



Ryegrass endophyte mycotoxin breakthrough

Groundbreaking research by Bio-Protection Centre scientists has identified a key genetic process contributing to ryegrass staggers in grazing animals.



Prof Barry Scott

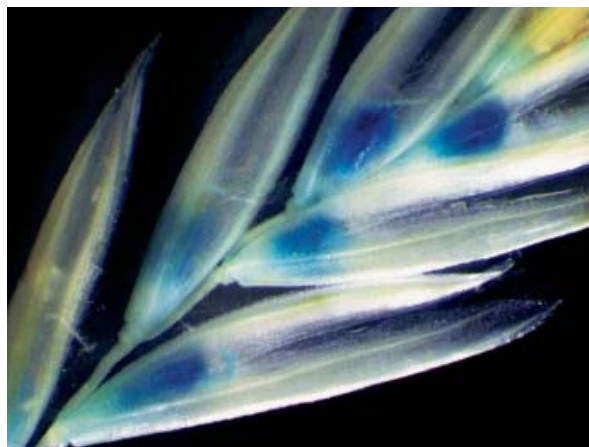
Carolyn Young, a PhD student working with Prof Barry Scott, has isolated a cluster of genes that regulate synthesis of lolitrem and related mycotoxins, which are synthesised by *Epichloë* endophytes in association with grasses. These chemicals are responsible for animal health problems

such as ryegrass staggers, but also benefit grasses by protecting them from attack by insects. The compounds are only produced when *Epichloë* endophytes grow within grass hosts, suggesting that their synthesis is symbiotically regulated.

The genes were absent from most genomic libraries because the chromosomal region they are located on is unstable in *E. coli*. The organisation of genes controlling synthesis of fungal secondary metabolites into clusters is a distinctive feature of fungal genomes, raising questions about their evolutionary origin and maintenance. Reverse transcriptase PCR analysis demonstrated that all of the genes are only expressed in plant tissue. The cloning of these genes has opened up several exciting new areas of research.

By systematically deleting these genes in *Epichloë*, and analysing the accumulated intermediates in ryegrass plants infected with each mutant, the pathway for lolitrem B biosynthesis is being resolved. This work was carried out by postdoc Dr Daigo Takemoto, in collaboration with AgResearch Grasslands.

To examine at what stage(s) in the ryegrass life cycle lolitrem are synthesised, postdoc Dr Kimberley May has made a gene fusion between the promoter of one of the lolitrem genes and the GUS reporter gene, allowing visualisation of expression in plants. Very high expression was observed in developing seeds, a role consistent with protection of seeds from herbivory. Kimberley has also made a 'knock-in' of GUS at the native locus of one lolitrem gene, and with MSc student Emma Brasell is using this to screen for *Agrobacterium* T-DNA induced mutants that fail to express the GUS. This method can identify genes that control expression of the lolitrem genes, which will give



GUS activity in a ryegrass inflorescence infected with a transformed endophyte

important insights into symbiosis signalling between grass hosts and endophytes.

Cloning of the lolitrem genes makes it possible to use PCR to assess the biosynthetic capability of different endophyte strains, to identify those producing anti-insect compounds but not the mycotoxins that harm grazing animals. Commercialisation of novel endophytes is managed by Grasslanz®, a subsidiary of AgResearch, and this PCR screen will enhance their ability to identify potentially valuable endophyte strains.

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Bayer/National Business Review Innovators Awards

Five Bio-Protection Centre researchers were nominated for these Awards in three categories. Agriculture and Environment, Prof Alison Stewart (Lincoln University) and Prof Stephen Goldson (AgResearch); Research and Development, Prof Tony Conner (Crop & Food Research); Science, Prof Nikola Kazabov (Auckland University of Technology) and Dr Trevor Jackson (AgResearch).

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Prof Alison Stewart

From the Director

Five years of hard work and commitment from Bio-Protection Centre researchers came to fruition in June, when the Tertiary Education Commission confirmed that the Centre will receive continued funding for the next six years. Together with our success in two large FRST programmes and the establishment of several industry initiatives, this places the Centre in a very strong and stable position. We have now secured average annual revenue of some \$8 million from these sources through to 2014. We do not want to rest on our laurels, however, and work is already in progress on two further FRST programmes and a major new business venture.

On a very sad note, Dr John Hunt (Agrimm Technologies Ltd), one of the Centre's key business partners, passed away in August after a short illness. John's fruitful interaction with the Centre over the last six years resulted in the commercial development of three biocontrol products – a major achievement by anyone's standards. I will miss him as a close work colleague and dear friend.

