2010-11 Annual Update / Final Report

Client report summary:

Key:	PROJ-14033-NEG-AGR C10X0815-CR-2
Project:	Exploiting Opprotunities from Forage Plant Genomics
Contract ID:	C10X0815
Investment process:	NEG 2006 Negotiated
Organisation:	AGR AgResearch Limited
IMS assigned to:	s9(2)(a)
Reporting period:	01/07/2010 to 30/06/2011
Contract total value:	$11,732,667.05

Annual Update 2010/11.

Outcome Benefits to New Zealand.

New Zealand faces a number of major environmental issues with the intensification of pastoral farming. For example, in the dairy sector due to the increased stocking rate and average cow live weight there has been a 57% increase in live weight over the last 30 years (1989-2010) – Dairy NZ, McDonald and Clark. The rate limiting step in our productivity is the feed supply and our pastoral grazing system is also the cause of a number of the environmental issues (GHG emissions and N leaching). Despite recent advances (*e.g.*, "high sugar" grasses and novel endophytes) we require multiple step changes in our forages to address these challenges as a substantial shift toward cut and carry feed based systems is uneconomic.

Our approach is to increase the quality of our forages by increasing the metabolisable energy (ME) and improving the rate of digestion. These targets cannot be achieved by plant breeding alone (even with marker assisted selection) so we have three targets: increased lipids; increased water-soluble carbohydrates; and an improved rate of cell wall digestion (these targets use biotechnology to assist breeding or involve genetic modification of perennial ryegrass or the ryegrass endophyte). We also have a programme focused on developing an improved endophyte that better protects the ryegrass crown and root system.

This programme is closely linked with the seed industry and builds on the research on the conventionally developed AR1 and AR37 commercial endophytes to apply biotechnology to provide increased knowledge and new options for endophyte development.

The most advanced area focused on ME is a GM technology termed DGAT1/Cysteine Oleosin that will increase the ME of forages by doubling lipid levels in vegetative tissue and potentially the available biomass by 25%. This technology has been implemented in perennial ryegrass (this contract), alfalfa and white clover (by PhytaGro Corp). Modelled benefits of a GM high ME perennial ryegrass include for the dairy industry:

- 6%-12% increase in Milk Solids production.
- 17% decrease in farm GHG emissions.
- $900 per ha increase in farm revenue leading to potential market benefit of up to $2B p.a. With a market adoption of 20% the potential benefit to the pastoral industries could be around $400M p.a.

We have also made progress with the development of forages with consistent levels of water-soluble carbohydrate (WSC). Last year we were able to dissect the strong Gene × Environment and Gene × Management regulation of fructan WSC in conventionally bred "high sugar" ryegrass varieties. This year Pastoral Genomics has co-

funded further development and we have produced GM ryegrass plants with increased levels of fructan WSC where the fructan levels obtained were as high as the best conventionally bred "high sugar" varieties. The major difference was that WSC levels were unaffected by the Gene × Environment and Gene × Management regulation seen in the conventionally bred lines. We have utilised the funding in this contract to cross these GM traits into a high sugar (high WSC) ryegrass genotype to determine the maximal levels of WSC that can be obtained.

Work on improving the rate of cell wall digestion is split into several funding streams and this contract supports those areas. We have expressed cellulases in model plants and the ryegrass endophyte. If these approached lead to applications in ryegrass that increase the rate of cell wall digestion in the animal this would have major environmental and production benefits for farmers.

Ryegrass containing an endophyte expressing significant levels of the bioactive lolines would benefit farmers through increased pasture persistence due to the demonstrated protective effects of lolines on the plant crown and root system. This would result in decreased insecticidal use and increased dry matter production especially in dry conditions (pasture with damaged root systems performs poorly when soil moisture levels drop). When endophytes from tall fescue (that normally produce high levels of loline) are transferred to perennial ryegrass the loline levels are only 1-10% of that seen in tall fescue (and insufficient for plant protection). We have identified the genetic switch that causes this effect and produced experimental lines of GM endophyte where levels in perennial ryegrass are equivalent or greater than that seen in tall fescue.

