Summary

Summary

Client report summary:

Key:	CONT-47267-CRFRP-AGR C10X1603-CR-3
Project:	Forages with Elevated Photosynthesis and Growth
Contract ID:	C10X1603
Investment process:	CRFRP 2016 Contestable Research Fund - Research Programmes
Organisation:	AGR AgResearch Limited
IMS assigned to:	Redacted
Reporting period:	01/07/2018 to 30/06/2019
Contract total value:	$11,500,000.00
Team:	

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Progress Reporting

Annual Update

2018-19 Annual Update

Introduction

This programme is focused on developing knowledge on the mechanism for enhanced photosynthesis in genetically modified (GM) High Metabolisable Energy (HME) ryegrass and supporting industry co-funding for overseas field trials and eventual animal nutrition trials of HME ryegrass. Several significant achievements in the last 18 months have advanced our understanding of this technology or have led us to refine our hypothesis of the mechanism for increasing carbon assimilation (photosynthesis). We also overcame some significant challenges in the breeding pipeline caused by the multicopy nature of the Gene Gun transformed HME ryegrass. The solution was to utilise *Agrobacterium* mediated transformation that has a higher frequency of single copy integrations of the HME Gene Cassette. We demonstrated that these plants have the expected HME phenotype of enhanced photosynthesis, increased growth rates and increased leaf fatty acid content. We have also altered the breeding strategy to minimise plant phenotypic variation inherent in perennial ryegrass germplasm and to utilise elite germplasm provided by the seed company partners. We have also greatly expanded our activity on raising farmer and industry awareness of HME ryegrass.

Detailed Summary of Main Focus Areas

Impact Statement 1: Carbon Dioxide Recycling in HME Ryegrass

A Novel Mechanism for Enhancing Photosynthesis

It had been our hypothesis that one of the mechanisms is reduced photorespiration (the process of carboxylation where plants with C3 photosynthesis fix oxygen and release carbon dioxide). This would therefore predict that in elevated atmospheric carbon dioxide, HME ryegrass would lose its growth advantage over non-GM ryegrass. However, experiments demonstrated that in elevated carbon dioxide while non-GM ryegrass had increased growth rates, HME ryegrass continued to maintain its growth advantage over non-GM ryegrass. This indicates that decreased photorespiration is only part of the explanation for the enhanced photosynthesis. We have made a major leap forward in our understanding of the mechanisms for enhanced photosynthesis in plants with the HME technology. Research in Impact Area 1 supported by our parallel SSIF project has led us to a revised hypothesis:

Redacted

Due to this finding we were invited to publish the study in a special issue of the *Journal of Experimental Botany,* and this will occur later in 2019. Photosynthesis is a complex 156-step biochemical process of interacting pathways. This is a well-studied process and international researchers have aimed to enhance photosynthesis via a step-wise improvement at specific stages of the pathway. Redacted dioxide recycling and reduced photorespiration contribute to enhanced photosynthesis, but they are only partial contributors.

This will enhance international efforts to improve crop yields and food security. The technology is expected to enhance photosynthesis in many crops with C3 photosynthesis, although each crop will need the technology optimised. This was identified by ZeaKal Inc. (AgResearch has licensed the technology to ZeaKal for row crops, biofuel crops and algae, all non-forage applications) in their soybean programme where they were able to enhance photosynthesis for part of the growing season, increasing seed yield by up to 5% and oil yield per ha by up to 17% but it is thought the potential is even greater. ZeaKal partnered with Corteva Agrisciences in early 2019 to increase the scope of the programme and access improved technology. We also believe it will be valuable to test the technology in crops with C4 photosynthesis (e.g. Corn).

Within Impact Area 1 we had planned to use carbon isotope partitioning to study the metabolic pathways relating to photosynthesis. Our Post-Doctoral fellow working on this project took another position 18 months ago and we have not been able to find someone with similar skills. This put the work behind schedule, but we are now getting this research back on track. Our solution was two-fold: We are developing a PhD project to continue some of this work that will be registered with the University of Otago. Secondly, we adopted the RNAseq technique to look at the changes in gene expression in HME ryegrass. This has helped improve our understanding of the mechanisms for enhanced photosynthesis and it helped us in refining the hypothesis for the mechanism leading to enhanced photosynthesis.

Impact Area 2: Nitrate Utilisation in HME Ryegrass and Other Species

Research in this area is split into grasses and legumes with grasses including ryegrass and rice (as a model species), and legumes including alfalfa and now soybean. The overall goal of this research has been to understand the nitrogen requirement of different species and their responses to different nitrogen forms, nitrate, ammonia and urea. We made significant progress in ryegrass and this was published at the end of 2018. The key findings were that HME ryegrass utilised all three forms of nitrogen, but the maximum growth responses were to reduced forms of nitrogen (ammonia and urea).

