BSES Limited

STUDY TOUR TO NEW ZEALAND

CONTROL MEASURES FOR CANEGRUBS

by

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SR06001

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SUMMARY

Kerry Nutt and Rachael Fox visited researchers at HortResearch (Auckland and Palmerston North), Crop & Food and AgResearch (Lincoln). The CRC SIIB 1Bii project group currently uses the avidin transgene with the PPI vacuolar targeting sequence developed by scientists at HortResearch. This trip was an opportunity to discuss the progress of our work with PPI-avidin, obtain methods for screening our plants and ideas for identifying promising plants earlier, as well as information to help progress plants to commercialisation. We were also able to learn about novel transgenes in the pipeline, at both HortResearch and AgResearch, which may lead to future collaborative research.

Associated with the visit to HortResearch was an opportunity for Rachael Fox to present the research of the CRC SIIB 1Bii project group to our collaborators. An invitation for Kerry Nutt to present the work of CRC SIIB 1Bii to researchers at Crop & Food and AgResearch was also extended.

The following actions will benefit the development and commercialisation of insect-resistant transgenic sugarcane, and the development of milky disease as a biocontrol agent:

- Source or establish a laboratory culture of *Spodoptera* sp. for early laboratory-based bioassays of transgenic plants.
- Monitor (information searches updated monthly) the progress of research in New Zealand with transgenic plants expressing the avidin gene – results of the research and information about regulatory approvals will aid us in conducting field trials and commercialisation of insect-resistant sugarcane expressing avidin.
- Maintain regular contact with Dr Christeller with the view to future collaborative research involving the nucleopolyhedrovirus chitinase and kiwifruit cysteine protease.
- Bioassay of papain (commercially purified cysteine protease from papaya) against greyback canegrub to test potential of using a cysteine protease as an insect-resistance gene.
- Check PPI targeting in sugarcane using a PPI-GFP construct and a non-targeted avidin construct.
- Measure the functional avidin expressed by current avidin transgenic sugarcane using the assay developed by Dr Christeller's group.
- Bioassay *Serratia* toxin complex against greyback canegrub to test its potential for use as a transgene.
    - Obtain recombinant vector with toxin complex genes and introduce genes into sugarcane.
- Re-examine the morphology of the parasporal body of the milky disease bacterium (*P. popilliae*) after re-isolation from an infected grub.
- Examine the effect of insect gut extract on the growth of *P. popilliae in vitro*.

1.0 BACKGROUND

Kerry Nutt and Rachael Fox visited researchers at HortResearch (Auckland and Palmerston North), Crop & Food and AgResearch (Lincoln), with the aim of establishing contacts for future collaborative research and to evaluate the progress of any relevant biotechnology research. Associated with the visit to HortResearch was an opportunity for Rachael Fox to present the research of the CRC SIIB 1Bii project group to our collaborators. An invitation for Kerry Nutt to present the work of CRC SIIB 1Bii to researchers at Crop & Food and AgResearch was also extended.

2.0 VISIT TO HORT RESEARCH

HortResearch is a fruit-science company, which shares with the CRC SIIB a desire to use environmentally sustainable production systems. HortResearch was established in 1992 as one of nine Crown Research Institutes (CRIs), arising from several Government departments. The institute is wholly government owned, but still gains revenue from commercial sources and competitive funding.

Like Australian sugar-industry research organisations, HortResearch is using genetic modification tools to maintain its horticultural science leadership position, but sees no current consumer demand for the transgenic products.

The largest research projects at HortResearch monitor the effects of genetically modified plants on beneficial insects, such as honeybees and predatory beetles. These projects are largely collaborative ones with AgResearch, under the umbrella of "Environmental Impacts of New Technologies". One of the most important projects looks at the impact of GM plants on native invertebrates and birds.

2.1 Mt Albert, Auckland

Mt Albert is the largest research site for HortResearch, housing around 200 staff. Our hosts at Mt Albert were Dr Elizabeth Burgess (Entomologist) and Ms Beth Stark (Commercialisation Manager).

The day began with Rachael Fox's presentation of our work producing transgenic sugarcane containing the PPI-avidin construct that was obtained under MTA from HortResearch. After Rachael's presentation, we met with the entomology group and discussed the differences between regulation of genetic modification of food crops in Australia and New Zealand.