A number of challenges face the commercialisation of GM crops in NZ including but not limited to the cost (approximately $20M for breeding, animal feeding trials and regulatory costs), the current challenging GM legislation under the HSNO act, containment and a concern by the pastoral industries for possible negative impacts on their markets. All these factors contribute to the issue of demonstrating that the high ME forages will have the expected nutritional benefits and subsequent increase in productivity and environmental benefits. We have developed a strategy in consultation with the NZ pastoral industries that includes overseas field trials and this is outlined in the next section on the implementation pathway.

Implementation Pathway.

AgResearch has well established relationships with all seed companies and has commercial relationships with a number of them. Grasslanz Technology Ltd, an AgResearch subsidiary, is a plant technology provider to farmers (end-users). Grasslanz is the world's premier source of grass endophytes and white clover, and a world leader in the development of temperate forage cultivars. Grasslanz works closely through the entire cultivar development process with its seed company partners. Grasslands Innovation, which is an incorporated joint venture between Grasslanz and PGG Wrightson Seeds (PGGWS) creates an exclusive pathway to market for perennial ryegrass cultivars. AgResearch underpins Grasslanz competitive advantage through exclusive access to conventional plant breeding, plant genomics, microbial genomics and a strong base of fundamental and applied plant and animal sciences. The subsidiary's main functions are to licence the use of AgResearch's seed products to seed companies, fund and manage R&D, produce nucleus seed and protect intellectual property.

Initial outputs from this contract can be in the form of molecular markers for complex plant genetic traits such as the water-soluble carbohydrate compounds the fructans. The knowledge is applied by the Markers 2 Market programme (AgResearch, Pastoral Genomics and the seed industry). This programme will ensure that the marker assisted selection technology we have developed over the last decade can help make positive improvements to complex forage traits and assist the breeders and seed companies to deliver improved cultivars.

The endophytes currently under development in this programme include both conventional strains selected for specific attributes and GM strains to improve plant biocontrol (increased lolines) and an improved rate of plant cell wall digestion. Conventional endophytes will be commercialised following the established route via Grasslanz Technology. GM endophytes face similar issues to GM forages and a similar strategy to that outlined next for GM forages could be used.

Longer term outputs potentially include genetically modified forages. We have made significant progress with one trait, high metabolisable energy (ME). As the technical aspects of this programme have progressed we have consulted

with the pastoral industries over the issues NZ faces with GM forages. The pastoral industries have not ruled out GM forages but expressed a desire that initial field trials be performed overseas. For the time being there is a desire for NZ to be a market follower rather than a market leader. As the timelines for delivery of GM seed to the farmer are lengthy (and costly), expected to be in 2021 we do not see this requirement as a barrier but rather an opportunity to share the risk with a larger AgBio player. Our strategy has therefore been to include high ME forages as a package in our two spin out companies AgResearch jointly owns with the San Diego based incubation firm Kapyon Ventures LLC.

Through the Kapyon-AgResearch partnership, the parties have formed two commercial spinouts - PhytaGro Corp (forages and bio-fuel crops) and ZeaKal, Inc (major row crops).

The spinouts are currently commercializing a novel plant yield platform with a specific focus on raising lipid content in both seed and vegetative tissue. The underlying portfolio has been shown to not only increase the oil yield/quality of traditional oil seed crops, but also extend seed-like oil production mechanisms into the vegetative tissue of second-generation feed and fuel crops that currently have low energy density. Both companies are being prepared for sale.

All the AgResearch IP in both PhytaGro and ZeaKal is licensed in and so will remain owned by AgResearch even after the two companies are sold. Forage species of importance to NZ will be included in the licenses that will be sold to a purchaser. In order to ensure that NZ farmers are not prevented from accessing these technologies in the future we will ensure that:

- The licenses exclude NZ, ensuring that AgResearch will always be free to develop the product for NZ;
- Any purchaser acquiring the Intellectual Property rights must develop the technology in the licensed forage species within a specified time frame or lose the right to do so;
- In the event that a purchaser does develop a new cultivar in a species important to NZ, they will make all endeavours to make the technology available in NZ.

As we progress with this strategy the companies will be offered for sale to NZ seed companies and regardless of the outcome we are working with both PGGWS and Agriseeds on a NZ focussed breeding strategy to ensure that when allowed, a high ME GM forage suitable for the NZ market will be available (estimated to be 2021).

Research, Science and Technology (RS&T) Benefits to New Zealand.

