Summary

Summary

Client report summary:

Key:	CONT-47267-CRFRP-AGR C10X1603-CR-4
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:	9(2)(a)
Reporting period:	01/07/2019 to 30/06/2020
Contract total value:	$11,500,000.00
Team:	

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Progress Reporting

Annual Update

2019-20 Annual Update

Introduction

High Metabolisable Energy (HME) ryegrass is being developed as a future option for grazing pastoral farmers in New Zealand and in temperate climates internationally, to help reduce environmental impacts of grazing ruminants while increasing farm efficiency and productivity. This programme underpins the pastoral industry funded field trial analysis of HME ryegrass. Field trials in the USA in 2017 and 2018 were designed to test that the environmental conditions were suitable for HME ryegrass field trials, develop the protocols for trials and analysis and identify HME ryegrass events suitable for detailed analysis. The 2019 Field trial in the USA provided strong evidence that the increases in fatty acids, gross energy and plant growth measured in a PC2 containment facility translated into the field. The 2017-2019 trials used first generation Gene Gun derived HME ryegrass. Subsequent research has moved to new *Agrobacterium* derived Commercial Ready HME ryegrass.

During 2018 and 2019 we developed over 100 *Agrobacterium* HME ryegrass events, the *Agrobacterium* system has the advantage of a high frequency of single copy T-DNA insertion events and 30 single locus insertion events were identified. We used whole genome sequence analysis to map the exact location of the transgene insertion in 18 events and confirm the intact status of the T-DNA insertion. These all have the HME phenotype (characterised using a relative growth assay) and a range of leaf fatty acids, and the plants have entered the breeding pipeline where we are presently developing the T_2 generation. In 2020 two events have been tested in the field in the USA.

We reported last year that we had identified a novel mechanism for the enhanced photosynthesis and in the last 12 months we have progressed our understanding, patented the new invention and published the mechanism. We have evidence for altered nitrogen use efficiency in HME ryegrass.

Impact Statement 1: Carbon Dioxide Recycling in HME Ryegrass

HME ryegrass has increased levels of lipids stored in the green tissues of the plant in stable micro organelles. Increased plant growth rates are also observed although the rate of increase is affected by competition for light in densely packed sward conditions. In 2020 we filed a new patent on the major breakthrough on identifying the novel mechanism for increased photosynthesis. The allocation of different sources of carbon (sugars and fat) in different tissues is altered leading to **reduced negative feedback** of photosynthesis. This enables the overall plant energy to be increased due to greater fixation of atmospheric CO_2. This was followed up by a publication in a special edition of the Journal of Experimental Botany.

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

We progressed our analysis of HME rice from that reported last year. The challenge had been identifying HME rice with the ideal level of expression. This year we have identified 4-6 that look ideal in homozygous progeny. These were under analysis when the COVID-19 lockdown occurred. Fortunately we were able to confirm we had homozygous plants with more appropriate expression and we harvested seed. We can perform photosynthesis analysis and a water use efficiency experiment over the next 12 months.

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

We have theorised that HME ryegrass in pastoral grazing may lead to reductions in the GHG nitrous oxide via two mechanisms. Firstly, a direct effect where an alteration in the plants nitrogen use efficiency means HME ryegrass responds better to reduced forms of nitrogen and therefore has the potential to reduce nitrous oxide emissions from urine patches. Secondly, nutrition models suggest the improved animal nutrition may lead to a reduction in urine N by 6% - 7% (requires animal nutrition trials) however, it is possible to assess the plants response to nitrogen in mesocosms in PC2 containment. We have shown a 50% reduction in nitrous oxide emissions. We will repeat this experiment with further HME ryegrass

material, publish in 2021 and extend the study to the field in 2022.

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

The plant breeding activities over the next 3 years are challenging as all steps need to be conducted in PC2 containment. The process is guided by an Advisory Group made up of seed company and University representatives. The seed industry has provided elite breeding material so that HME ryegrass can be delivered in the best available cultivar once it is deregulated. AgResearch has made a significant investment in a new PC2 glasshouse complex that will allow open pollination, required for producing 20kg of seed of both HME ryegrass and a control line required for the trial in 2023. In 2020 we are developing the T_2 generation of seed in two waves, the first with three events and the second with six events. We have developed a rapid homozygous production system to enable the measurement of the gene dosage effect in each event. It is anticipated that only two or three T_2 events will move onto the T_3 generation in 2021. Field trials in 2021 and 2022 will test the suitability of T_2 and T_3 families. We are taking through multiple events as it is unclear what is the upper limit of leaf fatty acid accumulation. In PC2 containment it is possible to grow plants with 7% DW fatty acid, double the level of control plants.