According to the entomology group, research into the environmental impacts of GMOs was largely driven by politics, arising from a need to secure a 'green' vote, and resulting in the introduction of the GM moratorium in 2000. Although the GM moratorium is no longer in place, there are few field trials for transgenic plants. This is due, firstly, to the expense of obtaining regulatory approval and, secondly, the dependence of New Zealand on export of a number of its horticultural products. Field trials of genetically modified 'sensitive' crops

are undertaken off-shore as collaborative research. Thus, the entomology group expressed excitement about our work with the PPI-avidin construct, because it is a chance for them to be involved in a field trial.

Figure 1 **Rachael Fox presents the results of our PPI-avidin research to staff at HortResearch, Mt Albert**

The research of the entomology group is focused on the environmental impact of GM plants produced either by HortResearch or other CRIs. Dr Burgess showed us some of the trials currently underway within the Mt Albert PC2 glasshouses. The first trial we examined was a collaboration with AgResearch, investigating the effect on earthworms of transgenic tobacco (*Nicotiana tabacum*) expressing the avidin gene.

Figure 2 **Dr Burgess and Rachael examining leaves of a transgenic tobacco plant expressing the avidin gene**

The second trial we examined was a 'public good' trial investigating how insects used for biological control are affected when their target organism has been feeding on a transgenic plant. In New Zealand, this is of particular interest to groups seeking to introduce new organisms for biological control purposes. The trial that Dr Burgess was involved in was investigating the tritrophic interactions of Monterey pine (*Pinus radiata*) expressing either a *Bt* toxin or altered lignin genes, a caterpillar pest (pine looper, *Pseudocoremia suavis*) and a parasitoid wasp (*Meteorus pulchricornis*).

Figure 3 ***Pinus radiata*** **expressing Bt toxin (left) and pine looper being reared on non-transformed pine needles (right)**

Dr Burgess' group have established laboratory colonies of pine looper and another native caterpillar, the leafroller (*Epiphyas postvittana*). Leafrollers are easy to rear in the laboratory, and are regularly used for early bioassay of pest-resistant transgenic plants. We

described to Dr Burgess and Mr Bruce Philip (research technician), the difficulties we have had with bioassay of our transgenic plants due to the seasonality of canegrubs. This led to a discussion about bioassay methods using alternative insect predators, and how to establish caterpillar colonies using insects collected from the field.

Figure 4 Inspecting a bioassay of pine needles expressing avidin with leafrollers

2.2 Palmerston North

The HortResearch facility at Palmerston North is located adjacent to the Massey University campus. Research at this facility includes plant breeding, and measuring and identifying aroma, flavour and bioactive compounds in foods, as well as protein chemistry and molecular biology research for improved pest resistance.

Our host at Palmerston North was Dr John Christeller (lead protein chemist), who has over 80 publications on topics such as insecticidal compounds in fruits, environmental effects of transgenic plants, and insect biochemistry.

Once again, Rachael Fox presented our work with the PPI-avidin gene to Dr Christeller's team. We were particularly interested in discussing with the group whether they had seen truncated products in their transgenic plants, similar to those we see in our higher expressing lines. We discussed what the products could possibly be – degraded products, early termination of translation/transcription – and how we might go about reducing their presence.

We were able to discuss with Dr Colleen Murray methods for examining the subcellular localisation of avidin in sugarcane expressing the PPI-avidin construct. Inaccurate targeting of the avidin protein may be causing the truncated products, although it is believed that, if the protein were being mistargeted, that no plants would regenerate. Dr Murray explained that they had been using GFP to examine the subcellular location of the avidin in tobacco, but there were some problems with the stability of the fluorescence under vacuolar

conditions. As an alternative, Dr Murray was attempting *in situ* hybridisation to detect avidin, but was having limited success.

The transgenic tobacco expressing avidin that we examined with Dr Burgess was prepared by researchers in Dr Christeller's laboratory. In tobacco, the avidin gene is expressed using a double 35S promoter, and is produced *in planta* at levels ranging from 0.5-1% total protein (leaves).

Figure 5 Richelle Marshall (research associate), showing Rachael some transgenic tobacco and *Arabidopsis*

Dr Christeller provided us with a method for measuring functional avidin from plant tissue, which we will be able to use as a pre-bioassay screening method for our transgenic cane. It is believed that this will significantly reduce the need for bioassays and allow us to identify promising lines earlier.