We had intended to use rice as a model in Impact Area 2.1.1, and generated over 100 HME rice plants in the parallel SSIF programme. When these were characterised, we discovered that the trait expression was significantly higher than we have ever seen in ryegrass. A majority of the plants had very high levels of leaf fatty acids and we think we pushed them too far. This is most likely due to us using the same gene construct used in ryegrass and the genes are regulated by rice regulatory elements (so very high levels of gene expression). We do have a subset of 15 plants with much lower levels of expression and we are developing homozygous see populations for further analysis. We hope to complete this work by 30 June 2020, this is later than the 30 September 2019 due date for impact area 2.1.1 (however it will not delay any other areas of work and Impact Statement 2 will be completed by 30 september 2020 as planned).

In the meantime, we have added PhotoSeed soybean (provided by Zeakal), as an extension to our model workl. This also allows us to look at rhizobium symbiosis. So far from field studies in the USA we have identified that rhizobium symbiosis is normal, and the plants can perform under different nitrogen regimes. As our glasshouse-based work relies on the brand new PC2 glasshouse facility at the AgResearch campus in Palmerston North (due to receive PC2 status by 30 September, 2019), we will be a little late finishing. It is anticipated we will also complete this phase by 30 June 2020, so still before the 30 September 2020 completion date for Impact Statement 2.

Impact Statement 3: Nitrogen and Water Use efficiency in HME Plant Species

The aim of this research area is to determine if HME trait expression in transgenic plants alters plant nitrogen metabolism. This goal is different from the research in Impact Statement 2 on nitrate utilization as it is more encompassing and focuses on overall plant nitrogen metabolism. We are also examining water use efficiency and other stress responses such as light and temperature.

Research Aim 3.1: Nitrogen Use Efficiency

We performed controlled environment experiments on HME ryegrass event ODR4501 and looked at its ability to utilize nitrate, ammonium and urea. HME ryegrass shoot dry weight increased across the entire nitrogen supply range regardless of nitrogen form, whereas the non-GM control ryegrass shoot dry weight did not significantly increase beyond 7.5 mM nitrogen supply. At 10 mM nitrogen supply, HME ryegrass shoot dry weight was 27-34% greater and root dry weight was 25-45% greater than in the non-GM control ryegrass. Total plant percent nitrogen and the shoot to root ratio was lower for plants supplied with nitrate than with ammonium or urea but did not differ between the non-GM control and HME ryegrass. This suggested that HME ryegrass has a similar nitrogen utilisation efficiency and biomass partitioning.

We will now be able to examine nitrogen utilization in a range of other species. Of particular interest is the legume species alfalfa and soybean. As these species can form symbioses with the nitrogen fixing bacterium *Rhizobium*, they are provided with a source of nitrogen in the form of ammonium.

Research Aim 3.2 Water Use Efficiency

HME ryegrass has increased stomatal conductance and an increased theoretical water use efficiency (WUE) referred to as intrinsic WUE. This research aim is focused on determining what the actual WUE is in controlled environment experiments.

This year we had planned to repeat these experiments with HME rice. The first set of HME rice were developed on 2016/17 and we developed homozygous populations this year. However, after completing this step we found that we had selected events where the expression was too high, and the homozygous plants had a growth penalty. Therefore, we have repeated this process and generated more HME rice and we are selecting lines with more appropriate expression. We should be on track to complete this research on time in mid-2019.

Impact Statement 4: Creating Genetic Material and Knowledge for Overseas Field Trial Assessment of Forages

Overcoming Major Technical Challenges

We have developed HME ryegrass using two different transformation techniques; the Gene Gun and *Agrobacterium*. The Gene Gun system was the method used to provide proof of concept of the HME technology in ryegrass. A negative aspect to the Gene gun is a high frequency of multi-copy insertions of the transgene in the plant chromosomes. When we initiated the breeding stage and started crossing our primary transgenic T_0 plants with elite ryegrass germplasm we identified that the multi-copy transgenes were segregating in the progeny. This made the breeding very challenging and the initial material used in the 2017 and 2018 field trials had a partial HME phenotype.

Our solution was to utilise the *Agrobacterium* transformation method. We had identified the multi-copy insert risk some time ago and in 2016-2017 had invested significant effort in getting an efficient ryegrass *Agrobacterium* transformation system up and running. This enabled us to develop over 100 *Agrobacterium* derived primary transgenic T_0 HME ryegrass events from 2017 through to 2019. Over 30% contained single copy insertions of the transgene cassette. A critical question was if a single copy insertion of the HME transgene cassette would be sufficient to confer an HME phenotype? We determined this was the case over the last 12 months.

A challenge we have encountered for several years is comparing different HME ryegrass plants developed with different transformation techniques over different experiments, with limited controlled environment room capacity. Therefore, we developed a Relative Growth Rate (RGR) Assay to enable the ranking of individual HME ryegrass events. This novel process has enabled us to prioritise plants going into the breeding pipeline.

The RGR assay synchronises plant growth and through replication we are able to consistently rank and compare different HME ryegrass events. The assay has enabled us to identify a balance between enhanced growth and fatty acid content. In ryegrass there is a “sweet spot” for optimum expression where we obtain maximum increases in plant growth when the leaf fatty acid content is 60% greater than control plants. We still have a growth advantage at higher increases in leaf fatty acids, but the growth advantage begins to decline. This has helped us to rank and select events for the breeding pipeline. It has also suggested that we need to progress a range of events as we may want to trade off some enhanced growth for greater levels of leaf fatty acids especially due to the benefit of increased dietary fat in reducing ruminant methane emissions.