Most ryegrass seed in New Zealand is sold with an endophytic fungus that provides insect resistance. We have tested the stability of the AR1 endophyte in HME ryegrass in containment and in the field in 2019. It performs in an identical manner to AR1 in non-GM ryegrass. Seed transmissibility, tiller infection rates, fungal biomass and protective alkaloid production are all identical in the lab and field (Richardson *et al.*, 2020).

Impact Statement 5: Increasing Farmer Awareness and Understanding of HME Forages

In collaboration with NZIER, AgResearch conducted a survey on farmer decision making and the key decision timeframes for a GM HME ryegrass. Key findings were:

- Some farmers are interested in the potential benefits of HME ryegrass. They may adopt it within a year or two of its commercial release. However, they want to know more about how it might perform.
- They are likely to want information early, through many channels. The best time to provide information will be after the field trials and before product release. Some farmers want information even earlier. Given the number of information sources that farmers use, and the time frames for decision making, the information should be available in many formats through many channels.
- The use of genetic modification (GM) technology creates additional complexity. Some farmers will not adopt HME ryegrass because it is GM. Other farmers would adopt HME ryegrass but recognise that GM technology is an issue with consumers and in their supply chains.
- HME ryegrass would likely be a minority of total pasture. Among potential adopters, HME ryegrass would likely be one of several cultivars used.

Implementation Pathway

One of the most significant changes to the implementation pathway is accelerating the development of *Agrobacterium* derived Commercial Ready HME Ryegrass, bred into elite industry cultivars ready for a New Zealand based plan. We currently have field trials running in the USA with this material and will conduct US based animal nutrition and greenhouse gas trials in 2023. We moved away from original Gene Gun derived HME ryegrass due to its complicated genetics which were not suitable for breeding. The Agrobacterium derived ryegrass has excellent breeding properties and we have a breeding plan co-developed with the seed industry partners.

The net benefit will be that at the end of 2023 there will be a comprehensive data package developed using HME ryegrass in New Zealand adapted cultivars for our stakeholders and the Environmental Protection Authority to assess.

Outcome Benefits to New Zealand

The research in this programme is future focussed and if HME forages are demonstrated to be a viable

product and are deregulated for use in New Zealand it would not be until 2025 or 2026. Predicted benefits are environmental including reduced greenhouse gas emissions and reduced nitrate leaching from pastoral agriculture. Production and efficiency benefits include increased animal liveweight gains, increased milk solids production in dairy and better feed solutions for farmers from higher energy forage and silage. Industry co-funders are currently defining the minimal viable product needed before they would pursue an EPA application for release.

Other Information

A new patent on sustaining photosynthesis may increase the patent portfolio life by an additional 10 years from 2029. This adds value to investors of the HME technology. It also increases the opportunity for utilisation of the technology in Pinus radiata as a future opportunity for greater plant growth and carbon capture.

MBIE/AgResearch meeting (06/08/19)

Recommendation: AgResearch to consider Iwi & Industry in the development journey. We discussed the need to plan our engagement escalation at the right time in the projects development and highlighted that 2021 was a critical year as we built towards formal EPA applications.

Publicly Available Information

High Metabolisable Energy (HME) ryegrass is being developed as a future option for grazing pastoral farmers in New Zealand and in temperate climates internationally, to help reduce environmental impacts of grazing ruminants while increasing farm efficiency and productivity. Many of the environmental impacts of pastoral farming arise from an imbalance of energy sources within grazed forages with excess nitrogen from plant protein being excreted in animal urine and dung leading to nitrate leaching and nitrous oxide emissions. In addition, the greenhouse gas methane is produced by methanogens in the animal rumen. To improve the nutritional imbalance and increase pasture energy density we have developed a genetically modified (GM) technology to produce fatty acid storing seed like micro organelles in the green tissue of forage plants. Serendipitously it was found that plants with this novel GM trait had enhanced photosynthesis and growth.

We identified a novel mechanism in 2019 and have now patented this and published the findings. All plants have exquisite regulatory systems for limiting photosynthesis and plants effectively snack on light as needed. One of the mechanisms of feedback is the carbohydrate balance in the plant. By producing additional fat in plant leaves we have reduced the negative feedback, allowing over 20% more carbon dioxide to be captured and converted into more plant biomass and energy.

