Bluggoe,' a cooking banana variety.

Race 3 is pathogenic to decorative floral and landscaping plants of the *Heliconia* species.

PHOTO: NORTHERN TERRITORY DEPARTMENT OF RESOURCES



Rachel Meldrum is investigating the mechanisms that have spread a new strain of banana wilt.

Foc Tropical race 4 is pathogenic to all commercial banana cultivars, including Cavendish, which make up 95 per cent of the \$350 million Australian crop.

Race 4 is further divided: subtropical and tropical race 4. While all races of Foc are important to the Australian banana industry, the threat of Foc tropical race 4 is considered the greatest; it kills otherwise healthy plants quickly and can spread rapidly on plantations.

"We know it is a soil-borne pathogen, so we already have some recommendations for growers about steps they can take to prevent it spreading," Ms Meldrum says.

Chief among these is the use of tissue-cultured banana plants to prevent the spread of infection. Ms Meldrum says that while it is possible for banana growers to regenerate their primary banana plants from suckers or daughter plants, the transportation of soil adhering to the plant can also transfer the pathogen.

Ms Meldrum is also investigating the role of the banana borer weevil as a possible disease vector. The borer is not a big problem for growers, particularly once banana plants are well established, but it can travel 30 metres in a day and has the potential to transfer infected soil between plants and crops.

Greater control of the borer may help eliminate one potential source of spread. Other quarantine measures being implemented include fencing for banana plantations to prevent wild animals transferring disease through the transfer of soil, and the use of washdown facilities and chemical washes.

As part of her research Ms Meldrum hopes to learn more about the epidemiology of the Foc TR4 pathogen, including how long it survives in the soil. "It is believed to survive in the soil for decades and we need to work out how it survives – does it use alternative host plants, or does it have hard spore cases that protect it?"

Tests are being conducted on two weeds common in the Northern Territory – Mission Grass and Gamba Grass – as possible alternative host plants that may be allowing the pathogen to survive long periods in the soil. Farmers also commonly grow sorghum as a cover crop on cleared land and this will also be tested as a possible host for Foc TR4.

Ms Meldrum says some banana species and cultivars with resistance to TR4 have been identified. However, none are acceptable as a commercial replacement for the Cavendish cultivars. "Our best long-term response may be to improve resistance in the commercial varieties," she says. ■

The answer is blowing in the wind

Tracking weather patterns is an integral part of research into fungal infections



By Dr Gio Braidotti

The microscopic spores produced by disease-causing fungi can become airborne, eventually spreading an outbreak as far as the wind can blow. By harnessing weather data, researchers are using computers to model the likely spatial pattern of dispersal and in the process, better direct control and eradication efforts.

As part of these efforts, CRC for National Plant Biosecurity PhD candidate Steven Coventry has built a wind tunnel at the University of Adelaide. There he studies the airborne behaviour of infectious spores, targeting ascochyta blight, a disease that devastated the chickpea industry in the 1990s in South Australia (SA), Victoria and New South Wales.

Mr Coventry says he places ascochyta-infected stubble at the head of the tunnel to replicate conditions in the paddock during a blight outbreak. "The wind blows on to the lesions that ooze the spores and they are then blown the length of the wind tunnel," he says.

"Wind speeds were between one and 4.7 metres per second, which is classed as a breeze. We could then follow how far spores travel by using spore traps. This involved using metal rods with double-sided sticky tape, placed at different lengths of the wind tunnel. Spores were dispersed at least 66 centimetres with the wind speeds available."

The wind tunnel provided Mr Coventry with crucial data needed to model spore dispersal using the commercially available modelling program, Mathematica. To check the accuracy of the resulting simulations, he also physically measured spore movement at chickpea field-trial sites in SA.

"We then used information about weather patterns at our field sites to see how closely the model correlates with the field studies," he says. "These comparisons were used to calibrate the software and we are now at the point where we can pretty much simulate the pattern of disease spread we see in the field. For one chickpea season in SA, the model achieved 90 per cent correlation with what happened in the field."

Although developed around a chickpea

PHOTOS: STEVEN COVENTRY



The wind tunnel used to study wind dispersal of disease-causing fungal spores at the University of Adelaide.



Pod lesions caused by ascochyta blight in chickpea.

disease, the software can be applied to model dispersal of many other diseases simply by altering information about the pathogen's biology. So ultimately, the ascochyta software becomes a template to simulate the spread of fungal disease in general.

"In terms of biosecurity, there are a lot of applications," he says. "It allows us to focus control and eradication measures along the dispersal pathways that spores are most likely to travel. We can help farmers better predict when they need to use fungicidal spray. If

we are dealing with preparations for the incursion of an exotic disease then we can run simulations as a precautionary measure."

Mr Coventry is now keen to trial his modelling tool in Queensland, where ascochyta blight first threatened the state's valuable chickpea crops in 2009. "Using their weather data and our model, we want to see how closely our simulation matches what happened in Queensland," he says. "I would like to do that next as part of testing and refining the modelling tool."

Also in the research and development pipeline are efforts to expand the software's capacity, particularly to take account of variable levels of genetic disease-resistance in commercially planted crop varieties.

"When you are modelling diseases there are so many things to take into account," Mr Coventry says. "Natural systems are never simple. I looked at the issue of the susceptibility level of plants as part of my project and did find that dispersal patterns are affected. We have other projects within the CRC looking at other aspects of modelling, spread and dispersal, as well as different surveillance tools. That integrated approach among many researchers helps us cope with the complexity."

These complementary efforts include a CRC project being conducted by David Savage from the University of Western Australia, who is taking a more

mathematically driven approach to modelling disease spread.

He works primarily with 'dispersal kernels' – a mathematical gizmo (made up of a probability density function) that can describe the distribution of spores or pollen at different times after a release event.

"Traditionally, you set up spore traps and measure the occurrence of spores in the field and fit your model to this data," Mr Savage says. "But a paper was published in 2005 that described a 'dispersal kernel' that you can derive using only information about the wind speed and direction and the terminal velocity of the modelled particle."

The 2005 paper, however, dealt only with a one-dimensional kernel, which measures only the distance of the dispersed spores from the origin by assuming uniform wind directions. So Mr Savage modified it to generate a two-dimensional kernel. It takes into account the distribution of wind directions and gives a better representation of the dispersal.

He then compares his dispersal pattern with the output from mechanistic models such as CSIRO's The Air Pollution Model (TAPM), which simulates pollutant dispersal using local meteorological, global terrain and land-use data as well as global synoptic

analyses. TAPM is used by 190 national and international users in 25 countries to model air quality.

"When I compare my dispersal patterns with TAPM, they match up quite well," Mr Savage says. "The kernel predicts the general shape of the dispersal surface, which gives a good indication of where the spores are more likely to be. But unlike TAPM, the kernel only takes a few minutes to calculate, rather than a few days."

Developed to model blackleg disease in canola crops, the kernel is constructed by specifying certain parameters, such as the height at which the spores are released. By altering these parameters, the same tool can be used to model other pathogens or even the pollen that cause allergies for many people.

"The spores themselves are considered essentially the same – tiny inert particles," he says. "The only bit of biology that matters in the air is that blackleg spores are degraded by ultraviolet light. So the longer the spores remain aloft, the less chance it has of being infectious. But another spore I am interested in modelling, which causes rust disease in wheat, contains a pigment that protects them from UV light."

Once again, the issue of complexity is seeing Mr Savage attempt to expand the

model's capacity. In the first instance that means integrating the 2-D dispersal kernel into more sophisticated modelling tools that can, for instance, take into account levels of UV light or differences in crop susceptibility to the modelled pathogen. The idea is to produce more informative models without losing computation speed.

One issue in particular has caught his attention. He explains that virulent strains of fungi that overcome the genetic resistance of crops are often present at low frequencies within the population. The introduction of new resistant cultivars acts as a selection pressure on these strains, causing them to become more prevalent. When this happens, the resistant cultivar breaks down and farmers incur substantial disease control costs or may even lose their crops. However, Mr Savage thinks it is possible to develop models that predict the breakdown's spatial pattern.

"The idea is to use these predictions to prolong the duration of resistance genes used in commercial cultivars," he says. "Ultimately, this model, and others that are being developed, can help Australian biosecurity agencies understand how incursions might unfold, and how they can most effectively use the control measures available to them." ■

Biodiversity the key to tracking pathogen evolution



Stripe rust is a fungal disease of wheat that costs Australia about \$130 million a year to control. This covers the cost of breeding disease resistance into wheat, as well as fungicide and monitoring costs. In the absence of these measures, stripe rust would eliminate \$1 billion annually from the Australian economy.

Astoundingly, this situation arose as a consequence of just two incursions.

The first introduction occurred in Victoria in 1979 and involved a European stripe rust strain probably brought over on travellers' clothing. It has dominated the east coast of Australia where it has been mutating to give rise to more than 20 new pathotypes. In 2002, stripe rust of a North American type was recorded in Western Australia, resulting in a widespread epidemic. This pathotype has also mutated and spread to the east coast where its descendants have become dominant.

To better understand the biodiversity in the Australian stripe rust population, CRC for National Plant Biosecurity PhD candidate Jordan Bailey is studying at the University of Sydney's Plant Breeding Institute (PBI). She is exploring variation in pathogenicity (the ability to cause disease) using DNA fingerprinting technology and the PBI's prodigious stripe rust collection, one of the best in the world.

"At the moment, I am using DNA fingerprinting to investigate the various pathotypes of *Puccinia striiformis* f. sp. *tritici*, within Australia, as well as their relationship to other forms of *Puccinia striiformis*," she says.