This programme has delivered the following benefits in the last 12 months:

- Increased knowledge of forage grass endophyte chemistry and genetics and genomics;
- Identification of the regulation of loline biosynthesis in endophytes and demonstration the endophytes can deliver valuable recombinant proteins;
- Increased understanding of lipid biosynthesis in plants with 3 patent applications or PCT filings forming the cornerstone technology portfolio of spin out biotechnology companies PhytaGro Corp and ZeaKal, Inc;
- Improved understanding of WSC production in forage grasses and approached to improve WSC levels in marker assisted breeding programmes;
- Development of the metabolomics capability for analysis of GM plants expressing output traits, and for analysis of plant cell wall polymers;
- Identification of plant cell wall degrading enzymes suitable for improving the rate of forage cell wall digestion and the opportunity to further utilise these in the production of cellulosic biofuels.

Publicly available information

New Zealand depends on agriculture for a substantial proportion of its income. In 2007, agricultural exports represented 48% of all export products. The pastoral sector exports alone were valued at $16B in 2007. This high dependence on agriculture will certainly continue well into the future as the world's burgeoning middle-class increasingly demands those high-quality food, fibre and health-related products that New Zealand is ideally placed to

deliver. The feed supply for New Zealand's agricultural animals is the biggest constraint to animal production. This is highlighted in the Strategic Frameworks of both the dairy and meat & wool industries.

We have initiated a new programme that follows from the successful 6-year contract C10X0203. This programme will build on the progress or the plant genomics platform and deliver new forage cultivars and endophyte strains for the pastoral industries. The objectives within our programme are specifically intended to address the feed constraint equally valid for the meat and dairy industries. These targets will not only have production benefits but will also have significant environmental benefits.

We will develop perennial ryegrass with consistently elevated levels of water-soluble carbohydrate (WSC).

Increasing the WSC content would provide the rumen microflora with a readily available energy source and reduce their fermentation of protein, which results in increased nitrogen supply to the ruminant and reduced nitrogen losses through excretion.

We will also increase the metabolisable energy of perennial ryegrass by up to 10% by developing lines that contain elevated levels of protected leaf triglycerides.

We also intend to increase the rate of fibre digestion by modifying the linkage of cell wall polysaccharides to lignin and other wall polymers. Increasing the rate of fibre digestion would not only provide more energy but also reduce the time required to pass through the rumen and therefore reduce nitrogen losses.

Endophytes for the Future Farm focuses on developing the next generation pasture endophyte to target insect pests above and below ground that are not affected by the AR1 endophyte (a commercial strain released in 2001). This will improve pasture productivity and resilience by both reducing insect predation and improving plant access to water and nutrients through healthier root systems.

This year we have made significant progress towards these objectives with the main highlights being:

Objective 1:

We are using biotechnology to understand how ryegrass makes the water-soluble carbohydrate fructan. To do this, experimental genetically modified (GM) ryegrass lines containing laminar (leaf) fructans at levels greater than the conventionally bred high sugar grasses have been developed.

This modification overcomes the strong gene x environment control of fructan levels normally seen in ryegrass that results in sub-optimal levels unless the plants experience a prolonged cold period. This project is now aligned with the Pastoral Genomics Consortium. We have crossed this trait into conventional high sugar lines to determine the maximal level of water-soluble carbohydrate that can be obtained. We are also utilising the knowledge gained in a marker assisted breeding programme to develop a conventional ryegrass variety with more reliable levels of water-soluble carbohydrate that what is currently available.

Objective 2:

We have demonstrated that is possible to double leaf triacylglyceride lipids in a model plant by encapsulating the additional lipids with a novel oleosin protein. This has been implemented in alfalfa, white clover, and perennial ryegrass. The ryegrass lines will contain 10% greater metabolisable energy.

Modelling has shown that a farm using this ryegrass would not only have increased productivity but also significant environmental benefits due to a 17% reduction in greenhouse gas emissions. This is a result of the increased quality of the animal feed. Currently there are no forage plant species that could deliver these benefits so a GM ryegrass like this would provide new options for farmers in New Zealand when it was eventually available.

We have formed two spin out companies, PhytaGro Corp and ZeaKal Inc to commercialise the novel oleosin technology in biofuel crops, forages, and major row crops in international markets.