High metabolizable energy ryegrass has been tested in the field in the mid-west of the USA. In 2019 we demonstrated that the plant energy, plant fatty acid and plant growth characteristics of HME ryegrass measured in PC2 containment growth chambers and glasshouses translated to the field. We also demonstrated that the endophytic fungus AR1 used in many commercial ryegrass cultivars was stable and performed identically in HME ryegrass to non-GM ryegrass.

Our research has shown that HME ryegrass has altered nitrogen use efficiency and has an improved tolerance for reduced form of nitrogen. This means it theoretically may better use nitrogen from animal urine and may reduce nitrogen run off and reduce nitrous oxide emissions.

We have continued a process of end user engagement and conducted a survey of farmer decision making in relation to the possibility HME ryegrass may be available in the future (if New Zealand decides to adopt GM). Several farmers said they would trial it on part of their farm to assess the benefits and suitability within their farming system. Some farmers raised the issues of consumers and supply chains. They said they would like more information on HME ryegrass so that they can make informed decisions. This highlights some of the uncertainty of the future use of GM technologies in New Zealand and the need for further public engagement.

Key Achievements

Sequence	Key achievements

1	**HME Technology in Ryegrass Leads to Improved Nitrogen Use Efficiency** We theorised that HME ryegrass in pastoral grazing may lead to reductions in the greenhouse gas nitrous oxide via two mechanisms. The first of these is a direct effect where an alteration in the plants nitrogen use efficiency means HME ryegrass responds better to reduced forms of nitrogen and therefore has the potential to reduce nitrous oxide emissions from urine patches. The second indirect mechanism comes from nutrition models that suggest the improved animal nutrition may lead to a reduction in urine N by 6% - 7%. The latter benefit can only be verified in animal nutrition trials planned for 2023. It is possible to assess the plants response to nitrogen in mesocosms in PC2 containment. We have completed an experiment using HME ryegrass plants expressing the HME phenotype and shown a 50% reduction in nitrous oxide emissions compared to the genetically identical control ryegrass. We will repeat this experiment with further HME ryegrass material and aim to publish in 2020 or 2021. Ultimately, we would like to extend the study to the field in 2022.
2	**Stability of AR1 Fungal Endophyte in The Field in HME Ryegrass** Most proprietary ryegrass seed in New Zealand is sold with a symbiotic endophytic fungus that provides insect resistance. An important question is, will the endophyte behave in a similar way in HME ryegrass and provide the same levels of protection against insect pests? We have tested the stability of the AR1 endophyte in HME ryegrass in PC2 containment and in the field in 2019 and have demonstrated that it performs in an identical manner to AR1 in non-GM ryegrass. The seed transmissibility, tiller infection rates, fungal biomass and protective alkaloid production are all identical in HME and control ryegrass in both the lab and field. We are testing the AR37endophyte in 2020 and this will be used in future breeding programmes.

3

Acceleration of Commercial Ready HME Ryegrass to Field in 2020 and Animal Nutrition 2023

Our 2016 Science Plan had two phases, firstly to progress Gene Gun derived HME ryegrass to proof of concept animal nutrition trials in 2021; and in parallel developing Agrobacterium derived Commercial Ready HME Ryegrass bred into elite industry cultivars ready for a New Zealand based plan starting at the completion of the US trial phase. We have accelerated the Commercial Ready HME Ryegrass phase and have field trials running in the USA and will conduct US based animal nutrition and greenhouse gas trials in 2023.

We reported last year that the complicated genetics of the Gene Gun derived HME ryegrass made breeding problematic. Our solution was to push forward the Agrobacterium derived Commercial Ready HME Ryegrass (Research Aim 4.2) into the USA field testing phase and use this for animal nutrition. While this would delay the USA based animal nutrition trials until 2023, the benefit was we will have a much earlier result with the Commercial Ready HME Ryegrass.

We have developed and validated a comprehensive breeding plan with the industry to produce the seed needed in early 2023 for animal nutrtion and GHG trials.

4

New patent on sustaining photosynthesis and publication on HME mechanism

HME ryegrass has increased levels of lipids stored in the green tissues of the plant in stable micro organelles. Increased plant growth rates are also observed although the rate of increase is affected by competition for light in densely packed sward conditions. In 2020 we filed a new patent on the major breakthrough on identifying the novel mechanism for increased photosynthesis. The allocation of different sources of carbon (sugars and fat) in different tissues is altered leading to reduced negative feedback of photosynthesis. This enables the overall plant energy to be increased due to greater fixation of atmospheric CO_2. This was followed up by a publication in a special edition of the Journal of Experimental Botany.

