

## Summary

## Summary

## Client report summary:

<b>Key:</b>	CONT-47267-CRFRP-AGR C10X1603-CR-2
<b>Project:</b>	Forages with Elevated Photosynthesis and Growth
<b>Contract ID:</b>	C10X1603
<b>Investment process:</b>	CRFRP 2016 Contestable Research Fund - Research Programmes
<b>Organisation:</b>	AGR AgResearch Limited
<b>IMS assigned to:</b>	B(2)(a)
<b>Reporting period:</b>	01/07/2017 to 30/06/2018
<b>Contract total value:</b>	\$11,500,000.00
<b>Team:</b>	

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## Progress Reporting

### Annual update

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#### 2017-18 Annual Update

#### Brief Overview 2017-2018 Summary

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 have advanced our understanding of this technology or have led us to refine our hypothesis of the mechanism for increasing carbon assimilation (photosynthesis). It has 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 also found that HME ryegrass grows effectively with nitrate as the sole nitrogen source. Our previous understanding was that it was only able to effectively utilise a reduced form of nitrogen (ammonium or urea). This is a positive outcome for any future HME ryegrass pastures in a New Zealand farming system.

The programme co-funding supports the current 2018 field trial in the mid-West of the USA. We genetically characterised the T<sub>2</sub> generation of seed for three HME ryegrass Events and developed new protocols for enabling our research partners to assess HME gene expression both in containment and in regulated field trials. The trials include both space plants (to assess growth of individual plants) and swards (to simulate pasture where plants are competing). We also continue the trait fixing process in Palmerston North and now have homozygous T<sub>3</sub> plants. These are needed to generate seed for 2019 trials.

#### Science

##### CO<sub>2</sub> Recycling

- Demonstrated that HME ryegrass still out-performs conventional ryegrass in elevated CO<sub>2</sub>. This means the mechanism of elevated photosynthesis may be more complex than previously thought and potentially means HME ryegrass could still out-perform conventional ryegrass under rising atmospheric carbon dioxide levels.

##### Endophyte

- AR1 grows normally in HME ryegrass and is stably transmitted in seed. This was reported last year, and over the last 12 months the team have been assessing endophyte alkaloid levels and assessing additional HME ryegrass events.

##### Nitrogen Use

- HME ryegrass can utilise nitrate, ammonia and urea. The greatest advantage is on ammonia and urea. These results are good news for performance in pasture and the potential for mitigation of environmental impacts of farming.
- This year, we supported a new MPI funded programme examining the fate of nitrogen in a system. The project will examine nitrate leaching and nitrous oxide emissions in lysimeters in a containment glasshouse. Significant analysis was needed prior to starting the experiment (June 2018).

##### Water Use

- We reported in 2017 that we measured a 9% increase in actual WUE meaning the plants grow the same using 9% less water. It is anticipated that we will assess this in the field at a future date

(perhaps 2020).

### Trait Fixing

- We now have T<sub>3</sub> generation which will include homozygous individuals. We are using a qPCR technique to identify homozygotes. This means we can start bulking up homozygous seed for later trials and the first batch of homozygous T<sub>4</sub> seed will be available in March 2019.

### In vitro Rumen assays

- 15-23% reduction in methane result fits with nutritional studies and provides lead-in to continuous flow fermentation study. The material from the Nitrogen use trial will be used for the continuous flow study.

### HME Alfalfa

- AgResearch is in discussion with MBIE about a variation (draft submitted for MBIE consideration 22/6/2018 – investment manager 9(2)(a) ) to the contract where the HME alfalfa project will be funded by SSIF investment and we will substitute this work with increasing industry end user engagement with farmer focus groups.
- The HME alfalfa has had over 200 transgenic events developed and we have identified that one of the genes regulated by soy legume promoters is not functioning as expected. We may also have gene silencing issues. A gene construct redesign is underway.

### End user linkages – Farmer focus groups

- The main purpose of the High Performance Grasses (HME Ryegrass) extension programme is to raise farmer, and their rural advisors', awareness and understanding of High Performance Grasses. Following the first set of meetings seven recommendations have been made for future work .

### 2018 Field Trial (Mid-West, USA)

- The 2018 field trial was initiated on the 14<sup>th</sup> June and runs to the end of October. Three HME ryegrass Event T<sub>2</sub> progeny were included in the trial.
- Significant analysis was required to characterise the transgene genotypes of the T<sub>2</sub> plants and to assess the HME phenotype (increased leaf lipids, increased photosynthesis and increased growth).

### Detailed Summary of Main Focus Areas

#### Impact Statement 1: Carbon Dioxide Recycling in HME Ryegrass

HME ryegrass plants have elevated photosynthesis up to 24% greater than non-GM ryegrass. Our hypothesis for the mechanism of elevated photosynthesis is reduced photorespiration. Photorespiration is a process in the Calvin Cycle where the enzyme RUBISCO fixes oxygen (oxylation) releasing carbon dioxide rather than fixing carbon dioxide (carboxylation) to release oxygen. In plants with C3 photosynthesis approximately 75% of reactions are carboxylation and 25% are oxylation. Photorespiration has historically been thought to be a wasteful byproduct of photosynthesis but in more recent times has been shown to be involved in nitrogen metabolism. When plants with C3 photosynthesis are grown in high carbon dioxide (700 ppm) the levels of the RUBISCO protein are reduced and the levels of chlorophyll are elevated. The plants need less Rubisco to fix carbon dioxide and are also more efficient and can capture more light, hence the elevated chlorophyll. This leads to increased crop yields via increased carbon assimilation primarily due to reduced photorespiration.

Evidence for decreased photorespiration in HME plants came from analysis of the expression of the HME genes (DGAT1 and Cysteine oleosin) when HME ryegrass was acclimated to a high photosynthetic state. We are using the RUBISCO and chlorophyll gene promoters. HME ryegrass seems to mimic plants growing in elevated carbon dioxide, as we saw a 20% reduction in level of the DGAT1 enzyme (regulated by the RUBISCO gene promoter and a 20% increase in the level of Cysteine Oleosin regulated by the chlorophyll AB (CAB) gene promoter.

Our research this year is to better understand the mechanism of increased carbon assimilation via carbon dioxide recycling in HME ryegrass using two approaches. Firstly, by measurement of ACI curves (assimilation of carbon dioxide per  $m^2$  per s), and secondly by assessing the growth of HME ryegrass in elevated carbon dioxide. We would predict a significant reduction in photorespiration and a change in the ACI curves.

### Research Aim and Critical Steps: IRGA Analysis in HME Ryegrass, Alfalfa and Rice

The method for measuring carbon assimilation is Infrared gas analysis. This method allows the measurement of net photosynthesis at different carbon dioxide levels. The first target species was HME Ryegrass and once we have further elucidated the mechanisms we can confirm these in HME alfalfa and rice once these plants are available.

**ACI Curves:** So far we have found only a small reduction in photorespiration. This means our hypothesis only partially explains the increase in photosynthesis. In addition, in experiments in controlled environments, we found that even at elevated carbon dioxide HME ryegrass maintained a significant growth advantage compared to control ryegrass. This further suggests our hypothesis only provides a partial explanation.

We need to confirm this in a legume species and had planned on using HME alfalfa as the target as it is a commercially relevant forage. The first HME alfalfa had sub optimal expression or an imbalance of expression of the DGAT1 and Cysteine Oleosin genes. We have discovered in our work in *Arabidopsis*, ryegrass and soybean that to obtain enhanced photosynthesis we need an appropriate balance of fatty acid synthesis and encapsulation. The solution is to redesign the alfalfa gene constructs and the likely issue is the pea CAB gene promoter we are using to drive cysteine oleosin expression. Unexpectedly, this is strongly expressed in young leaves but the expression drops in older leaves with the consequence that the newly synthesized fatty acids are not adequately encapsulated and are degraded. This leakiness leads to a futile fatty acid biosynthesis and catabolism cycle that costs the plant energy. Using an alternative CAB gene promoter with more appropriate expression will likely solve the issue. While new HME alfalfa is developed, we will utilize an alternative legume, PhotoSeed (the HME technology) soybean which will be provided by ZeaKal as a collaborating partner and will be imported in early September. We expect to have ACI curve analysis complete by the end of October 2018. This data will enable us to confirm if there are any differences between a C3 grass and a legume species in how the plants have increased carbon assimilation. We will then be able to confirm these results in alfalfa once the new set of HME alfalfa are developed.