This edition of Bio-Protection once again profiles some of our young postgraduate students and summarises a range of significant activities, research highlights and Centre-related achievements. Prof Steve Wratten organised a very successful Transferable Skills Workshop for our postdoctoral fellows. This is an excellent example of the added value that the Centre provides to our young researchers. Plans are under way to organise follow-up workshops focusing on specific skills, and a modified version of the workshop for postgraduate students is also being developed.

With the summer break rapidly approaching, this is an opportune time to thank all those with interests and collaborative links with the Bio-Protection Centre for your continued support. I look forward to catching up with you all again in the New Year

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CoRE Fund success for Bio-Protection Centre

The Bio-Protection Centre has secured funding through to 2015 in the latest Centre of Research Excellence (CoRE) funding round announced in June by the Tertiary Education Commission (TEC). The Bio-Protection Centre was one of six Centres in New Zealand to achieve continued funding, with one new Centre being established.

The funding will be used in 11 new research programmes across four revised research Themes:

- **World-leading Biosecurity**
- **Sustainable Bioprotection**
- **Plant Bioprotection Systems Biology**
- **Māori Bioprotection**

The TEC funding also provides for new capital expenditure, which will be used to expand the growth room and soil experimentation facilities in the New Zealand Biotron, further enhance equipment for the Gene Discovery Unit, and expand computational capability in the Decision Support Centre.

Since the announcement, Prof Alison Stewart, Caroline Pratt (Centre Research Manager), Prof Richard Falloon and Centre research leaders have been developing the new Centre contract in consultation with TEC personnel. This has involved aligning the Centre research programme, project budgets, and capital equipment requirements with the allocated TEC funding.

Two important new initiatives under the new contract will include the appointment of a Māori Development Officer, as part of a project to promote education in bioprotection, and a new cross-Theme initiative to



Caroline Pratt, Bio-Protection Centre Research Manager

provide economic and social analyses of research undertaken in the Centre.

Centre staff and support personnel see the success of the funding application as an endorsement of the Centre's success since it was established in 2003. Continued funding ensures the ongoing evolution of the original research strategies, and expansion of well-established national and international collaborations on fundamental bioprotection research. The TEC funding also complements the Centre's broad portfolio of applied and industry-funded research.

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Transferable Skills Workshop for Postdoctoral Fellows

In a first for Lincoln University, the Bio-Protection Centre hosted a Transferable Skills Workshop in September for its Postdoctoral Research Fellows. The 10 postdocs are all carrying out relevant and high-profile research, but also operate in a larger context which includes developing understanding of the funding environment, national and international political trends and end-user requirements.

Acquiring appropriate non-research skills is also an essential part of developing full competency for a research career. These skills include oral and written communication, working with groups, facilitation, project and financial management and career planning. The aim of the workshop was to raise awareness of these skills among research fellows, demonstrate their relevance for career progression, and help them begin to improve the skills.

The workshop began with a half-day Research Seminar in which postdocs each gave brief outlines of their work. This was followed by a day and a half of skills development, involving a range of specialists. The workshop was organised by Stephen Hill and Nick Early of Restore NZ Ltd, with assistance from Centre staff.

Speakers and topics included: Dr Peter John, Lincoln University's Research and Innovation Office (intellectual property),



Workshop participants: Centre postdoctoral fellows and senior staff

Dr Barbara Brown, FRST (the funding environment), Christine Toner, a consultant (working with groups), Shary Vargo, Vargo + Lewis Consultants (career management), Ian McKenzie, consultant (project management), Nick Early (dealing with news media) and Stephen Hill (effective presentations). Dr Garth Carnaby, GA Carnaby & Associates Ltd, gave a compelling overview reflecting on his career in science and business. Senior Centre staff participated in all aspects of the workshop.

The workshop was a resounding success, according to feedback from participants, who considered the initiative a positive expression of the Centre's aim to ensure that postdoctoral scientists have the best possible preparation for developing their careers in science.

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Virus-free kūmara plantlets in tissue culture

Kūmara: pests, production and people

New research into kūmara production will bring together traditional knowledge and scientific research to help ensure high quality, sustainable production of the crop.

The project, a collaboration between Dr Nick Roskrige (Massey University) and Dr Steve Lewthwaite (Crop & Food Research), aims to produce, collate and transfer current traditional and scientifically-attested information on kūmara production as part of FRST-funded studies on soilborne diseases of vegetable crops.