Beechy-Gradwell, Z., Cooney, L., Winichayakul, S., Andrews, M., Hea, S-Y., Crowther, T., and Roberts N., (2020) Storing carbon in leaf sinks enhances perennial ryegrass carbon capture especially under high N and elevated CO2. **J. Experimental Botany** 71:2351-2361.

The publication received international recognition and was supported by an accompanying review article by MJ Paul and PJ Eastmond, two recognised photosynthesis experts. Turning sugar into oil: making photosynthesis blind to feedback inhibition. **J. Experimental Botany** 71:2216-2218.

5	**Translation of HME Trait from Laboratory/Glasshouse into the Field** The industry funded 2019 field trial in the USA provided strong evidence that the increases in fatty acids, gross energy and plant growth measured in a PC2 containment facility translated into the field. The trial used Gene Gun derived hemizygous HME ryegrass at an intermediate T2 generation of the breeding process. Under controlled PC2 conditions the HME ryegrass progeny had 34% higher leaf fatty acids compared to the null controls. This delivered a 0.5 kj/gDW increase in herbage gross energy concentration and a 6-10% increase in herbage growth rate advantage in HME ryegrass mini-swards compared to null controls. In the field trial in the USA HME ryegrass swards exhibited a 15-24% higher mid-season herbage fatty acid content than null controls swards, and 29-30% higher end-of-season herbage fatty acid content. This coincided with a 0.3-0.5% kj/gDW higher end-of-season gross energy. Herbage growth rates were generally similar for HME and null control swards although we observed a 27% higher end of season herbage growth rate. Further evidence for trait efficacy comes from soybean where ZeaKal Inc achieved an 18% increase in oil and 2.6% increase in protein per acre in replicated field trials.

Project Deliverable Status

Click on the deliverable to enter a status

Sequence	Short title	Type	Due Date	Status	Reason	Action
1	Carbon Dioxide Recycling in HME Ryegrass	Impact statement	30/09/2020	Achieved	Patent filed on the novel mechanism for reducing negative feedback of photosynthesis. Publication of research in Journal of Experimental Botany in 2020.	
1.1	Infra-Red Gas Analysis	Research aim	30/09/2019	Achieved		
1.1.1	IRGA analysis of Ryegrass	Critical step	31/10/2018	Achieved		
1.1.2	IRGA analysis of rice	Critical step	30/09/2019	Not achieved	The first set of HME rice developed for this aim was not suitable, as expression had overshot the ideal expression window (reported last year). We developed HME rice that was more suitable and during the early part of 2020 the COVID-19 lockdown prevented us from performing any detailed experimentation, although we were able to harvest seed. We can repeat this with a goal to complete by mid 2021.	Repeat experiment by mid 2021. Contract variation request will be submitted.
1.2	Isotope partitioning of metabolic pathways	Research aim	30/09/2020	Achieved	This was achieved but we needed to use different methodology to achieve the outcome. We used RNAseq analysis and the knowledge obtained has helped the understanding of the novel photosynthesis mechanism. This will be published in the next 12 months.	
1.2.1	Isotope partitioning in model species	Critical step	23/12/2019	Achieved		
1.2.2	Isotope partitioning in forage species	Critical step	30/09/2020	Achieved		

2	Nitrate Utilization in HME Ryegrass and other species	Impact statement	30/09/2020	Achieved		
2.1	Nitrate utilization in C3 plant species	Research aim	30/09/2020	Achieved		
2.1.1	Nitrogen utilization in model species	Critical step	30/09/2019	Achieved		
2.1.2	Nitrate utilization in forage species	Critical step	30/09/2019	Achieved		
2.1.3	Appropriate Fertilizer Composition	Critical step	24/12/2019	Achieved		
2.1.4	Effects on rhizobium symbiosis	Critical step	30/09/2020	Achieved		
3	Nitrogen and water use efficiency in HME plant species	Impact statement	30/09/2020	Achieved		
3.1	Nitrogen use efficiency	Research aim	30/09/2019	Achieved		
3.1.1	Assess stomatal conductance in grass species	Critical step	28/09/2018	Achieved		
3.1.2	Measurement of NUE	Critical step	30/09/2019	Achieved		