"Since its introduction into Australia, wheat stripe rust has acquired the ability to also infect wild barley grass. So I am further testing the stripe rust collection against Australian barley grass collections, which is important as barley grass facilitates the spread of stripe rust."

She has found that of the stripe rust isolates collected in 1982, 25 per cent were able to infect barley grass. Two years later 75 per cent of isolates collected were capable of infecting barley grass. That means the host-specificity of Australian stripe rust is evolving.

"From a biosecurity perspective, the stripe rust story highlights the importance of quarantine to the Australia farming industry," she says. "The development of a molecular identification method for stripe rust would expedite investigation into new incursions. My study of historic collections of stripe rust may also identify additional incursion events that could have contributed to the current pathogen population."

– Gio Braidotti

PHOTO: JORDAN BAILEY



Stripe rust pustules on an infected wheat leaf.

Tomato pathway for potato infection

The CRC gets to know a new 'enemy' of the Solanaceae family



By Catherine Norwood

CRC for National Plant Biosecurity researcher Alison Mackie describes the ease with which potato spindle tuber viroid (PSTVd) infection has spread during her laboratory trials as 'surprising'.

As part of her PhD research project Ms Mackie has discovered that PSTVd – a quarantine pest of significance in Australia – is capable of surviving at least 24 hours without a host plant, confirming that the viroid is indeed robust and capable of spreading easily in field conditions on items such as machinery, clothing and leather boots.

There have been six outbreaks of PSTVd in Australia since 2001, in both greenhouse and field tomatoes, in New South Wales and in Western Australia. As a research officer for the Department of Agriculture and Food, Western Australia, Ms Mackie has been at 'ground zero' for several of these outbreaks.

PSTVd affects many members of the *solanaceae* plant family including tomatoes, potatoes, capsicums, eggplants and chillies, as well as other non-commercial species. Although it has not been found in Australian commercial potato crops for more than a decade, this is the industry at greatest risk. If

PHOTO: SIMON EYRES



Alison Mackie is researching viroid off-host survival capacity.

established here it would quickly jeopardise fresh and seed potato exports worth close to \$20 million in 2009 as well as damaging domestic production.

The viroid typically causes stunting in tomato plants and reduces productivity. In greenhouse tomatoes it has also resulted in uneven ripening of fruits. In potatoes it generally reduces the robustness of plants,

with lower yields and smaller potatoes, which will be wider at one end and narrowing to a finer point at the other – the typical spindle shape.

Ms Mackie says infected tomato seed was initially thought to be the source of the outbreak, but her research is designed to identify other potential infection pathways, including alternative host plants.

Before beginning her PhD, Ms Mackie discovered that PSTVd was present on roadside vegetation in the Carnarvon region of Western Australia, particularly in blackberry nightshade (also a member of the *solanaceae* family) and in a native daisy of the *streptoglossa* genus. These plants have since been destroyed and roadside verges treated with herbicides.

She will use molecular analysis of infected plants from different areas to determine whether these infections are related or represent different strains of the viroid.

To help growers respond to any further outbreaks, Ms Mackie will evaluate different cultivars of susceptible commercial crops to determine whether some offer greater resistance. She also plans to review how effective various chemical disinfectants available for washing down plant and equipment are in killing PSTVd. ■

Australian leafhopper species appear unrelated to exotic pests



New research shows that Australian leafhopper insects in the subfamily *Macropsinae* belong to a different group (genus) than those macropsine species overseas known to transmit bacterial diseases to plants.

Linda Semeraro is undertaking a PhD project for the CRC for National Plant Biosecurity to classify Australian macropsine species, particularly those that share the same generic name as the overseas pests.

Ms Semeraro says at least four macropsine species (in the *Macropsis* and *Oncopsis* genera) transmit phytoplasma diseases overseas, but it appears that Australia does not have any endemic species that are known disease vectors. Phytoplasma diseases include rubus stunt, which affects *Rubus* plants such as blackberry and raspberry; peach yellows, which affects peach, apricot and almond trees; and alder yellows, which affects alder trees. These diseases are not known to occur in Australia.

Based on this study, macropsine leafhoppers in Australia appear

to be distinct and readily identifiable from exotic macropsine pest groups from other countries, allowing native non-pest species to be distinguished from superficially similar looking pest species.

Ms Semeraro has examined pinned adult leafhoppers from insect reference collections around Australia and overseas, looking at adult specimens under the microscope, and focusing on dissected male genitalia. One female specimen was classed as belonging to the *Macropsis* group, but females can be difficult to identify based on structural analysis alone.

To overcome the problem, Ms Semeraro is analysing DNA sequence data, in the barcoding gene region, to match female specimens to males and help identify species. She is also using DNA sequence analysis to explore the relationships between Australian macropsine species and pest groups in other countries.

As part of the research, about 50 new leafhopper species have been discovered and Ms Semeraro is developing a guide to help identify specimens to the genus and species level.

– Nicole Baxter

Thrips: sinner or saint?

A beneficial insect for graziers, South African citrus thrips are a potential threat to fruit growers



By Melissa Marino

Light will be shed on the potentially conflicting interests of fruit growers and graziers in Queensland and northern New South Wales through research into South African citrus thrips. This research is being completed with the support of the CRC for National Plant Biosecurity top-up scholarship

First detected in 2002 in Queensland, where infestations have been helpful to graziers by stunting populations of the Mother of Millions (MoM) weed, the insect is also known in its native country as a pest to mango and citrus crops.

In Australia, populations of the insect have only been found in the field feeding on the weed and it has yet to emerge on fruit crops, but Department of Employment, Economic Development and Innovation (DEEDI) Queensland laboratory tests and the South African experience have shown it is a possibility.

Australian National University (ANU) PhD candidate Brian Garms is studying the thrips in more detail to help determine whether the opposing interests of fruit growers and graziers can be reconciled.

Among his objectives, he hopes to understand why, in the short time it has been in Australia, it has not been seen to

have established itself on agricultural crops – and whether it could in the future.

In the first phase of his research Mr Garms has confirmed the DEEDI findings by showing a thrips population in a greenhouse and climate controlled laboratory feeding, maturing and reproducing on the new shoots of mango plants, as well as chilli and grape.

This, he says, has established beyond doubt the species' polyphagous nature, meaning it can eat more than one plant variety, and suggests it could indeed pose a risk to crops. While there are no reports to his knowledge of the thrips causing damage to crops in the Australian environment to date, Mr Garms says it is possible it has simply not yet been positively identified. Other explanations could be that it has its own natural predator or is being eradicated as part of the general pesticide spraying process.

"It's hard to say for sure because Queensland is a big place and this is a very small insect," Mr Garms says.

The thrips, he says, only eat new shoots, sucking up the content of individual plant cells through a tiny needle of a mouth, leaving strips of dead cells and curled and scarred leaves.

Because they are so small, Mr Garms says the key to the damage they could inflict on

a plant lies in their population density and therefore their reproductive rate is an issue.

"One or two thrips won't cause a problem at all but if a female is leaving behind 100 juveniles you can imagine you could get a real explosion in a short time, so that's what you're worried about," he says.

This factor, among others, could be the focus of Mr Garms' future research in which he hopes to investigate the South African citrus thrips' performance with related thrips species with known pest status.

By investigating several species together on a range of host plants, he hopes to clarify the threat the new insect presents to Australian horticulture. "If, for example, we can say a native thrips with reproductive rate of 10 does not pose a problem and our new thrips corresponds to that you might say 'well we've got a grip on it', but if the new thrips' reproductive rate is much larger it could be a new problem," he says.

Additionally, he hopes to understand more about how the insects select and interact with their host plants and whether particular traits such as waxy leaves may play a role in a plant's protection.

For this work, he will be using a scanning electron microscope to build a clearer picture of what the insect is doing on the leaf. "While we know in a general sense that they are puncturing cells and sucking up cell content on a very fine spatial scale, we don't know if they can select particular parts of the cells, or individual cells that are good for them and ignore other things," he says.

Depending on his findings, the thrips could find itself reclassified as a pest, which could have implications for graziers who have been happy to use it as a biological weapon against MoM.

While the insect may have value as a weed biological control agent, Mr Garms says the risk it presents to crop growers in Australia must be further assessed.

"Basically what my research is saying is 'before we can embrace it we need to know a lot more about it because on a basic level it doesn't look very safe,' he says. "And we need further study because while it might be a pest, it could actually have a real benefit and we want to be really thorough to ensure the right decision is made." ■

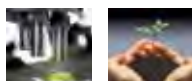


PHOTO: BRIAN GARMS

An adult thrips and a number of juveniles on a flushing red mango leaf taken through a microscope.

Nanotechnology heralds microscopic defences

Human genome technology is being adapted for plant disease diagnostics



By Dr Gio Braidotti

For pathologists charged with protecting Australia's plant-based economy – worth an estimated \$25 billion in 2009 – advances in molecular biotechnology can mean an opportunity to improve the nation's monitoring capability.

Previously, the unimaginable smallness of viruses that devastate food and fibre crops made them especially difficult to detect and control. But the virus size-advantage took a beating when scientists learned to apply nanotechnology to the problem. Operating at the scale of a billionth of a metre, scientists are custom-making molecules with desirable biological activities including the ability to identify viruses even if only minute amounts of virus material are present.

To ensure pathology can keep pace with technological innovations, the CRC

for National Plant Biosecurity researcher Dr Andrew Geering from Queensland's Department of Employment, Economic Development and Innovation is adapting the latest 'genomic' DNA technology – developed to sequence the human genome – into a new biosecurity diagnostic platform.