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

We are investigating whether or not the HME trait expression in transgenic plants alters plant nitrogen metabolism, specifically their ability to utilize nitrate. This can be tested by assessing growth on different forms of nitrogen (nitrate, ammonium and urea) in a controlled environment facility. The relevance of this research is firstly to estimate how HME plants will perform in the field with differing nitrogen forms and secondly due the relevance to providing a remediation tool for nitrogen leaching.

#### Research Aim 2.1 Nitrate Utilisation in C3 plant species

In the nitrogen trial we tested a single HME ryegrass event ODR4501 (This event was tested in the field in the US in 2017 and again in 2018). We describe ODR4501 as a medium level expressing event as it has a mid-range of leaf lipids (up to 6%), photosynthesis increase (15%) and increased growth rate (25%). We found in controlled environment experiments that ODR4501 outperformed the non-GM control ryegrass when grown with nitrate as the sole nitrogen source. The growth rate was 25 and 40% greater at 7.5 and 10mM nitrate which is the near optimal range for nitrate. Leaf fatty acid levels in both non-GM control and ODR4501 increased with increasing nitrate up to 10mM. However at 10mM control ryegrass fatty acids were 3.25% of the dry weight whereas ODR4501 was 5% demonstrating that the HME ODR4501 plants had 54% more leaf lipids.

These results provide positive evidence that HME ryegrass can effectively utilize nitrate and that it is likely the two valuable traits, increased plant biomass and increased nutritional quality will be present in HME ryegrass in pastures.

### 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.

#### 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 are able to 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.

In the previous report for the 2016/17 period we determined that actual WUE in HME ryegrass event ODR4501 was 9% greater than for the non-GM control ryegrass. This meant the HME ryegrass grew the same biomass using 9% less water. It is unclear what this means in field conditions and how it would relate to drought. This would potentially mean that HME ryegrass would provide a growth benefit in water limited environments.

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.

We also planned to conduct WUE experiments in HME alfalfa and encountered the opposite problem that the expression in the HME alfalfa was too low. Alfalfa transformation and regeneration takes longer than rice so while we develop new HME alfalfa we will switch to the PhotoSeed soybean provided by our partner ZeaKal Inc. and conduct experiments over the next 9 months. This will then enable us to confirm results in the newly developed HME alfalfa when they are available.

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

#### 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.

Another important step has been to assess the AR1 endophyte symbiosis and determine whether or not

we have a stable symbiosis. This work has provided evidence that the AR1 endophyte is able to form a stable symbiosis in HME ryegrass.

#### **Critical Steps 4.1.1 to 4.1.3: T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> Seed Generation**

This area has been a major focus over the last 12 months. We have successfully developed all the T<sub>3</sub> seed populations required for the trials. The major research effort has been characterizing the seed families for the trial. This has required the transgene genotype characterization in all families using Southern blots, and aligned this with the plant biochemistry and growth phenotype in the glasshouse and controlled environment rooms. We successfully completed this for three HME ryegrass T<sub>3</sub> events (described below) and these are now in field trials in swards and space plants.

#### **Material in the 2018 field trial: ODR6205**

This primary transgenic T<sub>0</sub> Event has the highest level of photosynthesis (20% greater than non-GM ryegrass) and fastest growth (40 to 50% faster than non-GM ryegrass). This plant may have eight insertions of the HME transgenes. The combination of these contribute to the rapid growth phenotype. Analysis of T<sub>2</sub> progeny showed they all contained three insertions. An interpretation of this result is that during the two recombination events to generate the T<sub>2</sub> generation the remaining 5 insertions segregated, and were lost. Three different ODR6205 T<sub>2</sub> families of seed have been assessed and have been shown to have 9-18% more growth than non-GM controls. Therefore, the progeny exhibit a partial phenotype compared to the primary transgenic parent but still have a measurable increase in growth rates. It is expected that when we characterise homozygous T<sub>3</sub> and T<sub>4</sub> progeny the trait expression will double.

#### **ODR4501**

This primary transgenic T<sub>0</sub> Event has an intermediate level of photosynthesis increase (15% greater than non-GM ryegrass) and growth rate of 20% faster than non-GM ryegrass. The T<sub>0</sub> primary transgenic plant has the most complicated transgene insertion genotype. The T<sub>2</sub> progeny have undergone extensive analysis of growth rates, plant morphology, nitrogen requirements, and photosynthesis. The increased growth rate in the T<sub>2</sub> progeny is due to an increased leaf extension rate.

#### **ODR6003**

The primary transgenic T<sub>0</sub> Event had an intermediate level of photosynthesis (10% greater than non-GM ryegrass) and growth rate of 20% faster than non-GM ryegrass. It contained three transgene insertions, although a possible fourth insertion is still being characterized as it may contribute to the growth phenotype. So far, the T<sub>2</sub> progeny do not seem to have an increased growth phenotype.

#### **Trial**

The seed for the trial was sent to the US at the end of March and germinated in a containment facility. The team then visited and performed an immunological based analysis to identify which plants were transgenic and expressed the Cysteine Oleosin gene and which plants were non-GM null segregating plants. This data was used to design the trial and both a spaced plant and sward design was used.

Transplanting occurred on June 14 and the first harvest of leaf material occurred on July 20.

The generation of homozygous T<sub>4</sub> seed has progressed as planned this year. We are able to perform one cross every 9 months although we also have PC2 glasshouse space constraints which means we have to stagger the development. The seed used in the 2017 and 2018 trials is hemizygous (the transgenes have not been duplicated through recombination to create a plant with a copy on each pair of a chromosome). We expect that in the homozygous state we will get an increase in transgene expression due to the gene dosage effect. We are still trialing the hemizygous T<sub>2</sub> seed as we can obtain valuable information on performance in the field. The homozygous seed is essential for any animal nutrition trials and we need to

generate significant quantities (10-15kg) to enable us to grow sufficient pasture for a nutrition trial. A new PC2 containment glasshouse will be completed at the end of September to enable the seed production.

#### **Research Aim 4.2 HME Alfalfa Trait Fixation**

For the first 200 HME alfalfa events generated and assessed for expression, we discovered that we had sub-optimal expression of the HME trait. This was based on the plant biochemistry and HME protein levels. This was a surprise as the gene constructs work in soybean. We believe that one of the regulatory elements in the genes derived from pea, is not functioning correctly or alternatively the pea CAB gene promoter functions poorly in alfalfa. We therefore need to develop new HME alfalfa events containing modified gene constructs. The consequence is that all critical steps will need to be pushed back by 1 year.

#### **Research Aim 4.3 Commercial Ready HME Alfalfa Trait Fixation**

The AgResearch SSIF fund is supporting the development of *Agrobacterium* derived HME ryegrass containing a commercial ready HME Gene construct. We have been successful in generating several single copy *Agrobacterium* HME ryegrass events with appropriate levels of expression. This means this Research Aim will be on track. We will be using elite breeding material provided by industry partners.

#### **Farmer Focus Groups**

The main purpose of the High Performance Grasses extension programme 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. This will help us lead into Maori Agribusiness engagement. We see this sort of engagement as part of the implementation pathway for delivering impact to the pastoral industries.

The goals were to a) ascertain the interest of New Zealand farmers in learning about the AgResearch high performance grasses research program, b) identify the particular parts of the research programme that would interest them most and c) to identify how they would prefer to learn about the components that interest them the most. The information from this study will be used to inform the development of a 'Raising farmer awareness and understanding' extension programme for year 2018-2019.

Focus group meetings were chosen as the most appropriate data gathering method to use in a small scale study of this nature, because they are an effective way to gather in-depth information. The focus group approach entailed a facilitator, Margaret Brown, meeting with each group of five to seven participants, for a semi-structured discussion lasting approximately one hour.

Four focus group meetings were held in May-June 2018 to coincide with the quieter time on farms. All participants came from either the Rangitikei or Manawatu regions. The four focus groups were; women in farming, young farmers, sheep and beef farmers and dairy farmers. It had been the intention to also hold a focus group for Maori farmers, but it was not possible to organise one before the end of June 2018.

