Project development and coordination for the improved management of perennial ryegrass toxicity

Milestone

A final report on the services provided identifying the gaps in MLA's research program on Perennial Rye Grass Toxicity and its links to pasture persistence and how this might be addressed in future.

Abstract

A successful Perennial Ryegrass Toxicoses Review Workshop was held on the 11th of July 2012. Working with Stephen Page a final report containing proceedings of the workshop plus overall recommendations was produced and provided to MLA and workshop attendees. A number of project proposals are under development which largely addresses all of the recommendations of the review and there is opportunity for good multi-institutional, multi-state collaborative activity along with excellent prospects for the involvement of the commercial companies including feed and seed companies. It is recommended that another review be conducted in 3-5 years in order to assess and present activity to date, revise the cost to industry and review recommendations and priorities in light of new information.

Project objectives

- Participate in the preparation and running of workshop to review PRGT that shall include industry representatives (producer and commercial) and as well as researchers
- Advise on the preparation of projects and applications in the areas of pasture production, persistence and toxicity for animal safety
- Collate and review recommendations and liaise with research organisation groups and commercial breeding companies to ensure recommendations are implemented
- Provide a monthly update to MLA detailing interaction with research organisations and breeding companies, as directed by MLA from time to time.

Success in achieving milestone
The following details outline the success in achieving/fulfilling the project objectives.

Delivery on Project Objectives:
Participate in the preparation and running of workshop to review PRGT that shall include industry representatives (producer and commercial) and as well as researchers
In partnership with Stephen Page a PRGT Review Workshop was successfully organized and conducted on the 11th July 2012. There were approximately 24 people in attendance including researchers (animal, veterinary and agronomic), Veterinarians, farm management consultants, seed industry representatives, feed supplement representatives, producers, Dairy Australia and Meat and Livestock Australia representatives. A report covering the workshops proceedings and recommendations and entitled "Perennial Ryegrass Toxicoses, Workshop to review current status and recommendations" has been prepared in conjunction with Stephen Page and presented to Meat and Livestock Australia.

Advise on the preparation of projects and applications in the areas of pasture production, persistence and toxicity for animal safety
Within the report mentioned above there were some key recommendations that are as follows:

There was general agreement on four main areas of need:
1. **Animal Production based research**
Areas for further study include production, reproduction, lifetime effects (mature stock and lambs, different breeds), development and validation of models (small and large animals), subclinical effects. The justification for this work was the absolute need to develop the compelling case for change, development of tools and testing of solutions.
2. **Therapeutic Options**
a. SHORT TERM OPTION - there is a case for a short term therapeutic agent that would assist in the rapid recovery of livestock from a staggers event – research has commenced and preliminary results look promising but further evidence of efficacy and safety is essential
b. MEDIUM TERM OPTIONS - primarily focussed on feed additives. There has been some work done to date, however, there was some discussion regarding what further work needs to be done. The exact mode of action of these agents appears to be unknown. Before such approaches can become available commercially it is necessary that each product is approved by APVMA. Further work should be guided by the requirements of APVMA and a meeting with APVMA may be invaluable in order to define more precisely the data needs to increase the likelihood of a successful APVMA application.
3. Awareness and Extension
a. Awareness and extension will need to be focussed on the key important areas of Gippsland, SW Victoria and Tasmania as these are the major areas for PRGT in Australia.

b. A key issue raised was the continued use of perennial ryegrass containing WT endophyte which is the cause of PRGT – this needs to stop wherever animal health and welfare is likely to be compromised

c. Awareness and extension will assist greatly in developing/presenting the compelling case for change and the current solutions as they are developed / available

d. Awareness and extension will operate as nodes for dissemination of information from other research activities such as in 1 and 2 above.