Dr Geering says molecular tools became an option for pathologists in the 1980s. First came custom-made antibodies used to recognise disease-causing micro-organisms in the diagnostic ELISA assay (or enzyme-linked immunosorbent assay). The 1990s saw the extremely sensitive polymerase chain reaction (PCR) target DNA to identify pathogens. More recently still, the development of nanosensors including DNA chips presented another step forward in technology. Robotic processing systems have further served to automate this technology, making it vastly more efficient than earlier detection systems.

It is this nanotechnology that Dr Geering is adapting and evaluating for use in plant pathology.

He says globalised markets, free trade practices and air travel are broadening the distribution of pathogens worldwide and already Australian pathologists are dealing with incursions every month. "The number of species and pathogens we are dealing with is so vast that we need innovations in diagnostics and surveillance to keep pace with the threats," he says.

In this quest for technological innovation, Dr Geering's team of pathologists joined forces with colloidal chemist Professor Matt Trau, from the Australian Institute for Bioengineering and Nanotechnology. Based at the University of Queensland, Professor Trau developed the OptoPlex™ fluorescent bead system that is being commercialised by spin-off company, Nanomics Biosystems.

Smut diagnostics and evolution



Once called the 'insidious black harvest', smut fungi infect commercially important plants such as wheat, and grasses more generally, to produce vast amounts of fine black spores. In addition to food security threats, the mere presence of spores in a shipment of grain can – and has – posed threats to Australia's export trade.

While smut fungi are very host-specific – infecting a narrow range of grasses – the spores from different species tend to look the same under the microscope. That makes diagnostics difficult to all but the most accomplished mycologists.

Alistair McTaggart, of the Queensland Department of Employment, Economic Development and Innovation, is undertaking a CRC PhD project examining 80 smut species in three genera to better understand their biodiversity – a project with important biosecurity applications.

Mr McTaggart says smuts form a huge taxonomic group containing more than 500 species. The morphological features used to classify smuts can create confusion, he says, and the current classification does not accommodate the vast diversity of smut species discovered over the years.

"It is my job to try and resolve this taxonomic muddle by finding natural groups for these smut fungi," he says. "To detect the natural groupings we have been studying morphology and using DNA sequence data to trace relationships based on similarities and divergences that occurred during evolution."

Of particular interest is a region of the smut genome that encodes ribosomal RNA – molecules required by the cell to make proteins. In this region is the so-called ITS (the internal transcribed spacer), a stretch of DNA that has been proposed as the 'barcode' for all fungal organisms. Parts of the ITS are known to change rapidly during evolution since they do not encode anything vital for fungal survival. These regions eventually become idiosyncratic among fungi. But the ITS also contains sequencing that is conserved among closely related smuts. This is useful for identifying groups or genera in phylogenetics studies.

Since the ITS can distinguish both groups and species, Mr McTaggart has sequenced the ITS region of all 80 smuts in his collections.

"In my groupings, you can see smuts co-evolving with grasses at the tribe and sub-tribe taxonomic levels," he says. "So smuts of cereals like wheat, oats, and barley that are closely related also have similar morphological characters. Smuts that occur on outback spinifex grasses similarly form a closely related group."

While Mr McTaggart has identified morphological features that can distinguish among smuts – an achievement people previously did not think was possible – it takes an accomplished microscopist to make an identification. So instead, Mr McTaggart is using the ITS region to design diagnostic probes to trial for use with nanobead technology. He is also testing a novel twist on that technology.

"In conjunction with molecular diagnostician Dr Paul Campbell, we are working on a never before tried variation of nanobead diagnostics," he says. "We want to attach Light Upon Extension (LUX) probes to the nanobeads and attempt to do PCR directly on the beads, streamlining the diagnostics even further."

– Gio Braidotti

The project centres on viruses that are damaging Australia's horticultural industry. High on their target list is a virus that first appeared in Australia in 2006 and is now crippling tomato production in southern Queensland – tomato yellow leaf curl virus (TYLCV). A related virus that is endemic to the Northern Territory and predates European colonisation – tomato leaf curl virus (ToLCV) – was also tested along with potato and banana viruses.

The new diagnostic system uses tiny 'nanobeads' that are impregnated with fluorescent dyes that emit light of different colours. Custom-made molecules capable of binding to a specific virus are then attached to the beads. These 'probe' molecules take the form of a short piece of DNA, although an antibody-based assay is also under development. The idea is to attach probes that detect different viruses to beads that fluoresce light of different colours.

DNA from the plant sample is then amplified by PCR and a light-emitting molecule (or 'fluorophore') is incorporated into the amplified DNA. This forms the 'target' molecule in the diagnostic test.

The target and nanobeads are then mixed together and passed through a 'flow cytometer' that creates a flow of single beads down a fine capillary tube where they intersect with light from two laser beams. One laser is used to read the colour of the bead; the other checks for the presence or absence of the target fluorophore.

"A positive hit involves two bursts of colours from the capillary tube – one from the bead and one from the target DNA," Dr Geering says. "A negative result involves just one light emission – from the bead alone. So the advantage with this technology is that we can use a mixed population of diagnostic beads, dyed different colours, in the same assay to routinely detect 20 pathogens or as many as 100 in a single assay."

The new technology is also faster at just two hours to run, compared with a full day for an ELISA assay. Although the project ran for just three years, the adapted technology has proved viable against a number of horticultural viruses.

"One of the most significant pathogens of tomatoes worldwide is tomato yellow leaf curl virus or TYLCV," Dr Geering says. "It is causing growers grief in south-east Queensland at the moment. This virus arrived in Brisbane in 2006 and has since spread west to Toowoomba and north to Bundaberg, one of the biggest tomato production regions in Australia."

PHOTO: SHARON VAN BRUNSCHOT



Sharon Van Brunschot has helped to develop diagnostic tests for silver leaf whitefly species and the tomato yellow leaf curl virus that whitefly transmit.

Since the virus is transmitted by the silverleaf whitefly, a common glasshouse pest, efforts have also been made to improve whitefly detection. CRC PhD candidate Ms Sharon Van Brunschot explains that the so-called 'B biotype' has been present in most Australian mainland states since the early 1990s. Since then, the 'Q biotype' has emerged overseas and it is causing pathologists concerns since it is resistant to insecticides. That makes the whitefly – and the virus it transmits – especially difficult to control.

With the assistance of a leading whitefly expert, CRC program leader Dr Paul De Barro of CSIRO Ecosystem Sciences, Ms Van Brunschot has designed and developed novel diagnostic assays that can rapidly detect and distinguish the B and Q whitefly biotypes as well as the Australian strains of TYLCV and ToLCV.

The detection technique she uses is called 'real-time PCR', a diagnostic tool that adds an extra level of sensitivity to the PCR assay by using a short stretch of custom-made DNA to help ensure any DNA undergoing amplification is truly specific for the target species.

"A major advantage of this technology is that assays can be multiplexed – that is, run at the same time, in the same tube – streamlining sample processing time and costs," she says. "This suite of assays allow us to test plant samples for both viruses at the same time, and also to test whitefly specimens for their biotype and if they are carrying a virus."

She is now hoping to transfer her probes for use with the fluorescing nanobead technology.

"I have been awarded a travel scholarship by the CRC to travel to the

Netherlands to collaborate with researchers from Wageningen University, where I will be working with an alternate bead technology called Luminex xMAP," she says. "Together we hope to develop a Luminex array for the detection of various tomato-infecting viruses and biotypes of whiteflies."

To date the emphasis has been on DNA-based diagnostics. But another young scientist, CRC PhD candidate Ms Jenny Vo, is hoping to trial the nanobead technology using antibodies as probes. She is isolating the genes that encode antibodies to banana streak myoscore virus, banana bunchy top virus and banana bract mosaic virus. The genes are then expressed in bacteria, which act like biological factories to produce large amounts of the antibodies. She hopes to be working with the fluorescent nanobeads late in 2010.

"While virus-specific DNA probes are easier to make than antibodies, the test itself is more difficult to run than with antibodies," Ms Vo says. "So initially the antibody work is more challenging but once we are working with the beads, this approach becomes more straightforward."

Dr Geering says he is pleased with the progress made to develop new diagnostic platforms, but says that more validation work is needed before it is rolled out to pathology laboratories.

"Techniques like PCR and ELISA have been around for many years, while nanobeads are at the same stage ELISA was in 1978," he says. "The technologies we develop now will hopefully be used in non-specialist laboratories in the future. So this project was really about investigating new platforms that pathology could use to better restrict the movement of viruses and protect trade." ■

Detection outside human vision

Hyperspectral detection of plant infections offers an edge in controlling outbreaks



PHOTO: ANTONIO ROBLES-KELLY

A hyperspectral image acquisition session on grapes in Rutherglen, Victoria.

By Dr Gio Braidotti

Well before symptoms are visible to the human eye, diseased plants undergo subtle changes that affect the way they reflect light across a broad range of wavelengths, from visible through to the near infrared. Digital cameras capable of photographing colours beyond the range of human vision, creating what are called hyperspectral images, may hold the key to a new, faster approach to diagnosing plant-disease emergencies.

The idea, says plant pathologist Dr Shane Hetherington, is to derive digital 'signatures' – from within the pixels of hyperspectral images – that are unique to disease-causing plant pathogens. These diagnostic signatures can then be hardwired into simple, hand-held devices for use as rapid, frontline diagnostic tools.