3.2	Water use efficiency	Research aim	30/09/2020	Achieved		
3.2.1	WUE in Ryegrass	Critical step	29/06/2018	Achieved		
3.2.2	WUE in model grass species	Critical step	30/06/2019	Not achieved	The first set of HME rice developed for this aim was not suitable, as expression had overshot the ideal expression window (reported last year). We developed HME rice that was more suitable and during the early part of 2020 the COVID-19 lockdown prevented us from performing any detailed experimentation, although we were able to harvest seed. We can repeat this with a goal to complete by early 2021.	Perform WUE experiment on HME rice by 31 March 2021.
3.2.3	WUE in commercial ready ryegrass	Critical step	30/09/2020	Achieved		
4	Creating genetic material and knowledge for overseas field trial assessment of HME forages	Impact statement	30/09/2020	Achieved	Refer to specific details on 4.2 which will be achieved within the contract.	File variation to reflect later date of completion.
4.1	Ryegrass HME Trait Fixing	Research aim	31/05/2018	Achieved		
4.1.1	T1 Generation	Critical step	31/05/2017	Achieved		
4.1.2	T2 Generation	Critical step	22/12/2017	Achieved		
4.1.3	T3 Generation	Critical step	31/05/2018	Achieved		

4.2	Commercial Ready HME Ryegrass trait fixation	Research aim	30/09/2020	Not achieved	We have a very robust and successful breeding plan that uses elite industry germplasm. Part of the delay was due to the new PC2+ glasshouse taking 1 year longer to complete than planned. COVID-19 has not impacted the breeding as the timing of lockdown was during seed development.	Do a contract variation to reflect the actual timelines. This work can be achieved in the timeframe of the contract.
4.2.1	T1 Generation	Critical step	30/06/2019	Achieved		
4.2.2	T2 Generation	Critical step	31/03/2020	On track with issues	1/3 waves of T2 seed production are complete. The final waves will be complete by March 2021. We have a very robust and successful breeding plan. Part of the delay was due to the new PC2+ glasshouse taking 1 year longer to complete than planned.	
4.2.3	T3 Generation	Critical step	30/09/2020	Not achieved	We will not complete until 30 December 2021. However we have a very robust and successful breeding plan. Part of the delay was due to the new PC2+ glasshouse taking 1 year longer to complete than planned.	Make a variation to contract to reflect the actual timeline.
4.3	In vitro digestion and GHG assays	Research aim	30/09/2020	Achieved		
4.3.1	Analysis of first generation Ryegrass	Critical step	29/06/2018	Achieved		
5	Increasing farmer awareness and understanding of HME forages	Impact statement	30/09/2021	On track		

5.1	Farmer focus groups	Research aim	30/09/2021	On track		
5.1.1	Farmer focused groups	Critical step	31/12/2019	Achieved		
5.1.2	Establish wider industry linkages	Critical step	01/12/2020	On track		
5.1.3	Design of a farmer-led, Farmer Awareness and Understanding Raising Programme	Critical step	31/12/2019	Achieved		
5.1.4	Stakeholder Feedback	Critical step	01/10/2020	On track		
5.1.5	Monitoring and evaluation of the Farmer Awareness and Understanding Raising Programme	Critical step	30/09/2021	On track		

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
Achieved

Reason

Patent filed on the novel mechanism for reducing negative feedback of photosynthesis. Publication of research in Journal of Experimental Botany in 2020.

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
Achieved

Reason

Action

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
Not achieved

Reason

The first set of HME rice developed for this aim was not suitable, as expression had overshot the ideal expression window (reported last year). We developed HME rice that was more suitable and during the early part of 2020 the COVID-19 lockdown prevented us from performing any detailed experimentation, although we were able to harvest seed. We can repeat this with a goal to complete by mid 2021.

Action

Repeat experiment by mid 2021. Contract variation request will be submitted.

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
Achieved

Reason

This was achieved but we needed to use different methodology to achieve the outcome. We used RNAseq analysis and the knowledge obtained has helped the understanding of the novel photosynthesis mechanism. This will be published in the next 12 months.

Action

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
Achieved

Reason

Action

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
Achieved

Reason

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
Achieved

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
Achieved

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
Achieved

Reason

Action

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
Achieved

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
Achieved

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
Achieved

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
Achieved

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
Not achieved

Reason

The first set of HME rice developed for this aim was not suitable, as expression had overshot the ideal expression window (reported last year). We developed HME rice that was more suitable and during the early part of 2020 the COVID-19 lockdown prevented us from performing any detailed experimentation, although we were able to harvest seed. We can repeat this with a goal to complete by early 2021.

Action

Perform WUE experiment on HME rice by 31 March 2021.

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
Achieved

Reason

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
Achieved

Reason

Refer to specific details on 4.2 which will be achieved within the contract.