4. Longer Term Change in the Feedbase

This included development of long term (agronomic) solutions that reduced toxicity to livestock but with better persistence and longer term productivity.

a. Improved understanding of the insect (biotic) pest problems was clearly raised – and how it relates to persistence and long term productivity of pastures

b. Important that there be developed ryegrass/endophyte combinations that are not only safe for livestock, but also persist and improve productivity – thereby basing the argument for renovation in terms of greater overall productivity and not just on animal health reasons

There are a number of research projects currently in development that will address one or more of these recommendations. In brief these are:
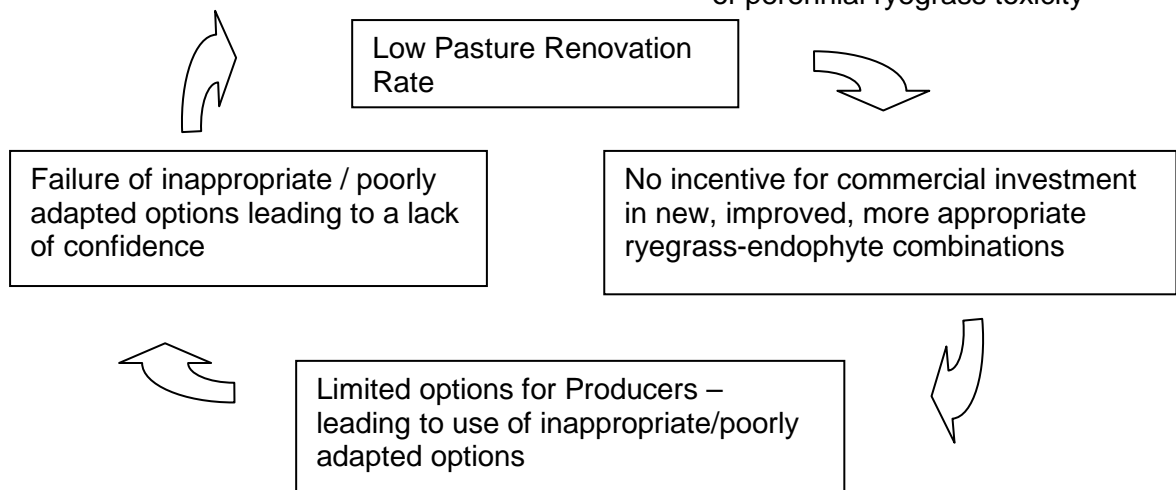
1. **PRGT Project Proposal**
   A collaboration between the University of Melbourne, Charles Sturt University, University of Sydney, MacKinnon Project and potentially the University of Tasmania. This project will specifically address recommendations 1, 2a, 3 and through recommendation 3, help to drive a longer term change in the feedbase (recommendation 4). A draft proposal is in production with delivery to MLA (and AWI) expected later in November/early December.

2. **Development of appropriate ryegrass genetics plus novel endophyte technology for the beef-sheep perennial ryegrass zone of Australia**.
   This has been under development for approximately 18 months and is entering the final stages of full proposal development. It is a long term project involving commercial partners. Low renovation rates plus producer resistance to adoption of new and improved plant (and endophyte) genetic options combine in this zone to make it commercially unattractive for commercial seed companies to invest without support. The delivery of appropriate plant-endophytes specifically developed and tested for this environment/zone in combination with other MLA activities directed at generating change in this region (eg PVTN) will be instrumental in breaking a cycle of poor "product" performance in this zone. This cycle is represented by:

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                          ┌──────────────────────┐
                          │ Low Pasture Renovation│
                          │ Rate                  │
                          └──────────────────────┘
┌──────────────────────────┐      ┌──────────────────────────────┐
│ Failure of inappropriate /│      │ No incentive for commercial   │
│ poorly adapted options     │      │ investment in new, improved,  │
│ leading to a lack of       │      │ more appropriate              │
│ confidence                 │      │ ryegrass-endophyte            │
└──────────────────────────┘      │ combinations                  │
                                   └──────────────────────────────┘
          ┌─────────────────────────────┐
          │ Limited options for Producers –│
          │ leading to use of              │
          │ inappropriate/poorly adapted   │
          │ options                        │
          └─────────────────────────────┘
```

The perennial ryegrass Beef-sheep zone (significant parts of which are designated high risk PRGT zones) covers approximately 80% of the total area sown to ryegrass, approximately 5M Ha.

This proposal is in the final stages of development and will be seeking to largely make use of the MLA donor company scheme. A second, smaller part of this project is seeking direct MLA and DA funding and will undertake insect survey work across the relevant region(s). Insects and insect protection are a significant issue relating to pasture production and importantly persistence and endophyte has a significant role to play in providing plant protection against insect pests.

Collate and review recommendations and liaise with research organisation groups and commercial breeding companies to ensure recommendations are implemented

This collation and review is represented by the report that was authored by Stephen Page and which included significant contributions from this author. In terms of recommendations being implemented, this author is actively working with research groups and commercial companies in order to put in place projects that will deliver on the recommendations over time. These efforts are outlined in the two main areas of work listed above.

Provide a monthly update to MLA detailing interaction with research organisations and breeding companies, as directed by MLA from time to time.

As previously mentioned, two main areas of work are currently underway.
1. PRGT Research.
2. Development of appropriate ryegrass genetics plus novel endophyte technology for the beef-sheep perennial ryegrass zone of Australia.

With respect to the first item, collaboration is being built between the major researchers in the PRGT space – this includes University of Melbourne, Charles Sturt University, University of Sydney (a relatively minor participant) and the

MacKinnon Project. In addition to this Kevin Reed has been asked to participate in this collaboration and is expected to be involved at an upper, advisory level plus the importance of PRGT in Tasmania has been recognized and preliminary discussions have been held with the intention of having the University of Tasmania also join the team. This represents a multi-agency, multi-state approach to addressing PRGT. A preliminary proposal is being developed for delivery to MLA in late November/early December.

With respect to the second item, significant consultation has occurred and the project proposal is well developed with commercial partners (PGGW, Grasslanz, AgResearch) and industry RDC's. MLA has participated in a round of meetings in New Zealand where there was significant consultation and fact finding with respect to understanding the capability and opportunity represented by these commercial partners. The meetings went well and this project is very close to submission of the final full proposal. In addition to this, a subset of this major proposal is focussed on improving our understanding of the insect pests in the relevant region and Dairy Australia has expressed interest in assisting with this. On the advice of DA, there have been discussions with GippsDairy (a DA RDP) and DPI researchers that have been working with them on Red Headed Pasture Cockchafer (RHPCC). This part of the project proposal has been drafted and is near to completion however it is still waiting on further discussions with the DPI researchers.

Overall progress of the project
There has been very good progress with a number of major projects under development that will directly address the recommendations arising from the PRGT review workshop. The workshop report is a good summary of current information in the PRGT space and it is clear that work currently underway will inform this space significantly.

Recommendations
In addition to the recommendations arising from the PRGT Review Workshop, the following recommendations are offered:
- Conduct another PRGT Review workshop in approximately 3 years as a way of reviewing progress to date, examining recommendations in light of new information and progress to date, and re-setting as necessary recommendations and priorities for future research.