The research of Dr Christeller's group is aimed at transgene discovery for pest-resistance, within a non-Bt niche. Currently, the group is promoting the use of avidin, in conjunction with the patented PPI-targeting sequence. However, they are investigating the potential of other compounds for use as transgenes, for example a nucleopolyhedrovirus chitinase and cysteine protease isolated from kiwi fruit.

Additional research within the laboratory includes that of Dr Robert Simpson (protein biochemist) who was examining the proteomic differences between caterpillars (light brown apple moth, carpet moth and a *Spodoptera* sp.) infected and not-infected with nucleopolyhedrovirus. Dr Simpson was also undertaking a general proteome investigation of moth moulting fluid for the purposes of identifying protein targets for insecticides (conventional or transgenic).

Working on the genetics of pest insects are Drs Heather and Lawrence Gatehouse, the final two members of Dr Christeller's team. Drs Gatehouse and Gatehouse are preparing an EST library of gut tissue from the New Zealand grass grub (*Costelytra zealandica*), a scarab in the same subfamily as Australian canegrubs. The purpose of the library is to study insect gene expression during infection with amber disease (*Serratia entomophilia*), and to learn

more about how the bacterium inhibits trypsin synthesis by the insect during infection. This is a collaborative research project with AgResearch, in particular Drs Trevor Jackson and Mark Hurst.

3.0 VISIT TO CROP & FOOD

3.1.1 Crop & Food

Crop & Food is another of the nine CRIs, and like HortResearch, the institute is government owned, but funded through a mix of local and international industry and government sources.

Crop & Food provides research services for arable, vegetable, dairy, food and ornamental crops, as well as the forestry and seafood industries. The research is divided into five key areas, also referred to as Centres of Innovation:

- Sustainable land and water usage
- High performance plants
- Personalised foods
- High value marine products
- Biomolecules and biomaterials.

3.2 Lincoln

Our host at Crop & Food in Lincoln was Dr Grant Smith, Team Leader Sustainable Productive Environments. The role of the Sustainable Productive Environments group is to develop sustainable systems for a range of land uses using skills in crop physiology, crop protection and soil and water management. The group has a broad research portfolio with projects in soil and water management, on-farm productivity, controlling pests and diseases, and biosecurity and bioprotection, the latter two areas being of particular interest.

Kerry Nutt presented an overview of the work within CRC SIIB project 1Bii, prior to a presentation on her PhD research to scientists from both Crop & Food and AgResearch (located in the same research park). Following the presentation, Dr Smith showed us around the facility and introduced us to some of the researchers in his team.

Within the Controlling Pests and Diseases group, research is being undertaken to develop resistant or tolerant plants, but at Crop & Food the focus in on conventional breeding. Work with transgenic plants is focused on the environmental impacts, in particular the effects of GM plants on native pollinators. Crop & Food does have a small transformation group working on production of bioplastics, which may be of interest to the CRC SIIB. Dr Smith told us that the group was producing biopolymers of commercial interest in plants, and the research was progressing well.

We visited the soil microbiology laboratory, where researchers are developing molecular tests for diagnosis of soil-borne pathogens. Crop & Food is interested in the molecular

characterisation of both pathogens and other microbes in the soil as a means of monitoring soil health (organism diversity).

Dr Smith introduced us to Dr John Marshall, leader of the Biosecurity and Bioprotection group. This group develops and utilises methods for detection of pests and diseases in plants and soils, contaminants in product lines and genetically modified organisms in foodstuffs. We also met with one of Dr Marshall's PhD scholars, Mr Matthew Galbraith, who described his work analysing the biochemistry and genetics of toxic metabolites produced by *Erwinia herbicola*, a potential biocontrol agent for fireblight (*Erwinia amylovora*).

4.0 VISIT TO AGRESEARCH

4.1 AgResearch

AgResearch is New Zealand's largest Crown Research Institute, employing around 950 staff at five campuses. AgResearch has three research groups: Agriculture and Environment, Applied Biotechnologies and Food and Health.