Kūmara, known internationally as sweet potato, was the first significant crop grown in Aotearoa-New Zealand. Māori introduced kūmara at first human settlement, and with a deep understanding of production complexities produced kūmara as a large-scale principal food. Generations learnt to both respect and transfer production information through ceremony, oral tradition and experience. Today, kūmara crops remain an important dietary component, in both domestic and international markets.

The study will look at resistance to Sclerotinia rot, a destructive disease in commercial kūmara fields. It will cover a range of kūmara lines, including early pre- and post-European cultivars, commercial cultivars and promising breeding

lines. Information on disease resistance will assist in developing cultivar selection and crop husbandry disease control strategies for sustainable *Sclerotinia* management.

International reports suggest that planting virus-free material can improve kūmara production, but this approach is as yet unproven with local cultivars and conditions. Selected lines from virus-free and virus-infected sources will be evaluated in a field trial to determine the effects of these pathogens on kūmara yield and quality.

The latest local and international research on aspects of kūmara crop production and bioprotection will be incorporated into a growers' manual, catering for Aotearoa-New

Zealand conditions, including some content in both Māori and English. The manual will facilitate further Māori involvement in the industry and advance the development of sustainable bio-protection systems.

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Sclerotinia rot on a kūmara storage root

Microbes enhance pine tree health

Bio-Protection Centre research has led to the development of a product to boost disease resistance in Radiata pine seedlings.

ArborGuard™, a *Trichoderma*-based product, has been developed by Dr Robert Hill (BioDiscovery NZ Ltd), who leads a forestry research objective in the Bio-Protection Centre's FRST programme 'Bio-protection of New Zealand's productive ecosystems'.

ArborGuard™ is a mixture of *Trichoderma* isolates selected after efficacy assessments on seedlings and cuttings. The product is designed to create Radiata pine seedlings and cuttings with increased resistance to *Armillaria*, an economically significant disease problem in New Zealand forests. *Trichoderma*-based products have been used in horticulture for many years, but until recently no products were available specifically for forestry applications.

Dr Hill has developed ArborGuard™ in close partnership with PF Olsen & Co Ltd and GroChem NZ Ltd. Seedling stock treated with the product is available through PF Olsen & Co Ltd.

Dr Hill says that the formulation improves establishment, growth and health of nursery seedlings and cuttings. "This drastically reduces the need for fungicide applications, saving costs and providing a chemical-free product,"

he says. Novel formulations of various micro-organisms and natural products are also showing promise in recent trials. "The best treatments boosted seedling growth by over 30 percent, and improved root volume and health. ArborGuard™-treated trees in forest plantation trials had 20-50 percent less mortality from *Armillaria* infections than untreated trees."

A major focus of the ArborGuard™ project involves practical commercial issues, such as determining optimum timing, rate and mode of delivery of the microbial inoculants. This phase of the work has been augmented by a Technology New Zealand Expert Fellowship, which supported research by Dr Danilo Paderes.

The uptake of ArborGuard™ has increased sharply in the 2007 planting season, with more than six million pine and one million Douglas fir seeds being coated with the product at BioDiscovery NZ Ltd. Further research is in progress to develop improved microbial formulations for Radiata pine, with funding from FRST and the Forest Biosecurity Research Council.



Pine sapling killed by *Armillaria*. [inset: Pine cuttings treated with selected microbes (left) or untreated (right)]

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Insects as vectors of *Fusarium tumidum* for biological control of gorse

Emmanuel Yamoah has successfully defended his PhD thesis, which determined whether insects can vector *Fusarium tumidum*, a foliar fungal pathogen of gorse. The research aimed to see if insects could be utilised as an alternative approach to mycoherbicide delivery for control of this very important weed. Four insect species, gorse seed weevil, gorse pod moth, gorse thrips and light brown apple moth, were assessed for their ability to carry spores of the fungus. The micro-organisms naturally carried by the insects were identified using morphological and molecular methods. All four insects carried several common bacteria and fungi, including those pathogenic to insects.

Factors promoting pathogenicity of *F. tumidum* to gorse, and the pathogen loading required to infect and kill the weed, were also determined. Amendment of *F. tumidum* inoculum with gorse extract increased spore germination and germ tube length, and gave more host infection than without gorse extract.