- Along with this review, commission a new, revised assessment of the cost of PRGT to industry and ensure that up to date information on all aspects is utilized in the process. Current projects such as BAHE 0039 will have been completed by then and will provide further useful information.
- While not mentioned in this report, the value of Stephen Page's long term involvement and advice in the PRGT area has been highly significant. His retirement from this space is a significant loss and it is recommended that a replacement be found. Finding an equally rigorous, knowledgeable, balanced and independent replacement will be a challenge which I don't envy MLA. Stephens input and advice will be greatly missed.

- It is inappropriate for this report to contain recommendations on project proposals either not yet submitted or in which this author is participant/developer, however it is reasonable for there to be a recommendation for MLA to consider responding to the PRGT Review Workshop with some indication of possible future investment and priorities.

Appendices

Please find attached the PRGT Review Workshop report. This report has previously been delivered to MLA.

PERENNIAL RYEGRASS TOXICOSIS

Meat & Livestock Australia

WORKSHOP TO REVIEW CURRENT STATUS & RECOMMENDATIONS

Best Western Airport Motel and Convention Centre,

33 Ardlie St, Attwood, Vic, 3049

11 July 2012

PROCEEDINGS AND RECOMMENDATIONS

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4. Recommendations from 2009 Workshop
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INTRODUCTION TO WORKSHOP

PRGT has long been recognised purely as "ryegrass staggers"- an inconvenient neurotoxicity. Recent catastrophic events have reemphasised the importance of heat stress and production and reproduction losses highlighting the critical role of ergot alkaloid toxicity under Australian environmental conditions.

Wild-endophyte pasture can be removed and replaced. However, it is an enormous challenge to persuade farmers to renovate poor pasture, let alone well established PRG dominant pasture that appears to them to be good pasture. Adoption of toxin-safe pasture should increase considerably when accurate cost-benefit data are available at representative locations. The PRGT Steering Committee and Werribee Workshop recommended that animal production studies are needed to investigate the widely unrecognised spectrum of "sub-clinical" losses associated with PRGT.

MLA, Grasslanz Technology, Biomin Australia and Feedworks have supported a number of recent dairy and sheep production studies. Available results highlight the importance of management (supplementary feed, time of mating, breed) and flock/herd size in obtaining statistically significant results. Future research will greatly benefit from current experience and allow improved economic modelling of the impact of wild-endophyte pasture.

OBJECTIVES OF WORKSHOP

1. Summarise and review the PRGT Steering Committee recommendations for Research, Development, Teaching and Extension, the priority list of arising from the MLA PRGT workshop held at MU Vet School, Werribee, 30 OCT 2009, and all R&D arising.
2. Review and update the economic assessment of PRGT described by Sackett and Francis, (2006) in MLA report B.AHW.0089
3. Identify gaps in knowledge and expertise, develop recommendations to fill gaps, and prepare guidance on key criteria for prioritisation.

KEY POINTS

[Refer to Appendix 6 for copy of presentations]

INTRODUCTION

Johann Schröder

- The MLA Animal Health and Biosecurity project portfolio has particular focus on endemic infectious diseases (internal and external parasites, bacterial, protozoal and viral diseases), exotic infectious diseases, and natural toxicants, especially toxic plants.
- Perennial ryegrass toxicosis has been and remains an area of special attention by MLA and a number of projects have been supported over the last 10 years
- The current workshop is especially important as a forum for sharing information, identifying data gaps and considering recommendations for further work.

REVIEW OF RECOMMENDATIONS, PRIORITIES AND OUTCOMES

Stephen Page

- The objectives of the workshop are threefold:
    1. Summarise and review the PRGT Steering Committee recommendations for Research, Development, Teaching and Extension, the priority list of arising from the MLA PRGT workshop held at MU Vet School in 2009 and all R&D arising
    2. Review and update the economic assessment of PRGT described by Sackett and Francis, (2006) in MLA report B.AHW.0089
    3. Identify gaps in knowledge and expertise, develop recommendations to fill gaps, and prepare guidance on key criteria for prioritisation
- Work supported by MLA includes:
    - 2003/N07 Determining Presence of Toxins in Rye Grass and Fescue Pastures
    - Perennial Ryegrass Toxicosis in Australia. Proceedings of a Symposium held on Friday 18 March2005 at Attwood, Victoria
    - PRGT Steering Committee (2005-2007) (see Appendix 5)