#### **Related Research from Industry Co-Funding**

##### **2018 Field Trial in US Mid-West**

This year we built on the successful 2017 pilot field trial in which the first HME ryegrass population (ODR4501) was tested in a 9-week study and we demonstrated that the plants grew well in the environmental conditions and in addition, transgenic HME ryegrass had 10% more photosynthesis than null control plants. The industry funded trials are currently evaluating three independently developed HME ryegrass Events (ODR4501, ODR6003 and ODR6205). Over the last 12 months this programme has supported the preparation of material for the field trial including development of T<sub>2</sub> generation seed (two cycles of reproduction beyond the initial T<sub>0</sub> primary transgenic plants), determination of the transgene genotype, transgene expression, and plant phenotype including leaf lipid levels, photosynthesis and plant growth characteristics.

This year was particularly technically challenging and required a number of innovations to ensure that sufficient seed with characterised genotypes were available for the trial. A complication is that the initial

HME ryegrass plants were generated using Gene Gun biolistic transformation technology. This method is prone to multi copy insertions of the transgene and it is also possible to get more than one locus of integration (in layman terms the HME genes can be inserted into more than one position in the plant chromosomes and in some cases more than one copy can be inserted in these positions). Each transgenic Event has a different insertion pattern and therefore needs individual characterisation both in the T<sub>0</sub> plant and in any progeny.

It is important to note that *Agrobacterium* transformation derived HME ryegrass plants are in the pipeline and these will be bred into elite germplasm supplied by the industry. This transformation system produces a higher frequency of single inserts of the transgene than the Gene Gun system. So far 4/15 *Agrobacterium* derived HME ryegrass plants have been shown to have a single insert. This development is being supported in the parallel AgResearch SSIF funded programme.

## 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.

As an important step towards verifying the benefits in detailed animal nutrition trials, we are conducting a full season regulated field trial on three different HME ryegrass lines in the USA. These trials will help us select material for regulated animal nutrition trials planned for 2020 and 2021.

## Five key achievements

Sequence	Key achievements
1	<p><b>The first full season HME ryegrass trial is underway in the mid-West of the USA and involves three HME ryegrass events in spaced plant and sward trials.</b></p> <p>The 2017 field trial was conducted a full year earlier than planned as we had made faster than expected progress in developing appropriate seed material and our co-funders saw the value of performing a pilot trial to test the regulatory system and the environment at the site in the mid-West of the USA. We successfully completed the trial, during which we were able to measure enhanced photosynthesis in the ODR4501 event. The 2018 field trial is larger and runs for longer and contains three independently generated HME ryegrass Events. The trial was established in mid-June and runs to October 2018 and will enable a number of plant growth assays to be performed and allow testing of plant performance over the season.</p>

2	<p><b>Homozygous HME ryegrass seed has been developed for the first time and this material will be used to develop significant quantities of homozygous seed for later animal nutrition trials.</b></p> <p>While we were confident that we could develop homozygous HME ryegrass seed (based on this achievement in the model brassica, <i>Arabidopsis thaliana</i> and in the commercial legume crop soybean), we achieved this for the first time in 2018. Ryegrass breeding is challenging as it is self-incompatible and therefore the timelines are protracted. We are now in a position to prepare for developing uniformly homozygous T<sub>4</sub> seed populations and then the scale up to generate sufficient seed for larger scale pasture plots for animal nutrition studies.</p>
3	<p><b>The Science team was awarded the AgResearch science prize to recognise the achievements and publication of the development of this technology in 2013.</b></p> <p>The AgResearch Science prize is awarded annually for the publication expected to provide the greatest impact for New Zealand. It comes with a \$200,000 prize to enable the team to invest in an area important for their science. The team chose to invest this in a post-doctoral scientist focused on nitrogen metabolism in HME ryegrass.</p>
4	<p><b>We demonstrated that HME ryegrass grows well on the nitrogen sources ammonia, urea and nitrate although it performs the best on a reduced form of nitrogen such as Ammonium or Urea.</b></p> <p>The implication is that in a pasture the HME ryegrass will utilise the excreted nitrogen in the form of urea that is in animal urine. HME ryegrass has the highest growth rates on urea so this supports the concept that it will provide a tool for the farmer to manage the effects of excreted nitrogen (nitrate leaching and nitrous oxide emissions). When a reduced form of nitrogen is limiting, the plant is able to utilise nitrate.</p>
5	<p><b>The development and signing of a collaborative agreement with industry stake holders that means the programme will receive \$4,500,000 of industry co-funding from October 2016 to September 2021.</b></p> <p>This is an important milestone as now we have the seed company partners providing germplasm and breeding expertise. The companies cover the majority of the New Zealand seed market and also have significant overseas markets. DairyNZ provides leadership for the dairy industry which would be expected to be a major user of the technology if it is deregulated and commercialised in New Zealand.</p>

## Performance indicators

**End user relationship:**

On track

**End user relationship  
comment:**

The collaborative agreement signed in June brings DairyNZ, PGG Wrightsons Seeds, Grasslanz Technology and AgResearch together along with stake holder co funding and provides part of the implementation pathway (Dairy industry and Seed companies).

**Key personnel:**

On track

**Key personnel comment:****Research progress:**

On track

**Research progress  
comment:****Success story worth  
communicating has been  
generated:**

No

**Comment:****Has any change event occurred in the Reporting Year?**

Yes

**If YES when was MBIE advised?**

Draft Variation submitted 22 June 2018

## 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	On track		
1.1	Infra-Red Gas Analysis	Research aim	30/09/2019	On track		
1.1.1	IRGA analysis of Ryegrass	Critical step	31/10/2018	On track		
1.1.2	IRGA analysis of alfalfa	Critical step	31/10/2018	Off track	The first 200 HME alfalfa Events developed had low levels of HME trait expression and therefore were not suitable for the carbon assimilation analysis.	We are developing new Events with redesigned gene constructs. In the meantime we are importing PhotoSeed soybean with elevated photosynthesis so that we can initiate this work on a related legume. When the alfalfa is ready we can confirm results in these plants.  Soybean data obtained by 30 October 2018. Alfalfa 12 months later.
1.1.3	IRGA analysis of rice	Critical step	30/09/2019	On track with issues	First set of HME rice T <sub>0</sub> plants had good expression. When we developed homozygous T <sub>3</sub> plants the expression was too high and we had a growth penalty. We need to have expression in a well defined window to get the enhanced photosynthesis and growth phenotype.	Because rice is a relatively easy system we can generate new plants in a few months and catch up before 30/9/19. We have slightly redesigned constructs in the pipeline.

1.2	Isotope partitioning of metabolic pathways	Research aim	30/09/2020	On track	
1.2.1	Isotope partitioning in model species	Critical step	23/12/2019	On track with issues	The first post doctoral scientist (co-funded by AgResearch Science prize-award) left for another position.  We are preparing to recruit a replacement.
1.2.2	Isotope partitioning in forage species	Critical step	30/09/2020	On track	
2	Nitrate Utilization in HME Ryegrass and other species	Impact statement	30/09/2020	On track	
2.1	Nitrate utilization in C3 plant species	Research aim	30/09/2020	On track	
2.1.1	Nitrogen utilization in model species	Critical step	30/09/2019	On track	
2.1.2	Nitrate utilization in forage species	Critical step	30/09/2019	On track	
2.1.3	Appropriate Fertilizer Composition	Critical step	24/12/2019	On track	
2.1.4	Effects on rhizobium symbiosis	Critical step	30/09/2020	On track	

3	Nitrogen and water use efficiency in HME plant species	Impact statement	30/09/2020	On track	
3.1	Nitrogen use efficiency	Research aim	30/09/2019	On track	
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	On track	
3.2	Water use efficiency	Research aim	30/09/2020	On track	
3.2.1	WUE in Ryegrass	Critical step	29/06/2018	Achieved	
3.2.2	WUE in Legumes	Critical step	30/09/2019	On track with issues	As the HME alfalfa were not suitable due to low expression we have not yet started this work  We are developing new HME alfalfa and will also begin work on PhotoSeed soybean gifted by ZeaKal inc. These can be used to assess WUE then when the alfalfa is ready we can repeat experiments and confirm results.
3.2.3	WUE in model grass species	Critical step	30/06/2019	On track with issues	The first set of HME rice had too much trait expression. They had a growth penalty.  New rice is being developed with slightly modified gene constructs and we expect to get the work completed on time.
3.2.4	WUE in commercial ready ryegrass	Critical step	30/09/2020	On track	

4	Creating genetic material and knowledge for overseas field trial assessment of HME forages	Impact statement	30/09/2020	On track
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	HME Alfalfa trait fixation	Research aim	01/05/2019	Off track
4.2.1	T1 Generation	Critical step	30/11/2017	Off track
4.2.2	T2 Generation	Critical step	31/08/2018	Off track
4.2.3	T3 Generation	Critical step	30/04/2019	Off track
4.3	Commercial Ready HME Ryegrass trait fixation	Research aim	30/09/2020	On track

The first set of HME alfalfa had too little trait expression so we are developing new HME alfalfa with redesigned gene constructs.