To test the feasibility of deriving these signatures, plant pathologists from state agricultural departments in New South Wales

(NSW), Victoria and Western Australia (WA) collaborated with National Information and Communications Technology Australia (NICTA) physicists led by Dr Antonio Robles-Kelly from the Australian National University in Canberra.

Dr Hetherington says that plant-disease surveillance relies on observers scanning plants for visible symptoms in areas with a high risk of incursion. However, given the enormous range of hosts and diseases, observers cannot be trained to routinely discriminate from all the disease possibilities.

"As a result, diagnostic services during an outbreak of disease are often overwhelmed by large numbers of samples requiring further identification," he says. "This creates a bottleneck to effective containment and eradication. But by imaging hyperspectral light we are hoping to develop technology that not only detects disease but can discriminate between the major pathogens of greatest concern to Australia."

During a two-year CRC for National

Plant Biosecurity project, digital cameras were modified by NICTA to integrate components and software that allow the capture of light from the lower blue range of the spectrum (with a wavelength of 380 nanometre) through to the near infrared (at 1100 nanometre). Dr Robles-Kelly says each pixel in these images is extremely data rich, containing 66 bands compared with the three (red, green and blue) used in standard photography. These cameras were used to obtain images of plants infected with six known pathogens in glasshouses and in the field.

Individual pixels were then analysed, quantified and fed into a huge database. Using NICTA's supercomputers, these images were compared with those obtained from uninfected plants to determine whether discriminatory disease signatures exist.

"We deliberately structured the project so we had a range of fungal, bacterial and viral diseases, as well as range of perennial and annual crops, including apple trees, wheat

and lupin crops,” Dr Hetherington says.

To avoid the risk associated with testing exotic diseases, Dr Hetherington says that pathologists targeted diseases endemic to Australia that are analogous to exotic diseases. This included *Phylloxera* (an analogue of Pierce’s disease in grapes) and nutrient deficiency in citrus (an analogue of citrus greening).

A second group involved diseases of domestic quarantine concern such as apple black spot (exotic to WA at the time of the project so experiments were performed in NSW) and lupin anthracnose (exotic to NSW so experiments were undertaken in WA).

Since imaging technology generates enormous datasets, a lot of computing time was required to screen the data for potential signatures.

“The results are definitely promising,” Dr Robles-Kelly says. “We managed to detect disease quite early, in some cases weeks before the plants were visibly diseased and just one or two weeks after the plants were inoculated.”

Work was also undertaken to validate the signatures, work that has Dr Robles-Kelly optimistic the technology is robust enough to work as intended.

“Once the signatures are known, it is not hard to hardwire them into a device for use in the field,” Dr Hetherington says. “It is discovering the signature that takes raw computing power. So the limiting factor is how many signatures we can generate and not how many we can fit into one device.”

Should the technology be adopted for biosecurity purposes, the next 10 years could see the list of pathogens targeted for hyperspectral analysis vastly increased. That would present opportunities to send pathologists overseas to derive signatures for diseases that could prove devastating should they ever reach Australia. This includes eucalyptus rust – a fungal disease that first appeared in South American tree plantations that poses a unique threat to native vegetation in Australia – and Ug99, a fungal disease of wheat that emerged in Uganda in 1999 for which there is little genetic resistance in the world’s cultivated wheat varieties.

“I started the project as a sceptic but now believe the technology has a lot of potential,” Dr Hetherington says. “The technology essentially gives us the ability to pick up disease before somebody who is doing surveillance could visibly see it, which gives us so much more potential for disease control and eradication.” ■

Proteins provide identity clues

DNA-based strategies offer faster bacterial identification



PHOTO: I&I NSW



Citrus canker symptoms develop on a leaf during a diagnostic bioassay.

By Catherine Norwood

Dr Deborah Hailstones is leading research to improve bacterial diagnostics, testing new technologies that analyse bacteria’s functional molecules to find faster and more species-specific methods of identification.

In too many cases, the definitive diagnostic techniques for bacteria rely on bioassays – infecting host plants in a laboratory and growing the infection over a period of days, often a week or more.

“But it’s a time-consuming process, and the symptoms that develop in the laboratory are not always typical of those in the field, which can further delay or complicate diagnosis,” says Dr Hailstones, who works with Industry & Investment NSW.

Her CRC for National Plant Biosecurity research project is using proteomics to study different strains of the *Xanthomonas* bacteria, which cause a spectrum of diseases across a wide range of plant crops.

The present DNA-based tests for a particular strain often also respond to other strains, requiring the use of bioassays to confirm diagnosis of either. But through proteomics – the study of the full set of proteins encoded by a genome – Dr Hailstones is working to identify proteins unique to individual pathovars or subspecies.

“If we can work out what different proteins do, we can trace back to the genes that are responsible for encoding these specific proteins and develop better DNA-based tests. We might also be able to use this information to develop a model to apply to other bacteria, telling us where in the genetic sequence we should look for the relevant, distinguishing genetic information.”

The project is also using metabolomics – the study of the unique chemical fingerprints that specific cellular processes leave behind – to identify the molecules the bacteria produce for a range of purposes, which also might provide identifiable ‘signatures’ of infection by particular strains of bacteria.

The molecules that might be identified using these approaches include those that break down a plant’s defences in order to establish an infection.

The secretions required to break through a cotton plant’s protective layers will be different to those required to infect an orange tree. By ‘back-tracking’, she hopes to identify other genetic coding responsible for creating these proteins or metabolites.

Dr Hailstones says these techniques could result in tests that provide a more definitive diagnosis and within just 12 hours, rather than the best-case scenario of four days using current bioassays.

Being able to more quickly and specifically identify bacterial pathovars will ensure appropriate management and trade protocols, which in turn will provide more secure access to markets and confidence in the movement of produce between Australian states and growing regions.

She says it should also be possible to develop dipstick tests (similar to a pregnancy test) to check for the presence of a specific bacterium. New technology has made these tests much more affordable and is already widely used for the detection of viruses. Her project is expected to provide some of the work required to characterise bacterial proteins and develop antibodies that will respond to the targeted bacterial pathovars.

Dr Hailstones says the project is a collaborative effort; other researchers include Dr Dave Berryman from Murdoch University and Drs Jo Luck and Simone Rochfort at Department of Primary Industries, Victoria. ■

Research reveals new threat to native forests

It's not always deadly, but the sudden oak death pathogen could devastate Australia's native bushland



By Cassandra Humble

It could begin among the cool, damp rhododendrons and azaleas of suburban Melbourne – a few fungal spores clinging to leaves of an imported nursery specimen added to one of the many heritage gardens in eastern Melbourne's Dandenong Ranges.

From there rangers at a nearby state park might notice leaves and branches dying on manna gums, the trees on which koalas feed. Only some time later would the disaster be realised. Across south-eastern Australia, mountain ash trees would develop cankers on their trunks and soon even the strongest of the species – the tallest hardwood trees in the world, which take 70 years to mature – would begin dying.

CRC for National Plant Biosecurity PhD candidate Kylie Ireland says this possible scenario highlights the susceptibility of Australian flora to an incursion of *Phytophthora ramorum*, the pathogen responsible for sudden oak death disease.

Ms Ireland is based at Murdoch University in Perth and her PhD has revealed that a number of economically valuable Australian species are potentially susceptible to the pathogen.

Should the disease find its way to Australia it could result in "a potential epiphytotic" – an epidemic of the disease. Eucalypts and the *Nothofagus* genus (beeches) are the most susceptible species.

The *Phytophthora* genus is named from the Greek 'phyton' (plant) and 'phthora' (destruction) and has caused widespread damage in Europe and the United States, affecting wild environments, such as California's redwood forests, and ornamental nursery species.

It was first documented in the 1990s as simply causing twig blight on rhododendrons in Europe. However, research began in earnest once highly valued old oak trees – "a keystone species", as Ms Ireland points out – started dying, apparently overnight, hence how it got its common name sudden oak death.

P. ramorum affects plants in three ways: attacking the wood, causing cankers (sudden oak death); attacking the shoots or branches (ramorum dieback); or attacking the foliage (ramorum blight).

Asymptomatic infection can also occur in some plants.

Ms Ireland says when *P. ramorum* affects the leaves of the plant it is not deadly to the

host. This is also the only form of the disease that produces spores capable of spreading the infection. It makes foliar-affected plants high-risk carriers of disease.

Ms Ireland's research has been two-pronged:

- investigating the susceptibility of Australian species to *P. ramorum*, through laboratory work in California, and
- climate modelling to identify and target high-risk areas in Australia for early detection surveillance.

The first part of her research has shown that many Australian plant species may be susceptible to the pathogen. Of 69 species tested Ms Ireland found all were capable of being infected, with half moderately to severely affected. All the species are important in the nursery, cut-flower and forestry industries, or in native forests.

Highly susceptible foliar hosts identified included conesticks (*Isopogon cuneatus*), rose cone flower (*I. formosus*), alpine ash (*E. delegatensis*), shining gum (*E. nitens*), karri (*E. diversicolor*) and tea tree species (*E. viminalis* and *Leptospermum scoparium*).

Species capable of producing spores and infecting other plants included willow myrtle or peppermint tree (*Agonis flexuosa*),

Calculating benefits from plant biosecurity research



The fruit fly exclusion zone in Victoria's Sunraysia region is providing a case study and the basis for an economic model to evaluate the cost-benefits of investing in biosecurity research and development.

Leading the CRC for National Plant Biosecurity research project is Professor Ben White, from the University of Western Australia.