Action

File variation to reflect later date of completion.

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
Not achieved

Reason

We have a very robust and successful breeding plan that uses elite industry germplasm. Part of the delay was due to the new PC2+ glasshouse taking 1 year longer to complete than planned. COVID-19 has not impacted the breeding as the timing of lockdown was during seed development.

Action

Do a contract variation to reflect the actual timelines. This work can be achieved in the timeframe of the contract.

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 with issues

Reason

1/3 waves of T2 seed production are complete. The final waves will be complete by March 2021.

We have a very robust and successful breeding plan. Part of the delay was due to the new PC2+ glasshouse taking 1 year longer to complete than planned.

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
Not achieved

Reason

We will not complete until 30 December 2021. However we have a very robust and successful breeding plan. Part of the delay was due to the new PC2+ glasshouse taking 1 year longer to complete than planned.

Action

Make a variation to contract to reflect the actual timeline.

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
Achieved

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

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
Achieved

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
Achieved

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:

The commercial ready breeding programme is 1 year behind schedule mainly due to the 1 year delay completing the new PC2+ glasshouse complex. There was also a small impact due to the size and scope of the project. As we move forward, this is the major activity of the team. We have a very robust breeding pipeline that will deliver a viable HME phenotype in elite industry cultivars. The timing of the industry funded animal nutrition trial is 2023, this is 2 years later than the 2016 science plan. The new patent filed this year extends the patent protection for HME by 10 additional years.

Has any change event occurred in the Reporting Year?
No

If YES when was MBIE advised?

Work Programme Conditions

Outputs

Knowledge Transfer

ModifiedDate	Knowledge transfer type	Number of Events	Knowledge transfer comments (optional)
25/08/2020	Workshops and hui	5	We delivered five farmer/rural professional presentations over the past year. A number of additional presentations were scheduled for the March to June period and have been rescheduled for the next year due to COVID-19.

25/08/2020	Commissioned reports	1	In collaboration with 9(2)(a) and 9(2)(a) from NZIER, 9(2)(a) (AgResearch) led a process to conduct a survey on farmer decision making and the key decision timeframes for a GM HME ryegrass. Key findings were: • Some farmers are interested in the potential benefits of HME ryegrass. They may adopt it within a year or two of its commercial release. However, they want to know more about how it might perform: persistence, palatability, animal performance, cost, etc. • They are likely to want information early, through many channels. The best time to provide information will be after the field trials and before product release. Some farmers want information even earlier. Given the number of information sources that farmers use, and the time frames for decision making, the information should be available in many formats through many channels. Farmers get information from other people, printed material and online material (including videos). • The use of GM technology needs to be addressed. The use of genetic modification (GM) technology creates additional complexity. Some farmers will not adopt HME ryegrass because it is GM. Other farmers would adopt HME ryegrass but recognise that GM technology is an issue with consumers and in their supply chains. HME ryegrass would likely be a minority of total pasture. Among potential adopters, HME ryegrass would likely be one of several cultivars used.

Knowledge transfer type
Workshops and hui

Number of Events

5

Knowledge transfer comments (optional)
We delivered five farmer/rural professional presentations over the past year. A number of additional presentations were scheduled for the March to June period and have been rescheduled for the next year due to COVID-19.

Knowledge transfer type
Commissioned reports

Number of Events

1

Knowledge transfer comments (optional)
In collaboration with 9(2)(a) and 9(2)(a) from NZIER, 9(2)(a) (AgResearch) led a process to conduct a survey on farmer decision making and the key decision timeframes for a GM HME ryegrass. Key findings were:

- Some farmers are interested in the potential benefits of HME ryegrass. They may adopt it within a year or two of its commercial release. However, they want to know more about how it might perform: persistence, palatability, animal performance, cost, etc.
- They are likely to want information early, through many channels. The best time to provide information will be after the field trials and before product release. Some farmers want information even earlier. Given the number of information sources that farmers use, and the time frames for decision making, the information should be available in many formats through many channels. Farmers get information from other people, printed material and online material (including videos).
- The use of GM technology needs to be addressed. The use of genetic modification (GM) technology creates additional complexity. Some farmers will not adopt HME ryegrass because it is GM. Other farmers would adopt HME ryegrass but recognise that GM technology is an issue with consumers and in their supply chains.

HME ryegrass would likely be a minority of total pasture. Among potential adopters, HME ryegrass would likely be one of several cultivars used.