The programme co-funding supported the 2018 and current 2019 field trials in the mid-West of the USA. The 2018 trial confirmed the value of utilising mini-swards in the trial rather than space plants. This better simulates the conditions in pasture. We also identified one T_2 (second generation) family of HME ryegrass family ODR6205 that needed further analysis in the 2019 trial. The 2019 trial contains three separate experiments. One is focussed on the AR1 endophyte and the other two focus on the ODR6205 family. We have one experiment where we cloned plants to increase replication and have seven replicated swards of transgenic and seven replicated swards on null siblings. The third experiment contains additional families of ODR6205. The goal is to determine if the high lipid phenotype seen in containment is also seen in the field. A secondary goal is to determine if there is a growth advantage of HME ryegrass in a sward situation.

Research Aim 4.1: HME Ryegrass Trait Fixing

This research aim supports the industry funded field trials for HME ryegrass in the mid-West of the USA. Perennial ryegrass is an obligate out crossing species and therefore requires two crosses to different parental genotypes prior to the cross used to generate homozygous seed. Each generation is designated as follows: T_0 is the primary transgenic plant; T_1 is the first progeny from a cross and so on until at T_4 we have uniformly homozygous seed. Uniformly homozygous seed is required as the HME trait has a gene dosage effect so that homozygous plants have up to double the expression of hemizygous plants. It is also important for plant breeding as we need to deliver a product where the trait is expressed in every seed.

We have basically completed this objective and developed T_3 and T_4 seed populations. We are now focussing on the *Agrobacterium* derived HME ryegrass plants. This has involved a further innovation to reduce the plant phenotypic variation seen in the breeding pipeline for the Gene Gun derived plants.

We had encountered significant plant to plant phenotypic variation in T_2, T_3, and T_4 progeny. This was expected however the initial plan was to make selections in the field and return them to the lab for further crossing stages. We were unable to do this as the trials were in the USA. Our solution was co-developed with the Technical Advisory Group and we used crossing into individual elite plants rather than a cultivar. We will be assessing the success of this new approach in the 2020 field trial. We also have a breeding pipeline with cycles that take plants through in batches so that we can progress as rapidly as possible in the constraints of PC2 containment.

Another component of this research (Impact Area 4.4) is examining the fate of the increased fatty acids in the animal. As we have yet to conduct animal nutrition studies, we are using *in vitro* Rumen assays. We reported on progress last year which demonstrated a 15-23% reduction in methane which is consistent with nutritional studies. In the last year we have completed further analysis including in vitro rumen assays on ensiled HME ryegrass. The ensiling process leads to major changes in the plant energy constituents. This is a fermentation process and one of the components that is altered is plant fatty acids. We identified that the additional fatty acids encapsulated in the artificial micro organelles is stable and resistant to biohydrogenation in the rumen. We see a 10-15% reduction in methane emitted. We have submitted the study for publication in the *Journal of Dairy Science* and expect this to be in press shortly.

In a related research programme funded by MPI we performed the first continuous flow fermentation study. The results of the study were inconclusive mainly as the plant material used did not express the full HME phenotype (due to the gene segregation issue we discussed earlier). This is going to be repeated in the next six months with HME ryegrass with the full phenotype (enhanced growth and 6-7% leaf fatty acids based on the dry weight).

Impact Statement 5: Farmer Focus Groups

The main purpose of the High Performance HME Grasses extension program is to raise farmer and their rural advisors' awareness and understanding of High-Performance Grasses. So that they can make informed decisions about possible use of the grasses, if and when they are released for public use.

The 2018-2019 raising awareness and understanding extension activities comprised: i) focus group meetings with Maori, sheep and beef and young farmer groups; ii) rural professional focus group meetings; iii) presentations to industry groups; and iii) investigation into and the development of further communication and extension tools.

In summary, the four focus group meetings produced very similar results to the focus group meetings held in 2018-2019. The key findings from two years of focus group studies were that:

i) the large majority of participants showed high levels of interest in the HME ryegrass research and learning more about it;

ii) the majority of participants would like to learn more about the HME ryegrass research;

iii) the preferred forms for learning about the research vary a little depending on the age of the person but did not differ significantly between industry sectors, or ethnicity;

iv) only women in farming questioned the possibility of there being harmful effects for humans who ate or drank products from animals that had eaten GM forages; and

iv) there were no significant differences between farmer and rural professional's interest levels, desire for further information and preferred information sources. One consistent point that came from across the different groups was, 'What are AgResearch doing, or going to do about a wider debate with the general public about the possible use of GM forages in New Zealand farming systems? This is addressed in the final paragraph discussing the ***AgResearch Gene Technology Impact Group***.

Investigation into this project having a 'closed' website as a repository for articles and to provide a discussion forum for farmers and rural professionals showed that farmers and rural professionals would like this. However, while it is technically possible to set up a password protected website, it raises questions around criteria for inclusion, transparency of the science and the work involved in keeping the site active and current and who would do this.