In experiments to determine the loading capacity of the different insects, it was found that the light brown apple moth carried and deposited more *F. tumidum* spores than the

other species. Conidia deposited on gorse by the insects (310 conidia/light brown apple moth) did not cause any infection. Many more spores (900) were required for disease development on gorse plants. Electron microscopy showed that the gorse pathogen penetrated plants only through open stomata, explaining why large numbers of spores are required for infection. Emmanuel's research has shown that a more virulent pathogen would be required for successful control of gorse using this novel 'lure-load-infect' concept.

"My experience at the Bio-Protection Centre and Lincoln University has given me skills enabling me to gain a position with MAF Biosecurity New Zealand," says Emmanuel. "My work will be as an adviser on eradication, containment and management of plant pests and diseases."

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Emmanuel Yamoah inspects gorse plants

'Smart Seeds' research initiative

The Bio-Protection Centre has won a \$5.2 million FRST research contract to develop bioactive agents for disease control in forage and vegetable crops.

The Smart Seeds for Export project is directed towards providing biocontrol of economically important diseases and pests of forage and vegetable *Brassica* spp. The research will specifically target bacterial black rot, Sclerotinia rot and diamondback moth, and aims to develop bioactive agents for delivery either endophytically or using appropriate seed coating technology.

The programme has four stages: identifying appropriate bioactive agents; developing prototype seed products; identifying innate and external factors affecting the biocontrol agents; and validating the final seed products.

Funding is for a six year period with the project managed by the Centre's Director, Prof Alison Stewart. The Science Leader for the work is Prof John Hampton, Professor of Seed Technology and Director of Lincoln University's Bio-Protection and Ecology Division. Research partners are Crop & Food Research and AgResearch, with industry participation and funding from South Pacific Seeds (NZ) Ltd, PGG Wrightson Seeds Ltd, and the Foundation for Arable Research. The successful programme application was developed with Lincoln Resource involvement, from a concept developed after wide consultation with the New Zealand seed industry.

Prof Hampton says this is the first time the New Zealand seed industry has attracted FRST funding. "Securing this research contract is a reflection of the Centre's internationally-



Prof John Hampton and Brassica seedlings from a germination test

respected track record in biocontrol," he says. "The Smart Seeds programme will be a significant contribution to New Zealand's export edge in products based on emerging strengths in biotechnology and bio-protection."

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Prof Steve Wratten, Bio-Protection Centre Deputy Director, talks with Prime Minister Helen Clark, along with Lincoln University Chancellor Tom Lambie and Vice-Chancellor Prof Roger Field, during a visit in October. The Prime Minister viewed sustainability research projects including studies into reducing nitrate leaching from dairying, food miles impacts on exports, and the 'greening' of the dairy industry. Prof Wratten outlined proposals to 'green' the Lincoln University Dairy Farm, applying biodiversity, ecosystem services and sustainable land use practices. The Prime Minister applauded the proposals, saying it was important to keep ahead of trends in consumer and market attitudes.

Dr John Hunt (1946-2007)

John Hunt (MSc, PhD) who died in Christchurch in August at the age of 61, was a well-respected scientist, innovator and entrepreneur. He will be greatly missed by the biocontrol research community both nationally and internationally.



Dr John Hunt

John was the co-founder of Agrimm Technologies Limited, a leading company in environmentally sustainable plant protection. Over the past decade, Bio-Protection Centre researchers

have worked in partnership with Agrimm Technologies to commercialise a number of plant disease biocontrol products for the horticulture sector. Two products are of particular note: Tenet®, based on *Trichoderma atroviride*, is registered for control of white rot of onion; and Sentinel®, also based on *T. atroviride*, is registered for control of *Botrytis* on grapevines and grey mould of tomato.

John was closely involved with the research required to establish these products in the disease control market, from early stage discovery through to full verification of efficacy and final development for registration and marketing.

As Agrimm's Technical Director, John was actively involved in collaborative scientific projects, working closely with Professor Stewart's research group. His commitment to the development of biological products (particularly those exploiting his beloved *Trichoderma fungii*) was unwavering and his enthusiasm infectious. John's contribution to the development of biological products for grapevine diseases was recognised worldwide.

John is survived by Marjorie, whom he married in 1969, and two daughters, Phillipa and Caro.