Professor White says an existing cost-benefit analysis of maintaining the fruit fly exclusion zone and Pest Free Area in Sunraysia found that it adds an extra \$379 million (present value) to net benefits for the region over a 20-year period compared to doing nothing. This analysis, prepared by the Department of Primary Industries (DPI), Victoria, also showed an annual cost of maintaining the zone of about \$4.6 million (based on 2007-08 figures).

The CRC project will add to this analysis by modelling changes in the costs and benefits that could accrue from the use of new technologies in maintaining the exclusion zone, Professor White says.

He says that data on outbreaks and existing surveillance technology is being used to develop the model and pilot analysis will

calculate the costs and benefits of simply increasing the number of fruit fly traps deployed.

"Doubling the density of trapping can more than double the costs of surveillance. But if outbreaks can be identified sooner, they can be eradicated more quickly. Producers gain by maintaining access to high value, fruit fly sensitive export markets for longer," he says.

The project began in mid-2009 and once the model has been developed, Professor White says inputs can easily be changed to reflect scientific advances, such as new 'smart traps' – also the subject of CRC research. This technology has the potential to provide remote monitoring. While each trap may be more expensive, smart traps could significantly reduce labour requirements, which are the largest costs in any monitoring program. This fruit fly case study and model are being developed in collaboration with DPI Victoria, Industry & Investment NSW and Horticulture Australia. Professor White is also developing a case study and adapting the model in collaboration with Western Australian bulk grain handler the CBH Group, to provide a cost-benefit analysis of investment to prevent stored grain insects developing resistance to the widely used fumigant phosphine.

– Catherine Norwood



PHOTO: MURDOCH UNIVERSITY

Murdoch University PhD candidate Kylie Ireland tests Australian species for susceptibility to *Phytophthora ramorum*.

red flowering gum (*Corymbia ficifolia*), spotted gum (*C. maculata*) and myrtle beech species (*E. delegatensis*, *E. viminalis* and *I. formosus* and *Nothofagus cunninghamii*). Her research found that *E. viminalis*, in particular, is capable of high levels of sporulation.

Preliminary results indicate a number of Australian species susceptible to trunk cankers, including mountain ash (*E. regnans*). The spores of *P. ramorum* like to travel in water to get to their next host and they prefer cool climates; the mists of the California redwood forests and the moist climate of Melbourne's Dandenong Ranges are both ideal.

Climate modelling has been an important part of Ms Ireland's research, incorporating information about environmental factors associated with infection and disease development into a model to work out the path *P. ramorum* might take across the Australian landscape.

"Risk predictions generated by the models and an understanding of the pathogen's host range will allow us to target high-risk areas for early detection surveillance," she says. "It will also help Australian regulators develop appropriate quarantine protocols."

Ms Ireland says the most likely route of arrival for *P. ramorum* would be an imported ornamental plant. ■

Fast data to feed response strategies



The speed with which a new plant pest or disease spreads can determine how difficult, and costly, it is to eradicate. This is why a rapid, effective response is crucial for averting a potential agricultural calamity.

Key to any response plan is accurately forecasting the spread of the organism in the environment in which it is found. But when a species is a new arrival, the information needed for simulation models to forecast spread may not be readily available.

To date, information gathering has been ad hoc, developed on a case-by-case basis, says Dr Michael Renton, from the University of Western Australia. Dr Renton is CRC for National Plant Biosecurity project leader for 'Forecasting spread for rapid response.'

He says most simulation models are species-specific and not easily modified to predict the spread of a new species. Until now.

"With recent advances in spread simulation modelling, real-time estimations can be made about the areas most likely to be inhabited by an invading species, given relevant biological parameters. This project will provide a system for quickly gathering biological information to generate the quantitative parameters required for simulating spread."

Dr Renton says when an incursion occurs, there is often little

information about how far the invading organism will spread in the new landscape.

"In some cases, it may be possible to use historical data from a different site with similar climatic and environmental conditions to parameterise a simulation model. But this is rare. Where there is little available data, expert knowledge is invaluable."

A list of questions to ask experts about an invading species is being developed that will allow available spread-related information to be gathered quickly and incorporated into forecasting models, which will assist with response decisions.

Information on an invading organism will be gathered using a multi-layered functional characterisation approach, instead of splitting species into specific functional groups where each organism is grouped separately for different types of characteristics. Each layer of characteristics will contribute to new parameters for spread models.

The planned release of biological control agents will be incorporated into the system to predict and monitor the spread of invading organisms in landscapes, and to validate the multi-layer functional characterisation approach and parameters of existing spread models.

Regulatory authorities and plant industries will be among the end users of the system when fully developed as it will allow them to rapidly characterise new invading pests and organisms directly to assess risk.

– Barry Pestana

It's in the mail: keeping test samples secure

New protocols will help maintain the biosecurity of plants, insects and soil samples in transit



By Kellie Penfold

It's not too often scientific research involves a combination of tall buildings, weight lifting gear and frozen mail, but that is what it has taken to test the biosecurity of packing for the transit of plant samples of high-risk pests and diseases.

United Nations and Australian standards for the packaging for high-risk animals and human biological samples have been in place for many years. However, CRC for National Plant Biosecurity's scoping study conducted by Dr Alan McKay and Jan Gooden, of the South Australian Research and Development Institute (SARDI), identified the need for protocols and improvements in commercially available packaging for the transit of plant pest samples.

Following this, the CRC started an 18-month research project at SARDI to develop national protocols for biosecure packaging.

Devising testing techniques provided some lighter moments for three SARDI researchers working on the project.

Project leader Barbara Hall, research officer Dr Pauline Glocke and technical officer Tanya Matic began by assessing just how samples were sent to each state's analytical laboratories. They then tested which was the safest way to ensure the sample arrived in the best possible condition for testing.

"We heard stories of plant samples arriving as mulch, of soil samples arriving with no soil left in the package. We knew packaging had to be tough to deal with everything Australia Post and courier services had to throw at it, be biosecure and it had to be commercially available," Mrs Hall says.

Then the fun began. Using commercially available products, the researchers tested packages' integrity by filling them with samples of seeds, soils, plant material, fruit and liquid and then dropping, throwing, crushing, freezing and thawing them out again.

Dr Glocke explains this involved simulating real-life stresses on packaging by improvising.

"Packages were dropped nine metres from the top of our building and then Tanya brought in her husband's weights to simulate the pressure of three metres of packages on top of a parcel," she says.

A variety of packages with samples were then combined to comply with the United Nations and International Air Transport Association (IATA) triple packaging regulations and sent for test runs to laboratories around the country. The receivers were asked to fill in an enclosed survey about the quality of the contents.

An example of triple packaging is to seal the sample in a zip lock bag – "they are surprisingly strong," says Dr Glocke – and remove the excess air. Then place it inside another secondary package that is secure in case the original package breaks, and then enclose these in an outer packaging, such as a post pack.

"Participants were enthusiastic about being involved in this part of the research and were quick with their responses. They too want to improve the packaging of samples," Dr Glocke says.

While numerous commercial sealed

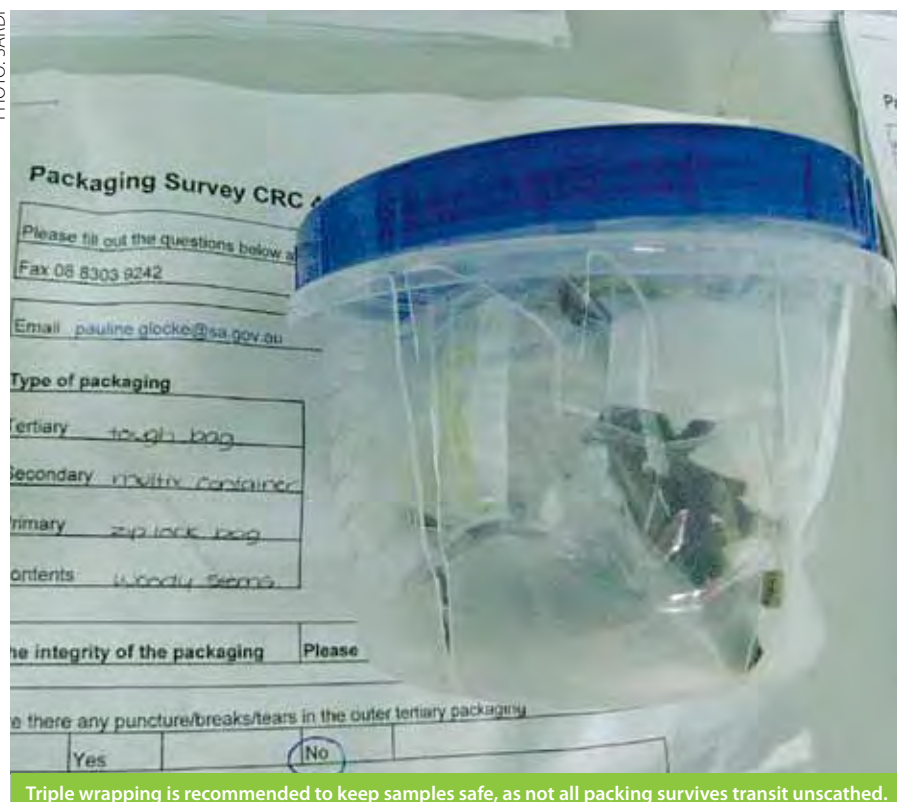
containers were trialled for holding liquids, most of them smashed during tests, so the research indicates the best container is a laboratory-supplied bio-bottle. Liquid samples should be packaged in a primary container then a secondary container plus sponge, scrunched up paper or other absorbent material to contain any leakage. These are placed into the bio-bottle then a box for postage.