Recommendations for Year 2019-2020 Extension Plan

- Focus on two regions- The extension plan work be concentrated in two regions that are readily accessible from Grasslands. Suggested regions, Manawatu/Rangitikei/ Wanganui and Wairarapa in an effort to build up a hub of interest and excitement, that will in time gather momentum which will spill over into other regions.
- Target young farmers- While keeping sheep and beef, and dairy farmers and rural professionals informed, really target young farmers across the sectors in these two regions because they are the people who are potentially going to benefit most from this new technology.
- Carry out a piece of research on Farmer Decision Making Timeframes. Because of the long timeframe of this extension program, it is very important that we understand how farmers make decisions about whether or not to adopt a new technology like the HME ryegrass. In particular, we need to identify the time it takes farmers to move through the key decision making phases as they decide whether to adopt the new technology. This information would allow us to develop a well informed, stepped and targeted extension program.
- Develop a full project, integrated Communications and Extension Plan with AgResearch communications. Decide whether this plan will include dissemination of project information to the general public. What role should AgResearch play in raising public awareness of GM products?

The team ended up making 10 presentations to various stakeholder groups over the last 12 months and supported the Dairy NZ Farmer workshops.

AgResearch has held discussions with MBIE during 2019 relating aspects of the social license of introducing GM crops, the regulatory aspects, communications and engagement with Maori. It has been agreed to expand activities and build on work in Impact Area 5 by bringing into the discussions other GM programmes on foliar condensed tannins in legumes and the ryegrass endophyte gene editing. The new initiative is called the ***AgResearch Gene Technology Impact Group***. The science teams are working with management and various stakeholders on how such a programme would be implemented.

Publicly Available Information

High Metabolisable Energy (HME) ryegrass has enhanced nutrition for grazing ruminants due to elevated lipid levels in the leaves from lipids stored in microscopic oil bodies. This is a genetically modified trait as no plant species including forages has the capacity to store lipids in this way in their leaves (although they do in seeds). The plants also have elevated photosynthesis and grow significantly faster than conventional ryegrass. The extra growth and improved nutrition will provide several benefits to pastoral farmers as they are expected to reduce nitrogen excreted by grazing animals. The increased lipids also lead to reduced methane emissions and this has been demonstrated via *in vitro* assays. We have also determined that HME ryegrass prefers urea, the form of nitrogen in animal urine, potentially providing a remediation tool to reduce nitrate leached into waterways.

We have made a major leap forward in our understanding of the mechanisms for enhanced photosynthesis in plants with the HME technology. We speculate that by behaving as uniquely stable micro-sinks for carbon, Cysteine Oleosin encapsulated lipid droplets can enhance the sink strength of leaves, reduce feedback inhibition of photosynthesis and drive greater plant growth.

The implications of this research finding support the application of this technology in other crops. The technology is licensed to the US based biotechnology compaany ZeaKal Inc. for several row crop species and is being commercialised in soybean.

This AgResearch programme is supporting industry funded field trials in the USA These trials will help us select material for regulated animal nutrition trials designed to determine if potential benefits predicted from supplementary feeding trials, animal nutrition models and *in vitro* (in the test tube) rumen assays are seen in animals fed HME ryegrass. The trials are in year three of five and are working towards identifying the best material to use for animal trials.

Key Achievements

Sequence	Key achievements

1

We have made a major leap forward in our understanding of the mechanisms for enhanced photosynthesis in plants with the HME technology. Research in Impact Area 1 supported by our parallel SSIF project has led us to a revised hypothesis:

We speculate that by behaving as uniquely stable micro-sinks for carbon, Redacted

Photosynthesis is a complex 156-step biochemical process of interacting pathways. This is a well-studied process and international researchers have aimed to enhance photosynthesis via a step-wise improvement at specific stages of the pathway. Redacted We continue to support the hypothesis that carbon dioxide recycling and reduced photorespiration contribute to enhanced photosynthesis, but they are only partial contributors.

This will enhance international efforts to improve crop yields and food security.

2

A manuscript describing the *in vitro* gas analysis of fresh and ensiled high metabolizable energy (HME) ryegrass for rumen fermentation profiling is in press in the Journal of Dairy Science.

We have shown that gross plant energy in HME ryegrass was 6-8% greater than controls. Incubation of both fresh and ensiled HME ryegrass in rumen fluid resulted in: a) less biohydrogenation of fatty acids compared to the control; b) a significant reduction in butyrate; and c) a 10-15% decrease in the methane proportion of the total gas production.

The findings on decreased methane emissions are in line with published studies on the benefits of dietary fat in reducing methane from ruminants. The *in vitro* studies are a step on the way to demonstrating the benefits of HME ryegrass and ultimately animal feeding studies are needed to verify this.

The greater gross energy is also a benefit that needs to be demonstrated in animal nutrition studies. This may translate into reduced nitrogen excretion and improved productivity.

The reduced biohydrogenation of fatty acids may translate into lower levels of saturated fat in animal products. Therefore a human health benefit may need to be investigated in future studies.