Objectives 4 and 5:

We have developed a 4 in 1 molecular assay to test endophyte viability in commercial seed. This will offer a cheaper and faster option for industry than the current immunological test. The new system is currently being beta-tested. We have also successfully increased the level of lolines in experimental strains of endophyte in perennial ryegrass. This is a major breakthrough as we have identified the genetic switch which has so far limited the levels we can obtain in ryegrass to about 10% of the required level for an effective biocontrol agent.

Objective 6:

We are exploring the use of the ryegrass endophyte to deliver novel traits in a ryegrass cultivar. Currently we are testing enzymes that increase the digestibility of the grass in the animal rumen.

These enzymes would be sequestered by the endophyte and only released during digestion. This year we demonstrated that the enzymes are active and the next step is to perform in vitro digestion assays of plant material to measure to increased rate of digestion expected.

Five key achievements.

1. Cysteine Oleosin - Oleosins allow oil bodies in seeds to become tightly packed organelles. Oils in the leaf are used as short-term intermediate metabolites and are catabolized in the cell. No one has yet achieved any significant increase in oil accumulation in plant leaves. Cysteine Oleosin addresses these shortcomings by creating an encapsulation system that enables the accumulation of neutral lipids in vegetative tissues (ryegrass) at all stages of development.

2. Lolines in Ryegrass - Two regulating genes for loline biosynthesis in the endophyte have been overexpressed and the GM endophytes introduced into ryegrass. The plants grown in containment have high levels of lolines, an order of magnitude greater than the non-GM strains. These levels if expressed in field conditions would protect ryegrass against a range of insect pests not currently affected by the commercial AR1 and AR37 endophytes.

3. A 4 in 1 diagnostic test for endophyte viability has been developed with supporting co-funding from Grasslanz Technology Limited for the seed industry. This is currently being beta-tested and if able to be scaled up, will replace the immunological test that is currently used. The compelling feature of this test is it can measure viability, strain, percent infection and detect any contamination in a single test.

4. AgResearch has a formal partnership with Kapyon Ventures LLC, a San Diego based technology incubation firm. Through this partnership, the parties have created two commercial spinouts - PhytaGro Corp (forages and bio-fuel crops) and ZeaKal, Inc (major row crops) to commercialize the IP portfolio in global agricultural markets. AgResearch is currently looking to sell both companies and is engaging with AgBio and seed companies both in New Zealand and internationally.

5. We have shown that the ryegrass endophyte can be used to deliver novel traits into ryegrass. Several GM strains that express functional plant cell wall degrading enzymes have been introduced into ryegrass. These plants are being grown in containment and will be used in in vitro digestion experiments to determine if the rate of cell wall digestion has increased. Endophytes are not spread by pollen so these strains would have a greater level of containment than a GM trait in ryegrass.

End-User relationship

AgResearch met in early July with representatives of the seed industry stakeholders PGGWS, Agriseeds, GTL and Pastoral Genomics for a technical meeting of the NZ Forage Consortium. AgResearch updated progress on developing High Metabolisable Energy (ME) ryegrass development programme. The High ME ryegrass will potentially provide significant environmental benefits (17% reduction in GHG) as well as increased farm productivity. The NZ Forage Consortium has a shared interest in developing GM forages to address requirements and provide opportunities for NZ pastoral farmers. Plans is in place to coordinate breeding and initial overseas field trial efforts.

Highlights include:

1) Development of Cysteine Oleosin technology to stably increase lipid levels in the vegetative portions of plants to increase metabolisable energy (ME).
2) Development of GM ryegrass, white clover and alfalfa with increase ME.
3) Development of a 4 in 1 diagnostic test for endophyte viability for seed industry (currently being beta tested).
4) Development of experimental endophyte lines with significant levels of lolines in ryegrass Through this program and through internal AgResearch investment and in collaboration with other research organizations across the world, we have developed a novel and well validated IP portfolio that has been shown to:
    - Increase the energy density of forage crops through the elevation and stable accumulation of neutral lipids in the vegetative tissue;
    - Potentially improve feed conversion by as much as 15%;
    - Increase carbon fixation and biomass yield by up to 50% in forages;
    - Elevate oil yields by up to 34% in seed crops;
    - Modify the property of oil bodies for delivery of high value oil products.

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