New HME alfalfa will be developed in 9 months. The need to generate new HME alfalfa will push the achievement dates back by 12 months.

Will now be 30/12/19

Will now be 31/08/20

Will now be 30/4/21

4.3.1	T1 Generation	Critical step	30/06/2019	On track	
4.3.2	T2 Generation	Critical step	31/03/2020	On track	
4.3.3	T3 Generation	Critical step	30/09/2020	On track	
4.4	In vitro digestion and GHG assays	Research aim	30/09/2020	On track	
4.4.1	Analysis of first generation Ryegrass	Critical step	29/06/2018	Achieved	
4.4.2	Assays on alfalfa	Critical step	30/09/2019	On track with issues	<p>The first 200 HME alfalfa events had low level expression of the HME genes and therefore maximum total plant fat achieved was 5%. This is too low to perform meaningful <i>in vitro</i> assays.</p> <p>We are developing new HME alfalfa with dedesigned gene constructs with the goal of achieving 6% total fat. This material will lead to meaningful <i>in vitro</i> assays. We still hope to achieve this by the completion date of 30/9/19.</p>

**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**

**Action**

**Click on the deliverable to enter a status**

**Short title**

Intra-Red Gas Analysis

**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**

IRGA analysis of Ryegrass

**Due Date**

31/10/2018

**Achievement measure**

No achievement measure available

**Status**

On track

**Reason**

**Action**

REFEASSED UNDER THE INFORMATION ACT

**Click on the deliverable to enter a status**

**Short title**

IRGA analysis of alfalfa

**Due Date**

31/10/2018

**Achievement measure**

No achievement measure available

**Status**

**Reason**

The first 200 HME alfalfa Events developed had low levels of HME trait expression and therefore were not suitable for the carbon assimilation analysis.

**Action**

We are developing new Events with redesigned gene constructs. In the meantime we are importing PhotoSeed soybean with elevated photosynthesis so that we can initiate this work on a related legume. When the alfalfa is ready we can confirm results in these plants.

Soybean data obtained by 30 October 2018. Alfalfa 12 months later.

---

**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**

First set of HME rice T<sub>0</sub> plants had good expression. When we developed homozygous T<sub>3</sub> plants the expression was too high and we had a growth penalty. We need to have expression in a well-defined window to get the enhanced photosynthesis and growth phenotype.

**Action**

Because rice is a relatively easy system we can generate new plants in a few months and catch up before 30/9/19. We have slightly redesigned constructs in the pipeline.

---

**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

**Reason**

**Action**

REFEASSED UNDER ATTENTION 45  
OFFICIAL INFORMATION

**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**

The first post doctoral scientist (co-funded by AgResearch Science prize award) left for another position.

**Action**

We are preparing to recruit a replacement.