4.2 Lincoln

Within the Agriculture and Environment group sits the Biocontrol and Biosecurity research team, headed by Dr Travis Glare. Dr Glare and colleague Dr Trevor Jackson have had a long standing relationship with entomologists from BSES Limited through their work on New Zealand grassgrub (*Costelytra zealandica*). Drs Glare and Jackson have extensive knowledge and experience in the area of scarab pathology.

During our visit to AgResearch, we met with Dr Jackson and Dr Mark Hurst. Together, Drs Hurst, Glare and Jackson have patented the use of genes encoding an insecticidal protein complex. The genes were isolated from *Serratia entomophila*, the bacterium causing amber disease in New Zealand grassgrub.

Dr Hurst has a recombinant vector containing the toxin genes from *Serratia entomophilia* and would like to collaborate with us to test the toxin complex on Australian scarab larvae. The toxin is a protein complex ~1.2 million Da in size, encoded on multiple genes that need to be expressed at the same time and in the same place. A challenging transformation proposition for *in planta* expression, but Dr Hurst provided us with some copies of posters showing *in planta* expression of similar toxin complex genes by researchers at Dow AgriSciences.

Dr Hurst also has a method for fishing-out toxin genes, and expressed interest in helping us isolate toxin complex genes should we discover a native *Serratia* strain in canegrubs.

We asked Drs Jackson and Hurst about their experiences with the milky disease bacterium, *Paenibacillus popilliae*, and discussed the difficulties that Lorelene Bowler (honours student) was having with visualising the bacterium's parasporal body. Dr Jackson

suggested that the morphology of the parasporal body may be influenced by the bacterium's time *in vitro*. He suggested that we re-inoculate the vegetative milky disease bacteria into a grub, and then examine the morphology of parasporal body. Dr Jackson said that they had not pursued milky disease given the difficulties with germination and sporulation, but he did suggest that the bacterium may need an environmental cue to germinate, for example the alkaline pH and enzymes of the insect gut.

Dr Jackson has also been involved in a phylogenetic study of native and introduced scarabs in New Zealand. He described how, using the cytochrome oxidase II (COII) gene and internal transcribed spacer 2 (ITS2) region of the 16S rRNA gene, it was possible to differentiate between the northern and southern populations of New Zealand grassgrub. There has been some work of a similar nature conducted on canegrubs, and Dr Jackson expressed interest in performing a phylogenetic analysis of canegrubs with scarabs from New Zealand when sequence information becomes available.

5.0 CONCLUSIONS

New Zealand is a model country with regard to environmentally sustainable agriculture. At the three CRIs that we visited, research for sustainability examines all levels of agricultural practice, from maintenance of soil health, through to the effects of farming practices on wildlife and the broader environment. In the words of Dr Burgess: they have to look after it, because it is all they have.

Agriculture is where the main body of scientific knowledge is invested in New Zealand, which is not really surprising given that New Zealand is an agricultural rather than industrial country. Despite the regulations imposed on biotechnology research by the New Zealand government, each of the CRIs that we visited had a strong commitment to agricultural biotechnology research and the application of this technology to benefiting the community.

One interesting distinction between our research community and those we visited was the broad avenues of research undertaken by a single research group. Even more interesting, was that although the scientists and resources appeared to be spread thin, the researchers still seemed to achieve a great deal. This could be a result of the collaborative research efforts between laboratories and CRIs.

New Zealand has just emerged from 10 years of competitive research funding, the end of which resulted in the loss of whole teams of expertise when the funding ran out. The shift toward collaborative research was positive in the laboratories we visited, but we were told that many research institutes/laboratories are still struggling with old 'non-sharing' attitudes.

Everyone that we visited was happy to share information, and all were interested in maintaining contact for future information exchange, collaborative research or even exchange of scientists on sabbatical. We are maintaining our close ties with researchers from HortResearch, who are helping us with safety information for the future field release of sugarcane expressing the PPI-avidin construct. We are interested in pursuing the toxin

complex genes with Drs Hurst and Jackson, which we believe, can be successfully introduced using the *Agrobacterium* transformation method developed in CRC SIIB project 2ai. Maintaining contact with Drs Hurst and Jackson is still important, as they have experience in the commercial production and application of bacterial biocontrol agents for soil-dwelling pests.

6.0 RECOMMENDATIONS

The following actions will benefit the development and commercialisation of insect-resistant transgenic sugarcane, and the development of milky disease as a biocontrol agent:

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