"If you have a sample for testing we recommend contacting the laboratory first to ask for the guidelines and suggested sample sizes or if they have a test kit they can send out, which some do," Mrs Hall says, adding quarantine, infectious or hazardous items come under the UN guidelines, which can be provided by the laboratory.

The packaging guidelines will be published in pamphlets, to be distributed through Australia Post and courier services.

"The best method remains to hand deliver the sample, but if it has to be sent then it need not be complicated. Quality packaging is available commercially and if the guidelines are followed it should arrive in good condition," Mrs Hall says. ■

PHOTO: SARDI



Building Australia's immunity to viruses

Molecular tests aim to reduce the risk of importing new pathogens along with new plant germplasm



By Kellie Penfold

Insects are easy to spot, fungus can appear quickly, but a virus – you can't see a virus, especially in seeds or grains, when symptoms only develop in the plant.

A CRC for National Plant Biosecurity project is on target to change this situation, making diagnosis of plant and grain viruses faster and more accurate in quarantine through research involving the Grains Research and Development Corporation (GRDC) and the Victorian Department of Primary Industries (DPI), supported by the Australian Quarantine and Inspection Service (AQIS).

Mid way through Phase II (2009–11) of the 'Enhanced Diagnostic Platforms for Post Entry Quarantine and Market Access' project, AQIS will soon have access to validated molecular diagnostic procedures using polymerase chain reaction (PCR) molecular testing to potentially detect one-third of all known grain viruses, including five of the 22 viruses listed under Australia's *Emergency Plant Pest Response Deed*.

Phase I of the project concluded in 2008, producing reliable molecular diagnostic tests for the largest plant virus genus, a group of viruses known as the potyvirus genus. Additional tests have been designed for four virus genera important to the grains industry – furoviruses, hordeiviruses, tritimoviruses and rymoviruses. These tests are undergoing validation.

The AQIS senior manager south-west region, Mark Whattam, says AQIS facilitates the importation of more than 500 horticultural cultivars, 2,000 seed lines and 500 high-risk and 70,000 medium-risk ornamental plant lines/cultivars each year.

Globally, it is estimated more than 23 tonnes of wheat germplasm is shipped annually between breeders. Once wheat seed arrives in quarantine and after it is treated for insect risks and with hot water and fungicides, it is planted in post-entry quarantine areas and the resulting crops are assessed for virus symptoms.

CRC project leader Dr Brendan Rodoni, who is a senior plant virologist at the Victorian DPI Knoxfield Centre, says the development of the molecular tests provides

PHOTO: AQIS



AQIS staff inspect plants grown in quarantine for signs of infection.

AQIS with an accurate diagnosis rather than requiring staff to withhold a variety due to the suspicion of a virus.

"It will really make it easier for those poor pathologists working in the glasshouses trying to work out if or what virus is present," he says. "We know there are some awful viruses in other parts of the world – like wheat spindle streak mosaic virus which enters the soil, acts like a nematode and is hard to kill – that we don't want to let into Australia."

Although it will not necessarily speed up the quarantine process for winter cereals, Dr Rodoni says it will allow Australian agriculture and horticulture more security against viruses. It builds on gains already made in the horticulture quarantine processes, such as the reduction in approval time for new apple varieties from three years to 18 months.

He says it could also make a big difference by using DNA markers to analyse germplasm for the desirable agronomic traits plant breeders are seeking at the same time that molecular testing for viruses is undertaken.

This means some germplasm could be eliminated from the import process, saving a lot of time with further trials and testing.

Mr Whattam says AQIS is keen to provide feedback to the project in its endeavour to ensure relevant research results that can be used in its existing facilities.

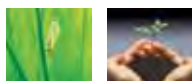
"The CRC has access to a critical mass of specialist researchers and has invested significant resources in the development of new diagnostic techniques for use in the biosecurity continuum," he says.

"AQIS is able to use the results of the research to better manage quarantine risks and support Australian exports. It is important to have the ability to test imported grain for viruses as it not only helps to protect Australian agriculture from exotic diseases, but also offers a rapid diagnostic test for Australian-grown grain, thereby protecting our valuable export markets."

Last year the new testing protocols were tried on 450 cereal varieties from the Industry & Investment New South Wales collection housed at Tamworth. All proved free of these viruses and no false positives were detected. This year Dr Rodoni will travel to the United States, where he will meet with virologists from the US, Canada and New Zealand to ensure the same testing procedures are used with main germplasm trading partners. ■

'Smart' traps target pest insects

Imaging technology used in mineral exploration could improve the detection of insects in crops



By Melissa Marino

The business of detecting problem insect infestations using trap grids, is, at present, a laborious one. It takes energy and money to manually check traps and the process is slow, meaning that by the time a problem insect is detected, it may be too late.

But high-tech imaging work being undertaken by the CRC for National Plant Biosecurity, National Information and Communications Technology Australia (NICTA) and other collaborators using hyperspectral sensing technology could soon change the way insect traps work, allowing pests to be identified instantly and remotely via computer screens.

These new automated 'smart traps' not only promise to make pest control more efficient at a local level, but open up new possibilities for biosecurity management on a much larger scale through networks integrated across vast distances and jurisdictions.

NICTA spectral imaging project leader and Australian National University Adjunct Professor Antonio Robles-Kelly says the technology could have implications not only for detection but also response and control.

"It does have implications in terms of local decisions on pest control, but it also may have wider potential for decision-making in terms of outbreaks and regional management of resources," he says. "If you

know what is happening in a small farm or area you can make intelligent decisions with information coming from the rest of the network, which is something you cannot do now because it's so fragmented."

Dr Robles-Kelly is supervising PhD candidate Pattaraporn Khuwuthyakorn, who is developing the complex imaging technology that operates within the hyperspectral range to identify pests in the trap – the first stage of a smart trap prototype.

It is work built on a four-year collaboration between NICTA, the CRC and the findings of CSIRO's Dr Louise Morin, who, in a completed CRC scoping project found it feasible to move forward with smart trap prototype development.

Hyperspectral imaging captures information from across the electromagnetic spectrum from the ultraviolet to the infrared, in a broad range of wavelengths invisible to the naked eye.

High-tech imaging work being undertaken by the CRC for National Plant Biosecurity, National Information and Communications Technology Australia (NICTA) and other collaborators using hyperspectral sensing technology could soon change the way insect traps work, allowing pests to be identified instantly and remotely via computer screens.

Commonly used in minerals exploration to detect deposits underground, it produces 'images' in the form of wavelength patterns that depict the 'spectral signature' of a target object, comprising its chemistry as well as colour and shape.

This is a crucial factor in the application of the technology for pest control given that pests lured to traps by specially developed pheromones will often resemble debris or have an almost identical appearance to benign insects, making the identification process by sight alone problematic.

"The main difficulty is to get reliable recognition so altogether, this makes a huge difference," Dr Robles-Kelly says. "The fact that we can look at shape and chemistry at the same time means we can quickly identify pests that otherwise would be difficult to find."

Dr Robles-Kelly says that because the 'spectral signatures' of target pest insects have not yet been identified, a statistical and computational framework for the classification and identification of selected insects must be created from scratch.

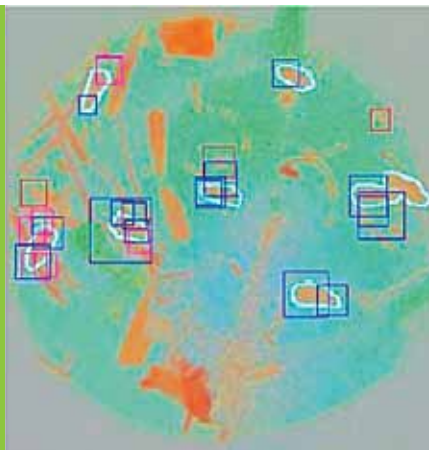
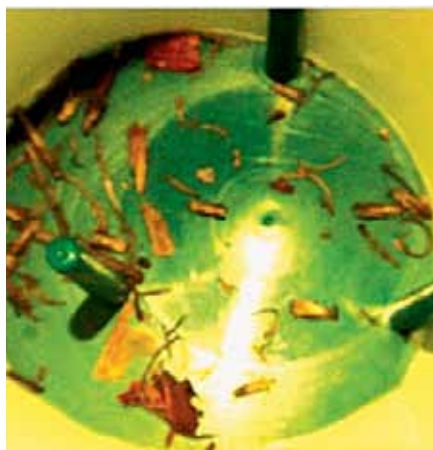
This is a focus for Ms Khuwuthyakorn, who is working to first isolate and identify the insects' spectral signatures and then develop the necessary techniques and tools required to recognise the target insects in real-time inside automatic traps.

Dr Robles-Kelly says that as Ms Khuwuthyakorn's research develops, work will progress on two other key elements of the prototype design – its power source and data transmission requirements.

If all the stars align, a prototype could be set for commercialisation within three or four years, he says.

The smart trap has potential for use in several industries in relation to a range of pest insects affecting grain and horticulture, both in the field and in storage.

"And of course there will be other advantages because it's not only the network, it's the data you gather," Dr Robles-Kelly says. "The data becomes extremely valuable because over a period of time you can start extrapolating future settings and providing services for decision support and so on. That's why it's so promising." ■



PHOTOS: NICTA

The pseudo-colour image acquired by the hyperspectral camera on a proof-of-concept trap which contains oriental fruit moths and debris (left) and sample recognition results (right).