3	During the year we had a variation to contract and added Impact Area 5. The programme based on increasing awareness and understanding of HME forages is aimed to help farmers and rural advisors to make informed decisions about possible uses of HME ryegrass. This comprised: i) focus group meetings with Maori, sheep and beef and young farmer groups; ii) rural professional focus group meetings; iii) presentations to industry groups; and iii) investigation into and the development of further communication and extension tools. Key findings were that: i) the large majority of participants showed high levels of interest in the HME ryegrass research and learning more about it; ii) the majority of participants would like to learn more about the HME ryegrass research; iii) the preferred forms for learning about the research vary a little depending on the age of the person but did not differ significantly between industry sectors, or ethnicity; iv) only women in farming questioned the possibility of there being harmful effects for humans who ate or drank products from animals that had eaten GM forages; and iv) there were no significant differences between farmer and rural professional's interest levels, desire for further information and preferred information sources.
4	In Impact Area 4 supported by the SSIF programme we modified our breeding approach to fix the HME trait in homozygous populations of ryegrass. The two issues we had encountered were a) the Gene Gun derived HME ryegrass plants were difficult to breed as we encountered segregation of the HME transgene across generations; and b) we discovered phenotypic variation due to the complex genetics of ryegrass. The second issue could have been solved by making selections in the field if the field trials were based in New Zealand. We solved the first issue by using Agrobacterium derived HME ryegrass plants containing single copies of the HME gene cassette. These plants have a similar range of HME trait expression as the original Gene Gun derived plants. This will greatly simplify the trait fixing and breeding. The second issue has been solved by accessing elite breeding genotypes from industry seed companies. We have produced the first set of crosses in an introgressive crossing programme where the aim is to reduce the proportion of background genetics inherited from the T_0 parent, and finish up with a HME line that is homozygous for the transgene, in an elite background and with AR37 endophyte present.

1	Carbon Dioxide Recycling in HME Ryegrass	Impact statement	On track	The major progress we have made is in our understanding of the mechanisms for enhanced photosynthesis in plants with the HME technology. Research in Impact Area 1 supported by our parallel SSIF project has led us to a revised hypothesis: *We speculate that by behaving as uniquely stable micro-sinks for carbon,* Redacted Photosynthesis is a complex 156-step biochemical process of interacting pathways. This is a well-studied process and international researchers have aimed to enhance photosynthesis via a step-wise improvement at specific stages of the pathway. However, photosynthesis is under exquisite control due to negative feedback regulation based on the plant carbohydrate balance and the carbon:nitrogen balance. Redacted support the hypothesis that carbon dioxide recycling and reduced photorespiration contribute to enhanced photosynthesis, but they are only partial contributors.	
1.1	Infra-Red Gas Analysis	Research aim	On track		
1.1.1	IRGA analysis of Ryegrass	Critical step	Achieved		

5	A challenge we have encountered for several years is comparing different HME ryegrass plants over different experiments with limited controlled environment room capacity. Therefore, we developed a Relative Growth Rate (RGR) Assay to enable the ranking of individual HME ryegrass events. This novel process has enabled us to prioritise plants going into the breeding pipeline. The RGR assay synchronises plant growth and through replication we are able to consistently rank and compare different HME ryegrass events. The assay has enabled us to identify a balance between enhanced growth and fatty acid content. In ryegrass there is a "sweet spot" for optimum expression where we obtain maximum increases in plant growth when the leaf fatty acid content is 60% greater than control plants. We still have a growth advantage at higher increases in leaf fatty acids, but the growth advantage begins to decline. This has helped us to rank and select events for the breeding pipeline. It has also suggested that we need to progress a range of events as we may want to trade off some enhanced growth for greater levels of leaf fatty acids especially due to the benefit of increased dietary fat in reducing ruminant methane emissions.

Project Deliverable Status

Click on the deliverable to enter a status

Sequence	Short title	Type	Status	Reason	Action

1.1.2	IRGA analysis of rice	Critical step	On track with issues	We conducted IRGA analysis on a set of HME rice plants and discovered these plants had overshot the ideal window or "sweet spot" for HME expression. We have have about 100 independently generated HME rce events and about 15 have lower levels of expression. So we are focussing on these and we are developing homozygous seed. We anticipate completing this work in early 2020 and so 4-6 months behing the 30 September completion date. This will not impact the overall Impact Statement 1 if a subset of the remaining 15 rice events are shown to have a HME phenotype.	Complete analysis of second set of HME rice by 31 March 2020.
1.2	Isotope partitioning of metabolic pathways	Research aim	On track with issues	18 months ago we lost the Post Doctoral fellow working on this project and we have not been able to find someone with similar skills. Our solution was two fold: We are developing a PhD project to continue some of this work that will be registered with the University of Otago. Secondly we adopted the RNAseq technique to look at the changes in gene expression in HME ryegrass. This has helped improve our understanding of the mechanisms for enhanced photosynthesis. With our alternative methodology we expect to resolve the issues and the research will be completed on time.	Establish the joint PhD studentship with the University of Otage and continue with the alternative approach of RNAseq analysis.
1.2.1	Isotope partitioning in model species	Critical step	On track with issues	We had intended to use HME rice but as described in Impact area 1.1.2 the first set of plants were not suitable due to excessive levels of trait expression. Therefore we are re-screening for a new set of plants and secondly importing PhotoSeed soybean provided by our partner ZeaKal. These plants contain the same technology and we will be able to use RNA seq to look at pathway changes and determine if they are similar to what we have seen in ryegrass. By adding an alternative species we expect to resolve the issues and the research will be completed on time.	Screen a second set of rice and the soybean plants using RNAseq. It is expected that we will complete this by 30 June 2020.