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Dr Maureen O'Callaghan



Dr Marco Jacometti

Awards to Centre personnel

Dr Marco Jacometti has received a 2007 MacDiarmid Young Scientist of the Year Award in the category "Adding Value to Nature" for his PhD research. His work showed that Botrytis bunch rot in wine grapes could be controlled with mulches and mown cover crops, by breaking the pathogen lifecycle and increasing vine resistance to the disease. Marco is currently a Postdoctoral Fellow in the Centre, and his research is supervised by Prof Steve Wratten.

Dr Maureen O'Callaghan was awarded the 2007 Crop & Food Research/New Zealand Institute of Management Memorial Scholarship, to attend a residential Senior Management course. As a soil microbiologist in AgResearch, she leads research on microbial control of key agricultural pests, and potential environmental impacts of new agri-technologies, particularly in soil ecosystems. Dr O'Callaghan was selected for the award from a number of excellent applicants across a range of science organisations.

Prof Barry Scott was awarded a 2007 Massey University Medal, recognising his contributions to supervision of postgraduate students. His progressive mentoring of research students, and introduction of a formal oral defence for doctoral candidates progressing to full registration - a practice now widely adopted at the University - were particularly noted in the award, along with the excellence of his research outputs and those from the group he leads.

Prof Stephen Goldson received the New Zealand Institute of Agricultural and Horticultural Science Jubilee Medal for 2007. The award citation noted his "exceptional contribution to primary resource science over a sustained career". His pioneering work on pasture pest weevils, and contributions to innovative approaches for protecting against biosecurity threats, including 'Sniffertech' technology to detect biological material in shipping containers, were particularly acknowledged.

Prof Richard Falloon has been elected Fellow of the New Zealand Institute of Agricultural and Horticultural Sciences. This award recognises his contributions in plant pathology and crop protection, across pastoral, arable and horticultural sectors. Prof Falloon's international leadership in the plant pathology discipline, as President of the International Society for Plant Pathology and Past President of both the Australasian Plant Pathology Society and the New Zealand Plant Protection Society, was also recognised in his Fellowship.

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Bio-Protection Centre Postgraduate Students

Profiles of postgraduate students associated with the Bio-Protection Centre, and their projects, are listed below:



Emma Brasell is a second year MSc student at Massey University. She completed a BSc at Massey University. Her MSc project is to identify genes that regulate the expression of the lolitrem toxin biosynthetic genes in *Epichloë festucae*. Her Masterate topic is "The expression of endophyte genes in planta". [Supervisor Prof Barry Scott].



Sam Brown completed his BSc in technology, biology and chemistry at Waikato University. His Masterate project is "Taxonomy and biology of *Carpophilus* spp. (Coleoptera: Nit: dulidae) with an emphasis on the South Pacific". [Supervisor Dr Karen Armstrong, Associate Supervisor Dr Rob Cruickshank, Advisors John Marris and Dr Rich Leschen (Landcare Research)].



Michael Cripps, from Canada, completed his BSc at the University of Guelph and MSc at the University of Idaho. His PhD project is "Multi-trophic interactions and the population dynamics of *Cirsium arvense* - a biogeographic perspective". [Supervisor Dr Grant Edwards, Associate Supervisor Dr Derrick Moot, External co-supervisors Dr Graeme Bourdôt (AgResearch), Dr Simon Fowler, and Dr Nick Waipara (both Landcare Research)].



Carla Eaton completed her BSc(Hons) at Massey University. Her PhD topic is "The signalling involved in maintaining the mutually beneficial association between *Epichloë festucae* and perennial ryegrass", and her research is supported by a TEC Top Achiever Doctoral Scholarship. [Supervisor Prof Barry Scott, Associate Supervisor Prof Jeremy Hyams].



Sara Mohan completed an MSc in Biotechnology at the Bharathidasan University, India. Her PhD project is "Development of durable late blight resistant potato cultivars by introducing resistant genes". [Supervisor Prof Tony Conner, External Advisors Drs Jeanne Jacobs and Meiyalaghan Sathiyamoorthy (both Crop & Food Research)].



Shengjing Shi is from China, and completed her MSc degree at the University of Nottingham. Her PhD project is "Influence of root exudates on soil microbial diversity and activity". [Supervisor Prof Leo Condon, Associate Supervisor Dr Eirian Jones, Advisory Team Prof Alison Stewart, Dr Maureen O'Callaghan (AgResearch), Dr Darren Smalley (AgResearch), Dr Christian Walter (Scion Research), Dr Alan Richardson (CSIRO Plant Industry), Dr Mark Osborn (University of Sheffield)].