---

OFFICIAL INFORMATION FOR AUCKLAND COUNCIL

**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**

**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**

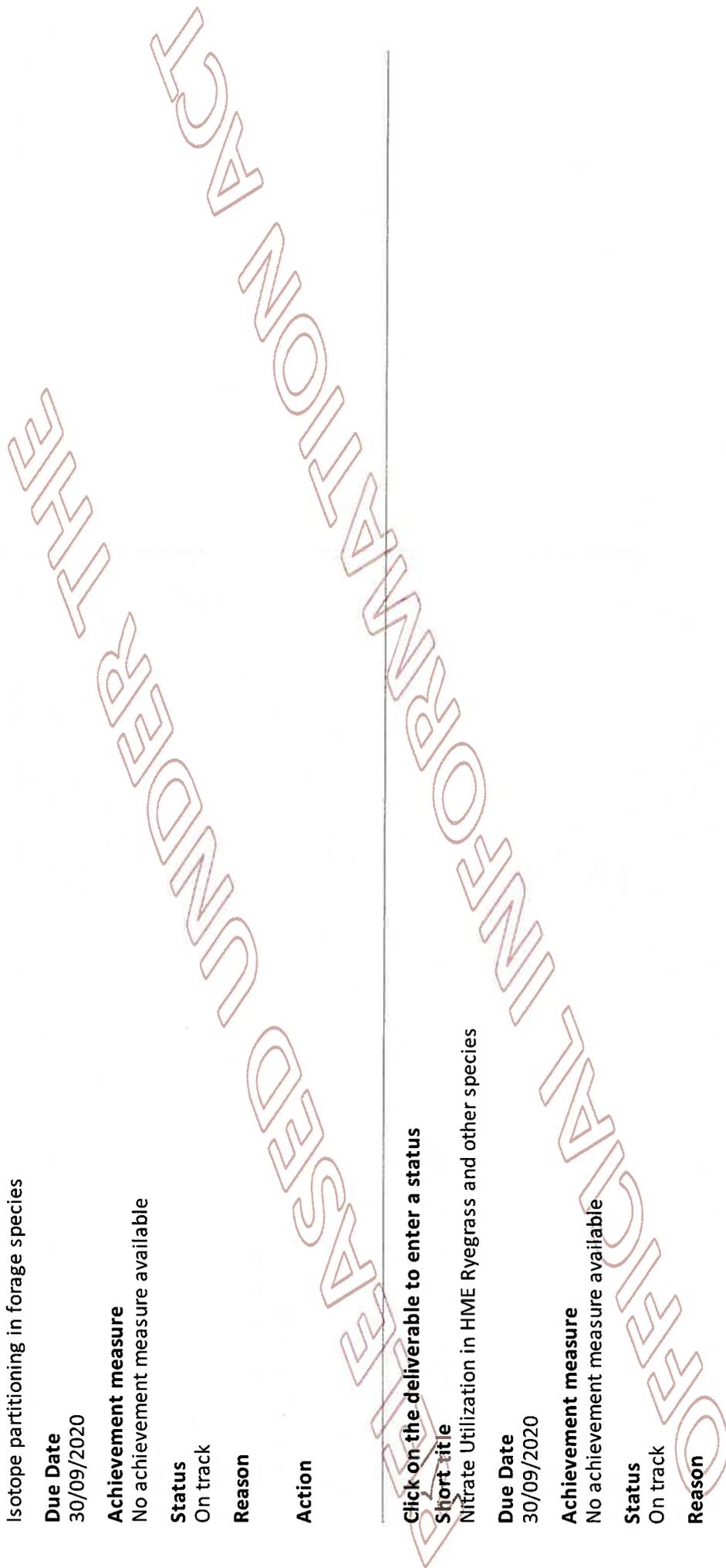
No achievement measure available

**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

**Reason**

**Action**

THESE ASSESSED UNDER MATTER INFORMATION 1545

**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**

On track

**Reason**

**Action**

**Click on the deliverable to enter a status**

**Short title**

Appropriate Fertilizer Composition

**Due Date**

24/12/2019

**Achievement measure**

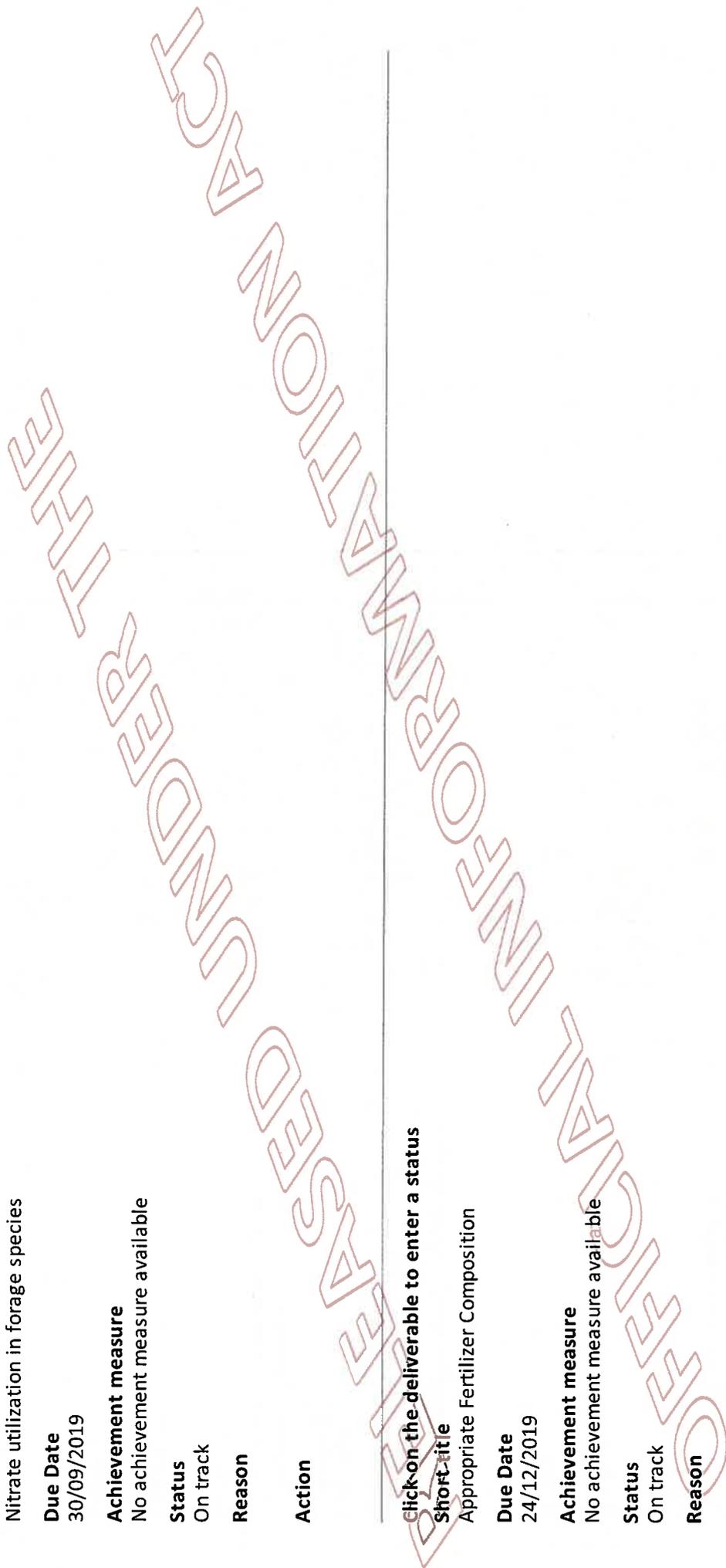
No achievement measure available

**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**

On track

**Reason**

**Action**

THE INFORMATION CONTAINED HEREIN IS UNCLASSIFIED

**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**

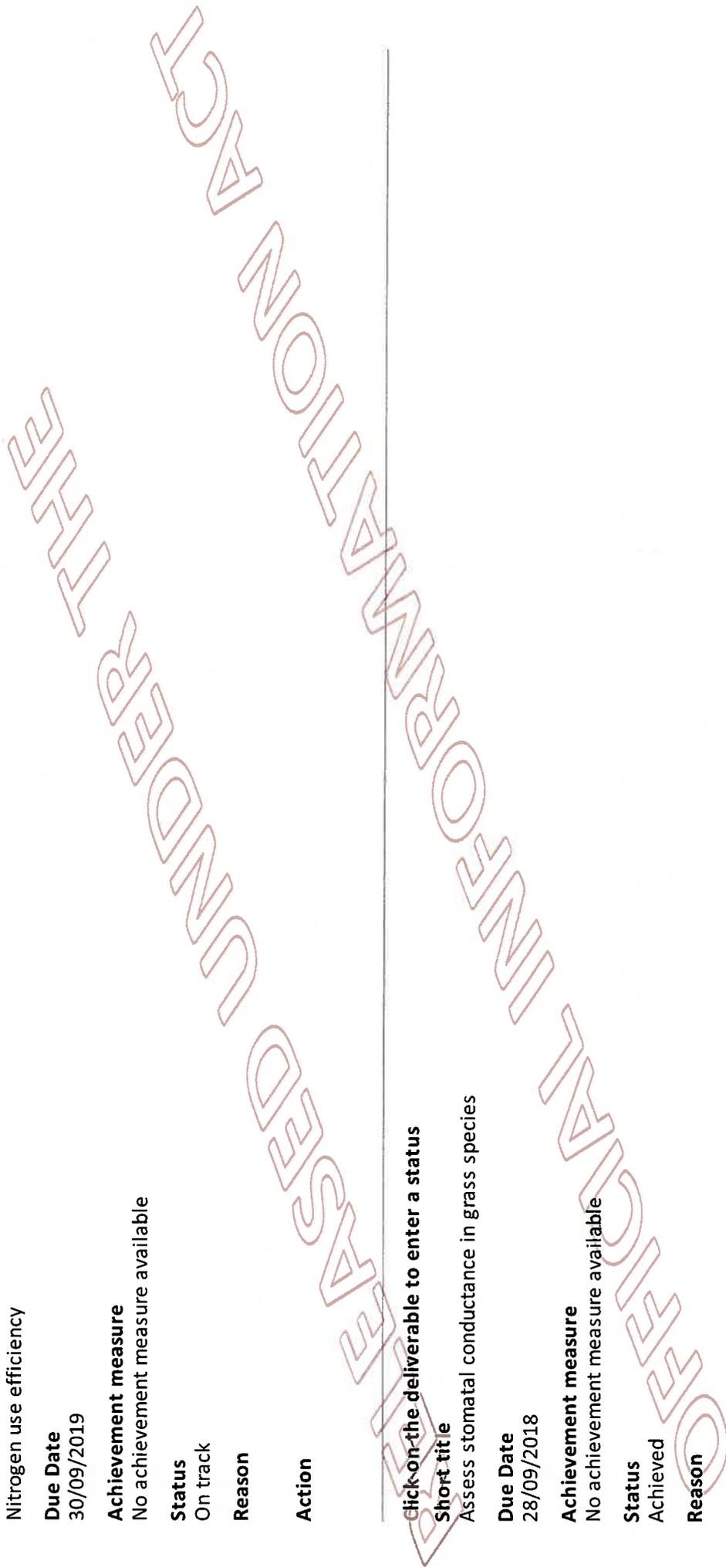
No achievement measure available

**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**

On track

**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**

THE INFORMATION CONTAINED HEREIN IS UNCLASSIFIED

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**

REFEASSED UNDER ATTENTION 45  
OFFICIAL INFORMATION

Click on the deliverable to enter a status

**Short title**

WUE in Legumes

**Due Date**

30/09/2019

**Achievement measure**

No achievement measure available

**Status**

On track with issues

**Reason**

As the HME alfalfa were not suitable due to low expression we have not yet started this work.

**Action**

We are developing new HME alfalfa and will also begin work on PhotoSeed soybean gifted by Zea kal inc. These can be used to assess WUE then when the alfalfa is ready we can repeat experiments and confirm results.

---

**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 had too much trait expression. They had a growth penalty.

**Action**

New rice is being developed with slightly modified gene constructs and we expect to get the work completed on time.

---

**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**

**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**

PLEASE ASSESSED UNDER MATTER THE FACTS

**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**

This was achieved for all 4 HME ryegrass Events.