1.2.2	Isotope partitioning in forage species	Critical step	On track	On track but using a revised technique to obtain the same outcome.	
2	Nitrate Utilization in HME Ryegrass and other species	Impact statement	On track		
2.1	Nitrate utilization in C3 plant species	Research aim	On track		
2.1.1	Nitrogen utilization in model species	Critical step	On track with issues	We have switched to using PhotoSeed soybean as our model due to problems identifying ideal HME rice plants. So far we have identified that legume symbiosis is normal and the plants can perform under different nitrogen regimes. As our glasshouse based work relies on the brand new PC2 glasshouse facility at the AgResearch campus in Palmerston North (due to receive PC2 statue by 30 September), we will be a little late finishing (this was due to be completed on 30 September 2019). It is anticipated we will complete this phase by 30 June 2020. This will not affect any other research areas and we expect to complete this work before the planned Impact Statement 2 end date of 30 September 2020.	Complete work by 30 June 2020.
2.1.2	Nitrate utilization in forage species	Critical step	Achieved		
2.1.3	Appropriate Fertilizer Composition	Critical step	On track		
2.1.4	Effects on rhizobium symbiosis	Critical step	On track		
3	Nitrogen and water use efficiency in HME plant species	Impact statement	Achieved		
3.1	Nitrogen use efficiency	Research aim	On track		

3.1.1	Assess stomatal conductance in grass species	Critical step	Achieved		
3.1.2	Measurement of NUE	Critical step	Achieved		
3.2	Water use efficiency	Research aim	On track		
3.2.1	WUE in Ryegrass	Critical step	Achieved		
3.2.2	WUE in model grass species	Critical step	On track with issues	The first set of HME rice selected had excessive levels of trait expression. We are developing homozygous seed for some events with lower levels of expression. The work should be complete by 30 June 2020.	Test new set of HME rice by 20 June 2020.
3.2.3	WUE in commercial ready ryegrass	Critical step	On track	We are making rapid progress. We need to generate homozygous commercial ready HME ryegrass and then complete this work. We have a pipeline of plants going through this process.	
4	Creating genetic material and knowledge for overseas field trial assessment of HME forages	Impact statement	On track		
4.1	Ryegrass HME Trait Fixing	Research aim	Achieved		
4.1.1	T1 Generation	Critical step	Achieved		
4.1.2	T2 Generation	Critical step	Achieved		
4.1.3	T3 Generation	Critical step	Achieved		

4.2	Commercial Ready HME Ryegrass trait fixation	Research aim	On track	The commercial ready HME ryegrass programme has taken a modified approach compared that used for the Gene Gun derived HME ryegrass carried out in Impact areas 4.1.1, 4.1.2 and 4.1.3. For the Gene Gun plant breeding we bred into populations rather than individual elite plants. This previous design intended to have in field selections of plants at each reproductive cycle. However we were unable to do this due to the regulatory requirements. We changed the breeding process to help to reduce the genotypic and phenotypic variation seen in the previous approach. The new breeding approach is working well and we have reported it as a key achievement. We are using elite industry cultivars and we have produced the first set of crosses in an introgressive crossing programme where the aim is to reduce the proportion of background genetics inherited from the T_0 parent, and finish up with a HME line that is homozygous for the transgene, in an elite background and with AR37 endophyte present.	
4.2.1	T1 Generation	Critical step	Achieved	We have the first set of crosses with seven HME events however this is designed to be an ongoing pipeline of overlapping steps as we have about 30 plants to progress. The limitation is space and capacity to analyse so we are doing this in batches.	
4.2.2	T2 Generation	Critical step	On track		
4.2.3	T3 Generation	Critical step	On track		
4.3	In vitro digestion and GHG assays	Research aim	On track		
4.3.1	Analysis of first generation Ryegrass	Critical step	Achieved	See Key achievement 2. We are publishing this work in the Journal of Dairy Science.	

Click on the deliverable to enter a status

Short title
Carbon Dioxide Recycling in HME Ryegrass

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

The major progress we have made is in our understanding of the mechanisms for enhanced photosynthesis in plants with the HME technology. Research in Impact Area 1 supported by our parallel SSIF project has led us to a revised hypothesis:

Redacted

Photosynthesis is a complex 156-step biochemical process of interacting pathways. This is a well-studied process and international researchers have aimed to enhance photosynthesis via a step-wise improvement at specific stages of the pathway.