Native vegetation reveals new fungal pathogens

A review of Western Australian pathogen collection has identified new species



By Catherine Norwood

Worldwide, many species of the fungal pathogen *Phytophthora* are well recognised as threats to agricultural crops and native vegetation. In Western Australia (WA), *P. cinnamomi* has been devastating native vegetation across the south-west of the state for many years.

With gene sequencing technology now more readily available, WA's Department of Environment and Conservation has been reviewing the *Phytophthora* isolates in its Vegetation Health Service culture collection, which have been gathered from native vegetation during the past 20 years or more.

The result is the identification of more than 10 distinct and previously unrecognised *Phytophthora*.

Murdoch University and the CRC for National Plant Biosecurity have been assisting with the review of species and CRC PhD candidate Alexander Rea has recently described and published one of these new species – *P. elongata*. He is in the process of submitting another two new species for publication, to be called *P. arenaria* and *P. constricta*.

In addition to the characterisation of these three, Mr Rea is now determining how

pathogenic they are and hopes to ascertain whether they are endemic to Australia or exotic.

"*P. elongata* occurs mostly in the jarrah forest, but has been found to be pathogenic to a species of *Banksia* that is widespread in the south-west of WA," Mr Rea says. "It appears to have been introduced to WA, and the closest identified relative of this species, *P. bisheria*, has recently been found in undisturbed old-growth forest in Taiwan. So there is a

possibility that *P. elongata* originated there."

At this stage pathogenicity tests are only being conducted on known host plants, although it is possible these *Phytophthora* species could spread to new hosts. For example, *P. bisheria* is known to be pathogenic to strawberries, raspberries and roses. Further pathogenicity tests are required to determine the host-range of *P. elongata* and the other *Phytophthora* species under investigation. ■

PHOTO: ALEXANDER REA



Oospores of *Phytophthora elongata* forming in oogonia with elongated stalks.

Infection process triggers distinctive protein response



Apple scab is caused by the fungal pathogen *Venturia inaequalis* and occurs in all major apple-growing regions around the world. Infection affects the appearance of fruit, resulting in downgraded or unsaleable produce. It also reduces the productivity of infected trees.

La Trobe University-based CRC for National Plant Biosecurity PhD candidate Daniel Jones is working on a relatively simple DNA test to identify the pathogen responsible.

"My project is still at a theoretical level, but I am aiming for a practical outcome. A DNA test for apple scab could identify infected plants or fungal spore samples within a few hours, rather than the several weeks involved in current tests, which require the fungus to be grown out to spore reproduction stage," he says.

At a more fundamental level, Mr Jones is working to characterise proteins the fungus generates during the process of infecting a plant, as well as proteins that apple trees generate during their resistance response.

"The stoma infection structure of the apple scab pathogen looks quite different to the normal growth structure, and the proteins I am studying are only produced using the infection process."

One year into his research, Mr Jones says it is unlikely he will get beyond characterising these proteins during the next two years. However, in the long-term, he says it is possible this information may prove valuable in developing apple trees that are immune to infection. It could also help to identify possible genetic modifications to commercial cultivars that could improve resistance.

"Some apple varieties have better natural resistance to apple scab than others, but most of them are wild apple varieties and they don't taste terribly good," he says. "I am hoping my research will help identify what it is that elicits an immune response from those varieties."

Mr Jones' project is part of a broader study of how the fungus infects apples headed by Dr Kim Plummer at La Trobe University. Researchers from the Department of Primary Industries Victoria, Department of Agriculture and Food, Western Australia, and Plant & Food Research New Zealand are also collaborating in study.

– Catherine Norwood

'Spies in the sky' set trap for fungal spores

Unmanned aerial vehicles track the spread of plant diseases



By Kenn Pearce

For years they have been the covert 'spies in the sky' for governments seeking intelligence about the enemy. Now hi-tech stealth machines – unmanned aerial vehicles (UAV) or 'drones' – have lowered their sights for missions against agricultural foes.

UAVs capable of accurate, detailed ground scanning from an altitude as low as 35 metres are the latest weapon against fungal spores.

The leader of the CRC for National Plant Biosecurity's 'Flying Spore Traps' project, Professor Rodney Walker from Queensland University of Technology (QUT), says mounting spore traps on remotely controlled UAVs will revolutionise the detection and combating of fungal spores. He sees their application transcending the capture of plant pathogens.

Professor Walker's team has taken a conventional farm-type spore trap and given it wings to provide unprecedented capture.

"As part of the CRC, we became interested in how we could use pilotless aircraft in the spore trap project," he says. "We fitted a modified trap to an aircraft and did a series of controlled experiments to understand how effectively it detected spores."

A senior agricultural research engineer with Queensland's Department of Employment, Economic Development and Innovation, Les Zeller, was pivotal in advancing the project. He designed a trap using standard plumbing fittings, an electric fan, a radio controlled servo-motor and microcomputer components. Mr Zeller's design expanded the project's scope to include adapting the spore trap for geo-referenced mobile spore sampling, using ground-based vehicles.

UAVs such as the Insitu ScanEagle, used by the United States and Australian armies, weigh 20 kilograms fully laden. They can fly 12-hour daylight missions at approximately 100kmh.

"In 12 hours you can cover a hell of a lot of ground," Professor Walker says.

The research challenge was overcoming design changes to enable traditional traps to move through the air at 40 metres a

PHOTO: ARCAA



Australian Research Centre for Aerospace Automation (ARCAA) researchers Richard Glascock (left) and Pritesh Narayan set up spore traps on an unmanned aerial vehicle for the CRC project.

second, and to pinpoint where spores were collected. The prototype trap spent time in wind tunnels as researchers gained an understanding of airflow patterns of trapped particles.

"In a rotating drum model, when the disease spore comes in you want it captured immediately," he said. "If the disease spore is allowed to move around to the side of the drum you'll end up thinking that spore was detected over Joe Bloggs' property instead of Fred Brown's and send a response crew to the wrong area."

"Through post-processing we can tell exactly which part of the paddock that spore was detected on."

QUT modified its Silverstone Flamingo UAV, fitting the spore trap to the top of the aircraft, and completed a series of flights at its test property at Burrandowan, Kingaroy.

Simulations run

"We used special test samples for the wind tunnel tests, chosen to be the same size and mass as spores we wished to detect. For the flight tests we used a 'smoke' generator (often seen in nightclubs), which releases sugar molecules at an appropriate concentration we could fly through and detect. We used smoke flares in some tests for better visualisation of the plume," Professor Walker says.

"We've been able to capture those

elements on the spore trap as the aircraft has flown through the plumes. We're quite confident that if we sent this out on a broad-acre surveillance mission we'd be able to detect plant pathogens if present."

Professor Walker believes UAVs have immense potential for Australia's agricultural industry. "Detecting the spread of disease could save growers money by identifying a disease before it destroys significant areas of crop," he says. "It also could protect the reputation of Australia agricultural exports; how do you put a value on that?"

"The most likely business model for provision of these aerial surveillance services is that regionally based companies will be established that can provide the service to farmers over regions up to 200km from home base."

"The UAVs will carry a multitude of sensors, from the spore trap, to conventional and multi-spectral imaging sensors. The farmers might request weekly imagery of their crops to estimate yield or moisture content. They may also request high-resolution standard imagery, so farmers can identify location of stock, condition of bores, dams or fence lines."

"It's part of an overall picture of a robotics surveillance service that will be flying, not only for the agricultural industry, but also for the power industry, road network managers and the mining industry." ■

A model to anticipate the unprecedented

Mathematics is helping to develop better responses to unprecedented biosecurity breaches



By Dr Gio Braidotti

When swine flu first emerged in Mexico in 2009, carefully laid biosecurity plans were activated worldwide and then questioned and critiqued. While the debate ensued as to whether the response was inadequate or overblown, the situation is one that perfectly encapsulates the problem that Professor Kerrie Mengersen and her colleagues work on.

Given the ability to anticipate biosecurity threats – as scientists were able to anticipate the outbreak of H1N1 influenza – how do you best prepare a response given the unprecedented nature of the event?

The problem, says Professor Mengersen, requires making the best use of limited information from all sources. That creates the need for a conceptual framework that can combine partial biological data, expert opinion and emerging facts in a transparent and repeatable fashion. As a professor at the Queensland University of Technology's School of Mathematical Sciences, she says there is a branch of statistics particularly suited to estimating risks, uncertainties and probabilities associated with biosecurity threats. It is called Bayesian statistics.

"Our job is to develop methods to formalise how we elicit expert opinion, the way we represent that information, and the way we combine and update it with other data," she says. "So we are not trying to change any of the information people use in dealing with incursions or impose a procedure that guides how experts respond. Rather we want to improve the way probabilities of events occurring are elicited and estimated."

She says the tools under development cover four broad areas:

1. Methods that formalise how experts estimate probabilities, which include the best way to solicit that information.
2. Modelling tools that allow information from various sources to be integrated into support decision-making.
3. Ways to translate expert opinion into a statistical distribution that can be incorporated into statistical models.

4. Designing how, where and when to collect measurements, and the trade-offs in terms of accuracy versus resources, especially in new problems where little is known beforehand.

Aware that statistics can be abused to the point where they can mislead, misinform or just plain lie, she says the Bayesian methods she uses were developed in the 1980s precisely to infuse real-world knowledge – and common sense – into statistical analysis.