**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**

THESE NOTES ARE UNCLASSIFIED INFORMATION

**Click on the deliverable to enter a status**

**Short title**

HME Alfalfa trait fixation

**Due Date**

01/05/2019

**Achievement measure**

No achievement measure available

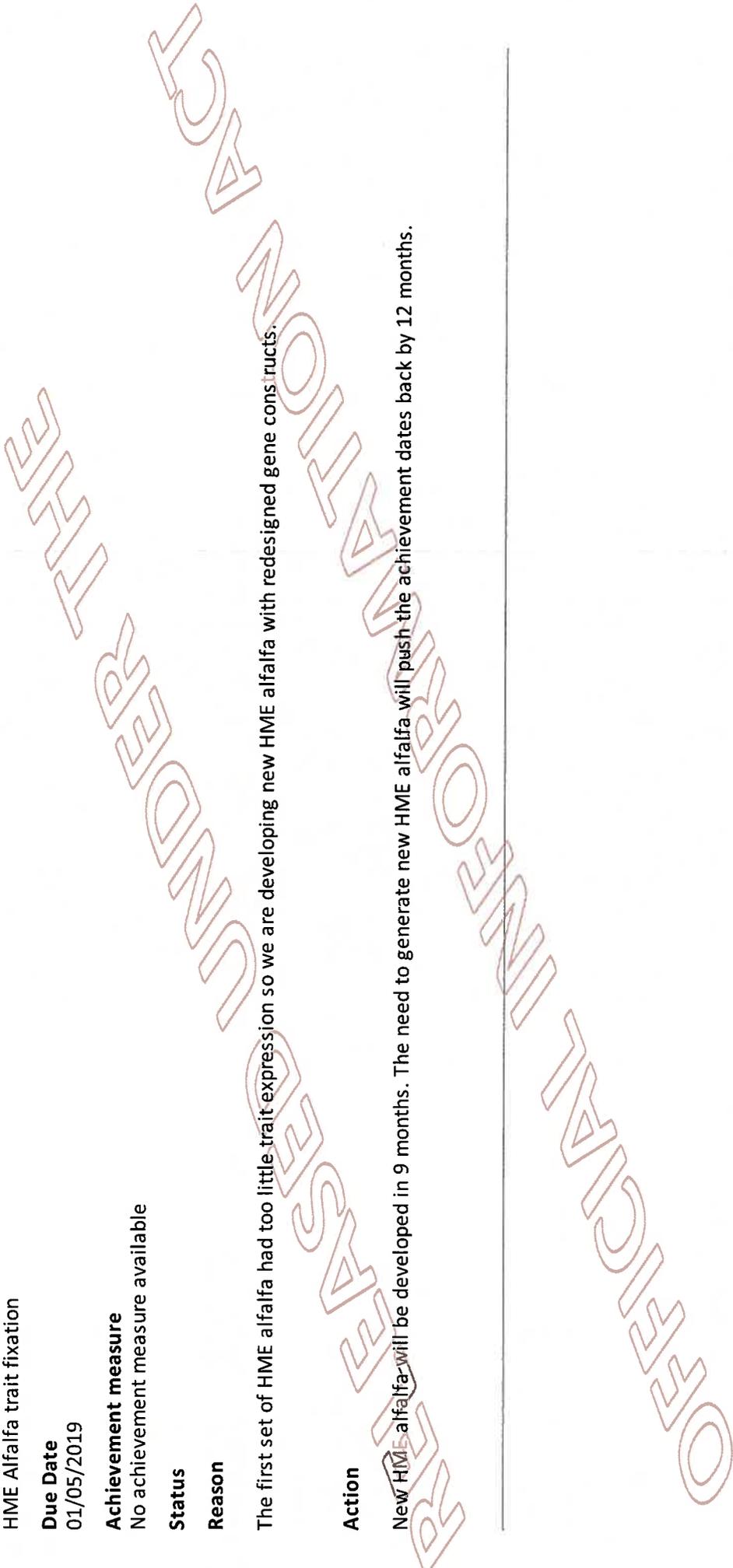
**Status**

**Reason**

The first set of HME alfalfa had too little trait expression so we are developing new HME alfalfa with redesigned gene constructs.

**Action**

New HME alfalfa will be developed in 9 months. The need to generate new HME alfalfa will push the achievement dates back by 12 months.



Click on the deliverable to enter a status

**Short title**

T1 Generation

**Due Date**

30/11/2017

**Achievement measure**

No achievement measure available

**Status**

**Reason**

**Action**

Will now be 30/12/19

Click on the deliverable to enter a status

**Short title**

T2 Generation

**Due Date**

31/08/2018

**Achievement measure**

No achievement measure available

**Status**

**Reason**

**Action**

Will now be 31/08/20

REFUSED UNDER THE INFORMATION ACT 431

**Click on the deliverable to enter a status**

**Short title**  
T3 Generation

**Due Date**  
30/04/2019

**Achievement measure**  
No achievement measure available

**Status**

**Reason**

**Action**

Will now be 30/4/21

**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**

**Action**

PREPARED UNDER MATRONS ATTENTION AT THE

Click on the deliverable to enter a status

**Short title**

T1 Generation

**Due Date**

30/06/2019

**Achievement measure**

No achievement measure available

**Status**

On track

**Reason**

**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**

THESE ASSES UNDERMATTER INFORMATION 45

**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**

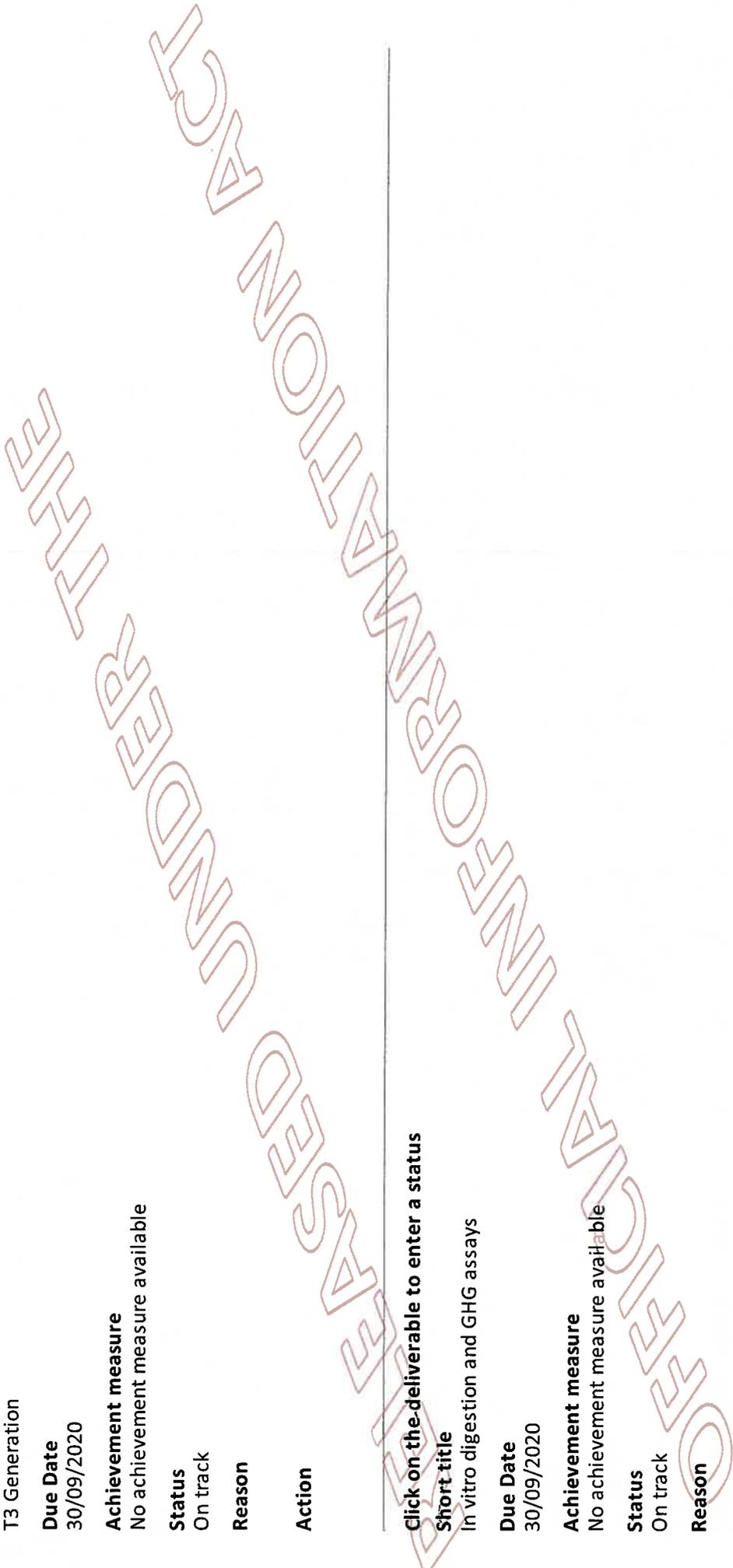
No achievement measure available

**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**

**Action**

OFFICIAL INFORMATION  
REFUSED UNDER THE  
ACCESS TO INFORMATION ACT

**Click on the deliverable to enter a status**

**Short title**

Assays on alfalfa

**Due Date**

30/09/2019

**Achievement measure**

No achievement measure available

**Status**

On track with issues

**Reason**

The first 200 HME alfalfa events had low-level expression of the HME genes and therefore maximum total plant fat achieved was 5%. This is too low to perform meaningful *in vitro* assays.