Redacted

Action

Click on the deliverable to enter a status

Short title
Infra-Red Gas Analysis

Due Date
30/09/2019

Achievement measure
No achievement measure available

Status
On track

Reason

Action

5	Increasing farmer awareness and understanding of HME forages	Impact statement	On track	The project has received increased organisational suport from AgResearch and its stakeholders. There is a desire to expand this work to include the Condensed Tannins and Modified Endophyte projects. So the approach is highly valued by Stakeholders.	
5.1	Farmer focus groups	Research aim	On track		
5.1.1	Farmer focused groups	Critical step	On track		
5.1.2	Establish wider industry linkages	Critical step	On track		
5.1.3	Design of a farmer-led, Farmer Awareness and Understanding Raising Programme	Critical step	On track		
5.1.4	Stakeholder Feedback	Critical step	On track		
5.1.5	Monitoring and evaluation of the Farmer Awareness and Understanding Raising Programme	Critical step	On track		

Click on the deliverable to enter a status

Short title
IRGA analysis of Ryegrass

Due Date
31/10/2018

Achievement measure
No achievement measure available

Status
Achieved

Reason

Action

Click on the deliverable to enter a status

Short title
IRGA analysis of rice

Due Date
30/09/2019

Achievement measure
No achievement measure available

Status
On track with issues

Reason

We conducted IRGA analysis on a set of HME rice plants and discovered these plants had overshot the ideal window or "sweet spot" for HME expression. We have have about 100 independently generated HME rce events and about 15 have lower levels of expression. So we are focussing on these and we are developing homozygous seed. We anticipate completing this work in early 2020 and so 4-6 months behing the 30 September completion date. This will not impact the overall Impact Statement 1 if a subset of the remaining 15 rice events are shown to have a HME phenotype.

Action

Complete analysis of second set of HME rice by 31 March 2020.

Click on the deliverable to enter a status

Short title
Isotope partitioning of metabolic pathways

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track with issues

Reason

18 months ago we lost the Post Doctoral fellow working on this project and we have not been able to find someone with similar skills. Our solution was two fold: We are developing a PhD project to continue some of this work that will be registered with the University of Otago. Secondly we adopted the RNAseq technique to look at the changes in gene expression in HME ryegrass. This has helped improve our understanding of the mechanisms for enhanced photosynthesis. With our alternative methodology we expect to resolve the issues and the research will be completed on time.

Action

Establish the joint PhD studentship with the University of Otage and continue with the alternative approach of RNAseq analysis.

Click on the deliverable to enter a status

Short title
Isotope partitioning in model species

Due Date
23/12/2019

Achievement measure
No achievement measure available

Status
On track with issues

Reason

We had intended to use HME rice but as described in Impact area 1.1.2 the first set of plants were not suitable due to excessive levels of trait expression. Therefore we are re-screening for a new set of plants and secondly importing PhotoSeed soybean provided by our partner ZeaKal. These plants contain the same technology and we will be able to use RNA seq to look at pathway changes and determine if they are similar to what we have seen in ryegrass. By adding an alternative species we expect to resolve the issues and the research will be completed on time.

Action

Screen a second set of rice and the soybean plants using RNAseq. It is expected that we will complete this by 30 June 2020.

Click on the deliverable to enter a status

Short title
Isotope partitioning in forage species

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

On track but using a revised technique to obtain the same outcome.

Action

Click on the deliverable to enter a status

Short title
Nitrate Utilization in HME Ryegrass and other species

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Nitrate utilization in C3 plant species

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Nitrogen utilization in model species

Due Date
30/09/2019

Achievement measure
No achievement measure available

Status
On track with issues

Reason

We have switched to using PhotoSeed soybean as our model due to problems identifying ideal HME rice plants. So far we have identified that legume symbiosis is normal and the plants can perform under different nitrogen regimes. As our glasshouse based work relies on the brand new PC2 glasshouse facility at the AgResearch campus in Palmerston North (due to receive PC2 statue by 30 September), we will be a little late finishing (this was due to be completed on 30 September 2019). It is anticipated we will complete this phase by 30 June 2020. This will not affect any other research areas and we expect to complete this work before the planned Impact Statement 2 end date of 30 September 2020.

Action

Complete work by 30 June 2020.

Click on the deliverable to enter a status

Short title
Nitrate utilization in forage species

Due Date
30/09/2019

Achievement measure
No achievement measure available

Status
Achieved

Reason

Action

Click on the deliverable to enter a status

Short title
Appropriate Fertilizer Composition

Due Date
24/12/2019

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Effects on rhizobium symbiosis

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Nitrogen and water use efficiency in HME plant species

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
Achieved

Reason

Action

Click on the deliverable to enter a status

Short title
Nitrogen use efficiency

Due Date
30/09/2019

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Assess stomatal conductance in grass species

Due Date
28/09/2018

Achievement measure
No achievement measure available

Status
Achieved

Reason

Action

Click on the deliverable to enter a status

Short title
Measurement of NUE

Due Date
30/09/2019

Achievement measure
No achievement measure available

Status
Achieved

Reason

Action

Click on the deliverable to enter a status

Short title
Water use efficiency

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
WUE in Ryegrass

Due Date
29/06/2018

Achievement measure
No achievement measure available

Status
Achieved

Reason

Action

Click on the deliverable to enter a status

Short title
WUE in model grass species

Due Date
30/06/2019

Achievement measure
No achievement measure available

Status
On track with issues

Reason

The first set of HME rice selected had excessive levels of trait expression. We are developing homozygous seed for some events with lower levels of expression. The work should be complete by 30 June 2020.