Non-peer Reviewed Published Articles

Number of non-peer reviewed published articles

2

Non-peer reviewed published articles comments (optional)
Two blog posts:

Firstly on the development of the technology by 9(2)(a)

https://www.zeakal.com/blog/an-unexpected-journey-the-winding-road-to-photoseed

and;

On genetic modification and plant improvement by 9(2)(a)

https://www.zeakal.com/blog/rethinking-gmos-the-past-present-and-future-of-genetically-modified-food

New Products, Processes and Services

Number of new products

0

Number of new processes

0

Number of new services

0

New products, processes and services (optional)

Science Quality

Peer-reviewed journal articles in the year they are accepted for publication

2

Number of books or chapters

0

Number of published conference proceedings

1

Awards for science achievement (not open internationally)

1

Awards for science achievement (open internationally)

0

Keynote presentations (not open internationally)

2

Keynote presentations (open internationally)

1

Number of masters or doctoral theses

0

Science quality comments (optional)

Event of special note- Eureka Awards

As a result of interaction with some of our HME ryegrass researchers last year, a school student, Lila Madden from Cashmere High School, Christchurch, won the finals of the Eureka Awards with her presentation on HME ryegrass.

Provisional Patent and PVR Applications

Number of Patent or Plant Variety Right (PVR) applications

0

Number of Patent Cooperation Treaty (PCT) applications

2

Provisional patent and PVR applications comments (optional)

Patent filed on sustaining photosynthesis

Patent filed on enhanced DGATs

Patent and PVR grants

Number of Patents or Plant Variety Rights (PVRs) that have been granted.

3

Name the countries in which you have been granted Patents or PVRs.

This patent portfolio covers three broad areas of plant improvement. The Cysteine oleosin and DGAT1 patents protect the HME technology and they are licensed to ZeaKal for row crops, biofuels and algae. They have generated substantial licensing and investment revenue to AgResearch (~$10M). The PeaPod patents are a future technology and this is being progressed using SSIF funding.

Total Patent Grants since 2009 and number of jurisdictions:

Photosynthesis/HME

Cysteine Oleosins: 26
Reducing WSC: 1
Increased CO2 and root oil: 6

DGATs

DGAT- N/C Chimera: 19
DGAT1 Modified N-term: 9
DGAT Zm-long: 6
Diarginine EDGAT: 1

PeaPod

Peapod in Monocots: 12
Peapod in any plant: 12

Jurisdictions

Indonesia
Iraq
India
Canada
Paraguay
Phillipines
Japan
Chile
Mexico
NZ
South Africa
Germany
Spain
France
China
AU
Brazil
Brazil
EPO
US
Argentina
India
Malaysia
Thailand
Uruguay
Venezuela

Revenue and Contracting

Co-funding and Subcontracting

Reporting financial year: 2019 (This report covers the period 01/07/19 - 30/06/20)

Select type	Organisation	Listed in the contract	Type	Cash or In-kind	Listed amount (NZD excl GST)	Actual amount (NZD excl. GST)	Comment
Co-Funding	Grasslanz Technology Limited	Yes	Direct	Cash	$50,000.00	$50,000.00	
Co-Funding	Dairy NZ	Yes	Direct	Cash	$750,000.00	$750,000.00	
Co-Funding	PGG Wrightsons	Yes	Direct	Cash	$100,000.00	$100,000.00	

Reporting financial year: 2019 (This report covers the period 01/07/19 - 30/06/20)

Organisation
Grasslanz Technology Limited

Select type
Co-Funding

Listed in the contract
Yes

Listed amount (NZD excl GST)
$50,000.00 (Excl. GST)

Type
Direct

Cash or In-kind
Cash

Actual amount (NZD excl. GST)

50,000.00

(Excl. GST)

Percentage of listed funding achieved:
100%

Comment

Reporting financial year: 2019 (This report covers the period 01/07/19 - 30/06/20)

Organisation
Dairy NZ

Select type
Co-Funding

Listed in the contract
Yes

Listed amount (NZD excl GST)
$750,000.00 (Excl. GST)

Type
Direct

Cash or In-kind
Cash

Actual amount (NZD excl. GST)
750,000.00
(Excl. GST)

Percentage of listed funding achieved:
100%

Comment

Reporting financial year: 2019 (This report covers the period 01/07/19 - 30/06/20)

Organisation
PGG Wrightsons

Select type
Co-Funding

Listed in the contract
Yes

Listed amount (NZD excl GST)
$100,000.00 (Excl. GST)