**Action**

We are developing new HME alfalfa with dedesigned gene constructs with the goal of achieving 6% total fat. This material will lead to meaningful *in vitro* assays. We still hope to achieve this by the completion date of 30/9/19.

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## Work programme conditions

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In accordance with clause 5.10 (e) of the Science Investment Contract, AgResearch Limited must report on the progress made towards giving effect to the Vision Mātauranga 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. If AgResearch Limited does not report on its progress towards Vision Mātauranga, then a Change Event will be deemed to have occurred and the Ministry may give Notice of a Change Event in accordance with clause 6 of the Science Investment Contract.

Our farmer engagement programme led by 9(2)(a) includes Māori stakeholder engagement with Māori agribusinesses. Initial discussions to date have led to the feedback that these groups currently have higher priority areas within their focus and that the HME forage will be considered at a later stage. We plan to maintain our communication with these groups so that we are ready to engage once this becomes a focus area.

We are also preparing to explore opportunities with Plant and Food Research in this important area and look for areas of common interest where we can work together. These meetings will occur later in 2018.

---

Outputs

Knowledge transfer

Modified Date	Knowledge transfer type	Number of Events	Knowledge transfer comments (optional)
02/08/2018	Workshops and hui	3	<ol style="list-style-type: none"> <li>1. NZBio Conference, Wellington 12 October 2017. The Future Farm: Can Forage Biotechnology Provide Environmental Benefit? <b>9(2)(a)</b></li> <li>2. DairyNZ workshop to rural professionals in the Waikato. Karapiro, March 27 2018. The Future Farm: Can Forage Biotechnology Provide Environmental Benefit? <b>9(2)(a)</b></li> <li>3. Ravensdown Advanced Pastures Course, Massey University 18 July, 2018. The Future Farm: Can Forage Biotechnology Provide Environmental Benefit? <b>9(2)(a)</b></li> </ol>

02/08/2018	Substantive information sharing and advice	11	<p>Progress Reports to Industry Programme Steering Committee (by Plant biotechnology team)</p> <ol style="list-style-type: none"> <li>1. 1. October, 2017</li> <li>2. 7 February, 2018</li> <li>3. 1 May, 2018</li> </ol> <p>Other</p> <ol style="list-style-type: none"> <li>1. Presentation to Fonterra Lactation Team [REDACTED], 2017</li> <li>2. Presentation to Government Ministries, [REDACTED] Wellington, 24<sup>th</sup> October 2017</li> <li>3. Presentation to Balance Innovation team, [REDACTED] 6 November, 2017</li> <li>4. Presentation at Science New Zealand Conference, [REDACTED] Te Papa Wellington, 21 October, 2017</li> <li>5. Presentation to Minister James Shaw [REDACTED], 6 April, 2018</li> <li>6. Presentation to Bayer Crop Science [REDACTED], 24 May, 2018</li> <li>7. Presentation to Cortiva [REDACTED], 24 May, 2018</li> <li>8. Presentation to MBIE SSIF Team [REDACTED], 28 May 2018</li> </ol>
03/08/2018	Workshops and hui	2	<p>Plant Science Conference, Massey University. Plants in a Changing Environment. 4th to 6th July 2017.</p> <p>[REDACTED] Presentation. A Novel Technology to Enhance Plant Growth Rates by Mimicking C3 Plant Growth Under High Carbon Dioxide.</p> <p>[REDACTED] Presentation. HME Ryegrass.</p>

**Knowledge transfer type**

Workshops and hui

**Number of Events**

3

**Knowledge transfer comments (optional)**

1. NZBio Conference, Wellington 12 October 2017. The Future Farm: Can Forage Biotechnology Provide Environmental Benefit? 9(2)(a)
2. DairyNZ workshop to rural professionals in the Waikato. Karapiro, March 27 2018. The Future Farm: Can Forage Biotechnology Provide Environmental Benefit? 9(2)(a)
3. Ravensdown Advanced Pastures Course, Massey University 18 July, 2018. The Future Farm: Can Forage Biotechnology Provide Environmental Benefit? 9(2)(a)

**Knowledge transfer type**

Substantive information sharing and advice

**Number of Events**

11

**Knowledge transfer comments (optional)**

Progress Reports to Industry Programme Steering Committee (by Plant biotechnology team)

1. 1. October, 2017
2. 7 February, 2018
3. 1 May, 2018

**Other**

1. Presentation to Fonterra Lactation Team 9(2)(a), 2017
2. Presentation to Government Ministries, 9(2)(a) Wellington, 24<sup>th</sup> October 2017
3. Presentation to Balance Innovation team, 9(2)(a) 6 November, 2017
4. Presentation at Science New Zealand Conference, 9(2)(a) Te Papa Wellington, 21 October, 2017
5. Presentation to Minister James Shaw 9(2)(a), 6 April, 2018
6. Presentation to Bayer Crop Science 9(2)(a), 24 May, 2018
7. Presentation to Cortiva 9(2)(a), 24 May, 2018
8. Presentation to MBIE SSIF Team 9(2)(a), 28 May 2018

**Knowledge transfer type**

Workshops and hui

**Number of Events**

2

**Knowledge transfer comments (optional)**

Plant Science Conference, Massey University. Plants in a Changing Environment. 4th to 6th July 2017.

9(2)(a) Presentation. A Novel Technology to Enhance Plant Growth Rates by Mimicking C3 Plant Growth Under High Carbon Dioxide.

9(2)(a) Presentation. HME Ryegrass.

**Non-peer reviewed published articles****Number of non-peer reviewed published articles**

0

**Non-peer reviewed published articles comments (optional)****New products, processes and services****Number of new products**

0

**Number of new processes****Number of new services****New products, processes and services (optional)****Science quality****Peer-reviewed journal articles in the year they are accepted for publication**

**Number of books or chapters**

**Number of published conference proceedings**

**Awards for science achievement (not open internationally)**

**Awards for science achievement (open internationally)**

**Keynote presentations (not open internationally)**

**Keynote presentations (open internationally)**

**Number of masters or doctoral theses**

**Science quality comments (optional)**

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**Provisional patent and PVR applications**

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**Number of Patent or Plant Variety Right (PVR) applications**

**Number of Patent Cooperation Treaty (PCT) applications**

29

**Provisional patent and PVR applications comments (optional)**

Ongoing prosecutions

<b>Title</b>	<b>Patent or Application #</b>	<b>Status</b>	<b>Country</b>
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	P100104029	Filed - examination requested	Argentina
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	46003/2010	Filed - examination requested	Paraguay
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	32994	Filed - examination requested	Uruguay
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	2010-001704	Filed	Venezuela
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	BR112012011464-6	Filed - examination requested	Brazil

Disulfide Oleosins (Cross Linked Proteins and uses thereof)	2778150	Filed - examination in progress	Canada
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	1068-2012	Filed - examination in progress	Chile
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	3491/DELNP/2012	Filed - examination in progress	India
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	PI 2012700229	Filed - examination in progress	Malaysia
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	T1201001996	Filed	Thailand
Enhanced DGAT1	P 13 01 03955	Filed - examination requested	Argentina
Enhanced DGAT1	2013340443	Filed - examination requested	Australia
Enhanced DGAT1	706832	Filed - examination in progress	New Zealand
Enhanced DGAT1	14/438758	Filed - examination in progress	United States
Enhanced DGAT1	201380069517.X	Filed - examination in progress	China P.R.
Enhanced DGAT1	MX/a/2015/005260	Filed - examination in progress	Mexico
DGAT1 N/C Chimeras	P 13 01 03956	Filed - examination requested	Argentina
DGAT1 N/C Chimeras	2013340445	Filed - examination requested	Australia
DGAT1 N/C Chimeras	BR112015009467-8	Filed - examination requested	Brazil
DGAT1 N/C Chimeras	2889985	Filed	Canada
DGAT1 N/C Chimeras	13851770.1	Filed - examination in progress	European Patent Convention
DGAT1 N/C Chimeras	706834	Filed - examination in progress	New Zealand
DGAT1 N/C Chimeras	201380069478.3	Filed - examination in progress	China P.R.
DGAT1 N/C Chimeras	3964/DELNP/2015	Filed - examination requested	India
DGAT1 Zm-Long	P 13 01 039600	Filed - examination requested	Argentina
DGAT1 Zm-Long	2013340444	Filed - examination requested	Australia
DGAT1 Zm-Long	706829	Filed - examination in progress	New Zealand
DGAT1 Zm-Long	201380069487.2	Filed - examination in progress	China P.R.