Action

Test new set of HME rice by 20 June 2020.

Click on the deliverable to enter a status

Short title
WUE in commercial ready ryegrass

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

We are making rapid progress. We need to generate homozygous commercial ready HME ryegrass and then complete this work. We have a pipeline of plants going through this process.

Action

Click on the deliverable to enter a status

Short title
Creating genetic material and knowledge for overseas field trial assessment of HME forages

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Ryegrass HME Trait Fixing

Due Date
31/05/2018

Achievement measure
No achievement measure available

Status
Achieved

Reason

Action

Click on the deliverable to enter a status

Short title
T1 Generation

Due Date
31/05/2017

Achievement measure
No achievement measure available

Status
Achieved

Reason

Action

Click on the deliverable to enter a status

Short title
T2 Generation

Due Date
22/12/2017

Achievement measure
No achievement measure available

Status
Achieved

Reason

Action

Click on the deliverable to enter a status

Short title
T3 Generation

Due Date
31/05/2018

Achievement measure
No achievement measure available

Status
Achieved

Reason

Action

Click on the deliverable to enter a status

Short title
Commercial Ready HME Ryegrass trait fixation

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

The commercial ready HME ryegrass programme has taken a modified approach compared that used for the Gene Gun derived HME ryegrass carried out in Impact areas 4.1.1, 4.1.2 and 4.1.3. For the Gene Gun plant breeding we bred into populations rather than individual elite plants. This previous design intended to have in field selections of plants at each reproductive cycle. However we were unable to do this due to the regulatory requirements. We changed the breeding process to help to reduce the genotypic and phenotypic variation seen in the previous approach.

The new breeding approach is working well and we have reported it as a key achievement. We are using elite industry cultivars and we have produced the first set of crosses in an introgressive crossing programme where the aim is to reduce the proportion of background genetics inherited from the T_0 parent, and finish up with a HME line that is homozygous for the transgene, in an elite background and with AR37 endophyte present.

Action

Click on the deliverable to enter a status

Short title
T1 Generation

Due Date
30/06/2019

Achievement measure
No achievement measure available

Status
Achieved

Reason

We have the first set of crosses with seven HME events however this is designed to be an ongoing pipeline of overlapping steps as we have about 30 plants to progress. The limitation is space and capacity to analyse so we are doing this in batches.

Action

Click on the deliverable to enter a status

Short title
T2 Generation

Due Date
31/03/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
T3 Generation

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
In vitro digestion and GHG assays

Due Date
30/09/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Analysis of first generation Ryegrass

Due Date
29/06/2018

Achievement measure
No achievement measure available

Status
Achieved

Reason

See Key achievement 2. We are publishing this work in the Journal of Dairy Science.

Action

Click on the deliverable to enter a status

Short title
Increasing farmer awareness and understanding of HME forages

Due Date
30/09/2021

Achievement measure
No achievement measure available

Status
On track

Reason

The project has received increased organisational suport from AgResearch and its stakeholders. There is a desire to expand this work to include the Condensed Tannins and Modified Endophyte projects. So the approach is highly valued by Stakeholders.

Action

Click on the deliverable to enter a status

Short title
Farmer focus groups

Due Date
30/09/2021

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Farmer focused groups

Due Date
31/12/2019

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Establish wider industry linkages

Due Date
01/12/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Design of a farmer-led, Farmer Awareness and Understanding Raising Programme

Due Date
31/12/2019

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Stakeholder Feedback

Due Date
01/10/2020

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Click on the deliverable to enter a status

Short title
Monitoring and evaluation of the Farmer Awareness and Understanding Raising Programme

Due Date
30/09/2021

Achievement measure
No achievement measure available

Status
On track

Reason

Action

Project Deliverable Status (cont)

End user relationship:
On track

End user relationship comment:

Key personnel:
On track

Key personnel comment:

Research progress:
On track

Research progress comment:

Has any change event occurred in the Reporting Year?

Yes

If YES when was MBIE advised?

We renegotiated a contract change, removing HME alfalfa and adding Impact Area 5. New Contract signed off.

Work Programme Conditions

Precontract condition - 37750

AgResearch Limited must report on the progress made towards giving effect to the Vision Matāuranga policy such as evidence of steps that have been taken to identify actual or potential Vision Mātauranga opportunities linked to the proposed research, and report on these efforts and results in the annual report to MBIE.

We have greatly expanded our work on the social license of genetically modified crops. This is reported in the work for Impact Area 5. As part of this increased focus on extension Maori stakeholders have been actively engaged with the programme. This emphasis on Maori engagement along with other interest groups including young farmers and women in farming as well as more mainstream industry engagement is all part of ensuring effective social engagement and two way knowledge transfer.