She says that for biosecurity applications, Bayesian statistics can be described as a way to update assessments as data becomes available about a real-world situation. It provides a flexible and informative way of

to solve calculations that an alternative approach was sought. In the 1930s, the mathematician who provided the statistical methods used in quantum mechanics, Ronald Fisher, developed the frequency-based approach routinely taught to this day. It was not until the 1980s when computers became available that statisticians returned to the more complete way of thinking about statistics and probabilities inherent to the Bayesian approach.

"The Bayesian framework allows us to do statistically what humans do in practice when assessing risk," she says. "We have an opinion about a risk, we get some information and we update our opinion."

In developing Bayesian-based biosecurity applications, Professor Mengersen and her colleagues are targeting three situations that are especially pertinent to the Australian context: incursions by exotic species, managing farm pests, and pre-border and post-border surveillance design. The idea is to develop conceptual, software and modelling tools.

There is so much interest in doing this work from so many sectors that the CRC is helping to ensure a critical mass of statisticians is available to work on biosecurity problems. Besides leveraging the existing skills of Professor Mengersen's extensive team, the CRC has developed a full-time position based at QUT – taken up by Dr Samantha Low Choy – with a special focus of developing expertise in biosecurity statistics relevant to the CRC's research.

Professor Mengersen says there are enormous applications that can come from applying statistical research to analysis, prediction and response strategies. Ultimately her interest extends to communities affected by biosecurity threats and to the general public and the struggle to interpret probabilistic risk assessments, be they about health threats or lifestyle choices.

"I firmly believe that educating people to interpret statistical analysis is the next step from teaching people to read," she says. "Originally, people were not taught to read, sometimes for fear of how the information would be absorbed and interpreted. We are well past that now of course. But I believe we need to travel the same road for statistics." ■

PHOTO: QUT



modelling, while allowing for more realistic or 'natural' interpretations. Importantly, unknown parameters that describe how a system works can be estimated numerically through a technique that produces a spread of values and a probability distribution of their occurrence – an approach that allows the researchers to assess the uncertainty of various estimations and predictions provided by the model.

"The probability distribution allows us to make much stronger statements about a situation," she says. "That includes making comparisons between parameters, the probability that a value is greater than a certain threshold, the probability that risk is above a particular level."

In fact, this is how statistics was originally conceived in the 18th century. Then, a lack of computational tools so restricted the ability

Filling nematode knowledge gaps

Improved diagnostics and understanding risk priorities are the focus of nematode PhD projects



By Kellie Penfold

Documented losses from plant parasitic nematodes in Australian agriculture total more than \$600 million annually, although to date our shores are free of some of the world's most destructive nematode species.

Establishing identification procedures and prioritising exotic nematode risk are the aims of two CRC for National Plant Biosecurity PhD projects under way to address the potential threat of these pests.

Matthew Tan is undertaking his PhD at Murdoch University, developing procedures for testing and early diagnosis of plant parasitic nematodes and building a database of nematode samples for ongoing Australian research. He hopes one of the outcomes of his work could be an easy-to-use test for Australia's quarantine services.

The destructive potential of exotic species such as the pine wood nematode is significant. This species is native to the United States, where it causes little damage, but in the early 1900s it migrated to Japan and subsequently destroyed

PHOTO: COURTESY SUNIGH SINGH



PhD student Sunigh Singh collecting soil samples for Root Knot Nematode analysis in his native Fiji.

What are nematodes?

Nematodes are small, worm-like animals that have digestive, nervous, excretory and reproductive systems, but do not have a respiratory or circulatory system. There are more than 30,000 known nematode species and about half of these are parasitic. The parasitic nematodes include species that infect humans, all types of animals and plants. In plants, nematodes generally attack the root system, but some infect leaves, stems and seeds.

Nematodes range in size from nearly 10 metres long to only a fraction of a millimetre. They are mostly worm-like in shape, but some are spherical, lemon-shaped, sausage-shaped and a host of other variations. In diameter, they range from a few millimetres to about five microns.

More information: www.ento.csiro.au/science/nematode.html

large parts of Japan's forests and is now spreading through Korea, China and Portugal.

"It would be impossible for all quarantine officers to know about every nematode, but a DNA-based test kit for on-the-spot diagnosis would dramatically reduce the risk of an unwanted nematode entering this country," Mr Tan says.

Mr Tan is using a MALDI-TOF mass spectrometer to develop standard procedures for identifying cyst and root lesion nematodes. This technology requires only small samples of nematode proteins and delivers a 99 per cent accurate diagnosis within a few hours.

He will further his studies through a CRC travel scholarship to visit a plant molecular biology laboratory specialising in root knot nematode parasites in agriculture at Hokkaido University, Japan. He will also undertake a field trip to gather samples of pine wood nematode and to find samples of soybean and potato cyst nematodes, which are not available in Australia. The genetic

profiles of each will be used to develop the MALDI-TOF testing system.

Fellow PhD candidate Sunigh Singh is focusing on building expertise in nematode biology, systematics and diagnosis to fill the gaps in the biosecurity system, working in conjunction with Charles Sturt University (Riverina) and CSIRO.

Mr Singh says his research will dovetail with Mr Tan's by identifying which nematodes pose the greatest risk and which hosts need closer examination pre-border, at border or post-border.

CSIRO research scientist Dr Mike Hodda is one of the handful of nematode specialists in Australia and is supervising Mr Singh's research. He says both PhD projects are attempting to answer important questions for Australian biosecurity and they have a clear direction and outcome in mind.

"The emphasis is on developing methods which simplify systems for our quarantine services in an era when the threat is great with so much more product entering our country," Dr Hodda says. ■

Risk analysis review to boost quarantine systems

An international comparison reveals areas where all can improve



By Melissa Marino

A world-first comparison of international biosecurity risk analysis systems is being undertaken as part of a project to boost the robustness and transparency of quarantine systems in Australia and around the world. The comprehensive review of the methods used internationally in biosecurity risk analysis compared systems from the United States, Canada, New Zealand and Australia.

Initially commissioned by the Australian Centre of Excellence for Risk Analysis, the CRC for National Plant Biosecurity project examined important aspects of the risk analysis approaches, including:

- how experts score risk
- how risk scores are combined according

to various expert opinions, and

- how uncertainty is incorporated in the risk evaluation process.

Project leader Professor Kerrie Mengersen from Queensland University of Technology says researchers were particularly interested to see how these issues were dealt with for a pest for which there was little or no data, and for which expert opinion was vital.

In addition to investigating theoretical issues, practical assessments were undertaken in collaboration with PRATIQUE, a European Union consortium of institutions that is developing the science to provide pest risk analysis.

While the project is yet to be finalised, Professor Mengersen says there is room for improvement in the quantitative approaches used for biosecurity risk assessment in all

countries. The project's conclusions to date include:

- countries need to recognise and acknowledge uncertainty and state what it is
- definitions of risk and methods for scoring risk should be consistent, and
- expert judgements employed in the assessment of risk need to be presented to decision makers transparently.

Professor Mengersen expects the report will be used as a resource for policy-makers both in Australia and internationally. "Considering the broader range of tools that are being used across countries can allow individual countries to improve their methods and that might then improve international policy and biosecurity standards," she says. ■

DNA provides faster fungal identification



New DNA-based techniques are improving Australian scientists' ability to identify fungal pathogens before they become widely established.

In the past, detection systems have relied on finding symptoms in affected plants – a time consuming process that requires specialist expertise, and by the time symptoms are found pathogens may already be well established.

Volumetric spore traps are among the most common alternative surveillance systems; they suck in air, catching particles on strips of adhesive tape wound around a rotating drum. The tapes can then be removed and examined under the microscope for specific pathogen spores.

However, the problem with this system, according to researcher Bonny Vogelzang, is the processing of the samples.

"Using a microscope to identify the different pathogens is very time consuming," Ms Vogelzang says. Pathogens collected in just one sampling could take half a day to process and expertise is required to identify the pathogens of interest as spores can be very difficult to distinguish from each other morphologically.

As part of her PhD research project for the CRC for National Plant Biosecurity, Ms Vogelzang has been using a recently developed process to identify fungal pathogens in spore trap samples, based on their DNA fingerprint. She says it is now possible to process 100 samples or more in just a few days.

"That makes spore trapping a viable strategy for identifying the presence of specific pathogens," she says. It also provides quantitative data, indicating the intensity of a fungal outbreak, not just that a particular pathogen is present.

Ms Vogelzang has been validating her DNA sampling techniques by using them to study the epidemiology of pathogens that cause blackleg of canola, ascochyta blight of peas and ascochyta blight of chickpeas.

Because spore trapping and DNA testing can be conducted without reliance on trap plants, or discovering infections in crops Ms Vogelzang has been able to provide new information about the way pathogens behave outside the growing season of their preferred host plants.

Her research will contribute to improved prediction models for farmers by providing more accurate advice about potential fungal infections, allowing them to take preventative action.

From a biosecurity perspective, she says it will improve Australia's ability to monitor any suspected outbreaks of exotic fungal pathogens, and to determine the spread of infection, or the absence of infection following a control or eradication campaign. It could also help verify area freedom of particular pathogens and facilitate exports.

- Catherine Norwood

PHOTO: CATHERINE NORWOOD



The pathogen responsible for blackleg of canola has helped to test new surveillance strategies.



The Cooperative Research Centre for National Plant Biosecurity is a collaborative venture between the following core and supporting organisations.

CORE PARTICIPANTS



Australian Government
Department of Agriculture,
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Department of
Agriculture and Food



SUPPORTING PARTICIPANTS



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