DGAT1 Zm-Long	MX/a/2015/005270	Filed - examination in progress	Mexico
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## Patent and PVR grants

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

2

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

Granted in last 12 months

Disulfide Oleosins (Cross Linked Proteins and uses thereof)	1/2012/500816	Granted	Philippines
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DGAT1 Zm-Long	14/438784	Granted	United States
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Summary of Patent grants in HME portfolia to date:

Title	Patent or Application #	Status	Country
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	2010313865	Granted	Australia
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	ZL201080058155.0	Granted	China P.R.
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	5934101	Granted	Japan
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	332575	Granted	Mexico
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	599429	Granted	New Zealand

Disulfide Oleosins (Cross Linked Proteins and uses thereof)	1/2012/500816	Granted	Philippines
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	8987551	Granted	United States
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	IDP000037613	Granted	Indonesia
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	10827209.7	Granted	France
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	10827209.7	Granted	Germany
Disulfide Oleosins (Cross Linked Proteins and uses thereof)	10827209.7	Granted	Spain
DGAT1 N/C Chimeras	14/438768	Granted	United States
DGAT1 N/C Chimeras	MX/a/2015/005271	Granted	Mexico
DGAT1 Zm-Long	14/438784	Granted	United States

## Revenue and Contracting

## Co-funding and subcontracting

Reporting financial year: 2017 (This report covers the period 01/07/17 - 30/06/18)

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: 2017 (This report covers the period 01/07/17 - 30/06/18)

**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)**  
(Excl. GST)

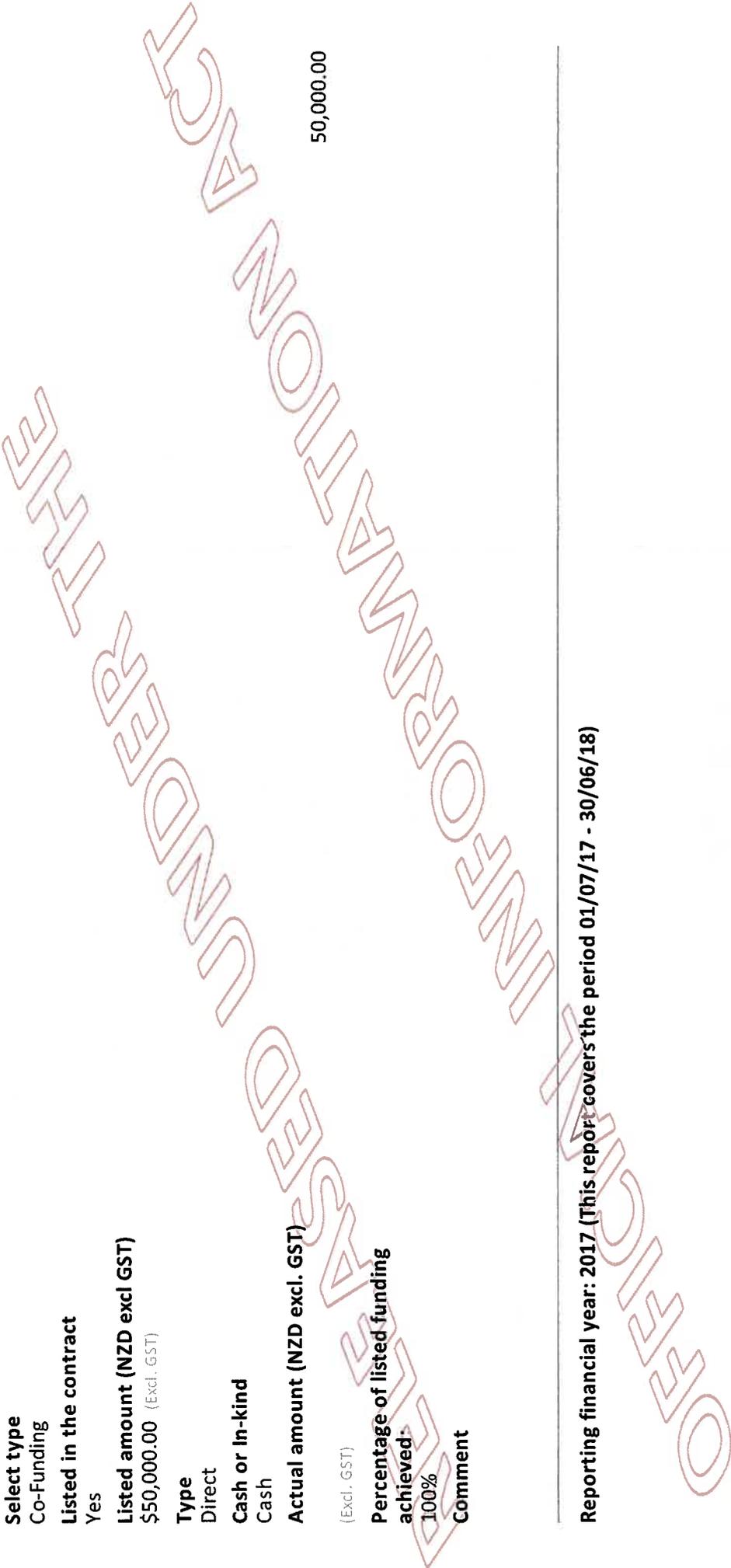
50,000.00

**Percentage of listed funding achieved**  
100%

**Comment**

---

Reporting financial year: 2017 (This report covers the period 01/07/17 - 30/06/18)



**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)**

(Excl. GST)

750,000.00

**Percentage of listed funding**

achieved:

100%

**Comment**

Reporting financial year: 2017 (This report covers the period 01/07/17 - 30/06/18)

**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)**  
(Excl. GST)

100,000.00

**Percentage of listed funding achieved:**  
100%

**Comment**

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## Formal Collaborations

## Collaborations by country

Country	Level	Number of collaborations	Comment
United States of America (the)	Strong	3	
Thailand	Medium	1	

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## Capability Building

### Students

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**Number of students obtaining Masterate qualifications**

0

**Number of students obtaining Doctoral qualifications**

0

**Number of students obtaining Post-Doctoral qualifications**

0

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### Secondments to or from end users

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**Number of secondments as FTEs from an end user organisation**

0

**Number of secondments as FTEs to an end user organisation**

0

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End User Relationships

End user contract details

Organisation	Briefly describe how you are working with this organisation	Contact person	Contact phone	Contact email
Dairy NZ	<p>Dairy NZ are co-funders and part of the collaborative agreement. They have a representative on the Programme Steering Group that has governance over the programme. DNZ work directly with dairy farmers, manage the Forage Value Index and work closely with processing companies. DNZ will be a key decision maker on how to proceed in New Zealand once the overseas field and animal nutrition trials are complete.</p>	9(2)(a)		
PGG Wrightson Seeds	<p>PGG Wrightson Seeds are co-funders and part of the implementation pipeline as they provide a route to market. They will have a representative on the programme steering group.</p>	9(2)(a)		

<p>Grasslanz Technology Limited</p>	<p>Grasslanz Technology Ltd are co-funders and part of the implementation pipeline as they provide expertise in endophyte commercialisation and the management of nucleus seed for the seed industry. They will have a representative on the programme steering group.</p>	9(2)(a)		

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## 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

Declaration

Declaration

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Click the check box to acknowledge that the information you have given is true, correct and complete

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