Type
Direct

Cash or In-kind
Cash

Actual amount (NZD excl. GST)
100,000.00
(Excl. GST)

Percentage of listed funding achieved:
100%

Comment

Formal Collaborations

Collaborations by Country

Country	Level	Number of collaborations	Comment
United States of America (the)	Strong	5	

Capability Building

Students

Number of students obtaining Masterate qualifications	0
Number of students obtaining Doctoral qualifications	0
Number of students obtaining Post-Doctoral qualifications	0

Secondments to or from end users

Number of secondments as FTEs from an end user organisation	0
Number of secondments as FTEs to an end user organisation	0

End User Relationships

End User Contact Details

Organisation	Briefly describe how you are working with this organisation	Contact person	Contact phone	Contact email
Dairy NZ	Dairy NZ are co-funders and members of the Collaborative Agreement around HME Ryegrass. They have a representative on the Programme Steering Group that provides governance for the programme. Dairy NZ works directly with dairy farmers, manages the Forage Value Index, works closely with processing companies and with Rural Professionals. Dairy NZ will be a key decision maker on how to proceed in New Zealand as the overseas field and animal nutrition trials provide knowledge of the benefits of HME ryegrass. This year we have contributed to several farmer and Rural Professional engagement/focus groups managed by Dairy NZ.	9(2)(a)		

PGG Wrightson Seeds	PGG Wrightson Seeds are co-funders and members of the Collaborative Agreement around HME Ryegrass. They have a representative on the Programme Steering Group that provides governance for the programme. They are part of the implementation pipeline and provide a route to market in New Zealand and overseas. PGG Wrightson seeds have provided elite ryegrass germplasm to the programme and this is being used in the HME ryegrass breeding pipeline. They also have a member on the Technical Advisory Group and we met in April to discuss the breeding plan.	9(2)(a)
Agriseeds Limited	Agriseeds Ltd are part of the implementation pipeline and provide a route to market in New Zealand and overseas. Agriseeds seeds have provided elite ryegrass germplasm to the programme and this is being used in the HME ryegrass breeding pipeline. They also have a member on the Technical Advisory Group and we met in April to discuss the breeding plan.	9(2)(a)

GrasslanZ technology Limited	Grasslanz Technology Ltd. are co-funders and members of the Collaborative Agreement around HME Ryegrass. They have a representative on the Programme Steering Group that provides governance for the programme. They are part of the implementation pipeline as they provide expertise in endophyte commercialisation and management of nucleus seed for the seed industry.	9(2)(a)		

Spinouts and Startups

Spinouts and Startups (super-users only)

Organisation	Current annual sales	Current annual export	FTE	Industry sector
Grasslanz Technology Limited	$11,441,493.00	$2,187,891.00	12	82 Plant Production And Plant Primary Products
Grasslands Innovation Ltd	9(2)(b)(ii)		0	82 Plant Production And Plant Primary Products
Farmax Ltd	9(2)(b)(ii)		6	83 Animal Production And Animal Primary Products
Phytagro Corp	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
Phytagro NZ Ltd	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
Phytagro Inc	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
EnCoate Holdings Ltd	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
AgResearch(USA) Ltd	$326,017.00	$326,017.00	2	82 Plant Production And Plant Primary Products
Covita Limited	$0.00	$0.00	0	83 Animal Production And Animal Primary Products
Phytagro LLC	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
AgResearch (PPGR-Consortia) Ltd	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
AgResearch (Pastoral Genomics Consortia) Ltd	$0.00	$0.00	0	82 Plant Production And Plant Primary Products
AgResearch (Johnes Disease Research Consortium) Ltd	$0.00	$0.00	0	83 Animal Production And Animal Primary Products
Celentis Ltd	$0.00	$0.00	0	83 Animal Production And Animal Primary Products

COVID-19 Information

COVID-19 Information

1) Please provide the amount (as a percentage) of work that was due in the reporting period that was:

a. not completed due to non-COVID-19 related reasons
5

b. not completed due to the impact of COVID-19
5

2) Please provide the amount (as a percentage) of work done in the reporting period that has a COVID-19 outcome
0

3) Has a contract variation request been submitted that has not yet been approved?
No

4) If 'yes' was selected for question (3), will the existing contract variation remediate all of the current issues with the project?

5) Is a future contract variation required to remediate any current issues with the project?
Yes

6) Would a 6-month no-cost extension* to the contract end date remediate all COVID-19 related issues with the contract (without the need for any other variations)?
No

Declaration

Declaration

The Contractor declares that:

I Agree