    - B.AHW.0089 Economic assessment of the impact of wild endophyte-infected perennial ryegrass (Lolium perenne) on the productivity of sheep and cattle and the profitability of Australian livestock enterprises (June 2006)
    - Tips & Tools: Perennial Ryegrass Toxicosis (NPA 21913/06)
    - Prograzier: Reducing perennial ryegrass toxicosis (Spring 2007)
    - Technical Bulletin: Perennial Ryegrass Toxicosis (updated 2010)
    - PRGT Workshop (University of Melbourne, Werribee)(30 October 2009) (see Appendix 4)
    - B.AHE.0039 Developing increased understanding, awareness and potential mitigation strategies for perennial ryegrass toxicoses in sheep production systems (Brian Leury)
    - B.AHE.0040 Identification of metabolites associated with severe cases of perennial ryegrass toxicosis (Kevin Reed)
- Presentations at this workshop will review the current status of knowledge and will be followed by a breakout session to discuss how the significant impact of PRGT can be most efficiently mitigated in the future.

MOLECULAR GENETIC TECHNOLOGIES IN PLANT BREEDING AND PRE-COMPETITIVE FACILITATED ADOPTION IN RYE GRASS

Felice Driver

- Application of genomic tools for managing PRGT
    - Identification of safe novel commercial endophyte strains and standard toxic strains (AR1 and NEA3). Other SNPs that can be validated; proxy for a toxin profile.
    - Examine host/endophyte symbiosis over time to monitor population dynamics, cultivar performance, cultivar adaptation and persistence, and incursions of foreign material. Systems biology and use of microarrays.
    - Develop selection signatures (allelic diversity) for persistence/adaptation and safety in an environment

WORKING TO MAKE THE FEEDBASE SAFE

Cameron Allen

- What improvements can be achieved and when?
- Elements of the evidence based case for future work include:
    - What is the probability of technical success?
    - What is the likelihood of adoption?
    - Are there effective alternative technologies to solve the problem?
    - What can MLA invest in to support private enterprise (for example, via the MLA Donor Company or MDC)?
    - Who can deliver the outcome?
- Is sufficient information and knowledge already available that needs a communication plan to enable adoption?

PASTURE MYCOTOXIN RESEARCH BY BIOMIN

Kevin Reed

- REPLACE TOXIC PASTURE?
    - Objective data is needed on costs/benefits of replacing pasture as the resowing rate <2% p.a. in many affected districts
    - Info. is needed on lifetime exposure - its impact on fertility, animal development, health & production, behaviour and lactogenesis
- MANAGING TOXIC PASTURE?
    - Studies with Mycofix® delivered to sheep in mash or loose lick from 2008 to 2011 found reduced adverse impact of PRGT on heat-stress, liveweight gain, faecal staining and fertility

B.AHE.0040. IDENTIFICATION OF METABOLITES ASSOCIATED WITH SEVERE PERENNIAL RYEGRASS TOXICOSIS

Kevin Reed/Wade Mace

- Why are mortalities and dam-crowding events in Aust. so much worse than in New Zealand?
- Two studies:

- Metabolite changes over the season (5 dates) in isogenic PRG (cv Samson WT) at 3 sites in Vic and 1 in NZ
  - In the first two seasons there were no obvious trans-Tasman differences in the identity of indole diterpenes (esp lolitrems) and ergot alkaloids
- Metabolites in PRG associated with acute cases of PRGT
- At present the cause of the trans-Tasman difference in impact of PRGT is unknown, it does not appear to be associated with differences in toxin concentration or toxin intake; it is possible that differences in unidentified toxins, solar radiation, ambient temperature, stage of PRG growth or botanical composition of pasture may play a role.

B.AHE.0039. EFFECTS OF PERENNIAL RYEGRASS ENDOPHYTE ON SHEEP: PHD AND MLA/GRASSLANDZ PROJECT

Michelle Henry / Stuart Kemp / John Webb Ware / Brian Leury

- Controlled environment temperature studies (thermoneutral and 35^0C) showed decreased DMI and increased temperature of rectum and skin on back with increased endophyte toxin exposure
- Dookie field study program (commenced February 2011) aims to:
  - Assess agronomic benefits of novel endophytes (AR1 and AR37) compared with WT endophyte-infected ryegrass over two seasons.
  - Assess physiological and productivity responses of young Merino ewes and pregnant and lactating crossbred ewes over two seasons.
    - Liveweight and rectal temperatures of Merinos (but not crossbreds) decreased in WT group summer/autumn 2011
    - Significant prevalence of staggers displayed first (day 35) by Merino ewes then later (day 44) by crossbred ewes.
  - Determine whether endophyte alkaloid consumption during pregnancy alters milk production and lamb performance.
- Elitox study at thermoneutral conditions
  - Rectal temperature and respiratory rate increased by alkaloid ingestion and magnitude of increase was reduced by Elitox at both doses studied (2g/d and 4g/d)
  - Skin temperature increased by alkaloid ingestion, but magnitude of increase not reduced by Elitox at either dose

PRGT CLINICAL INVESTIGATIONS: CURRENT FINDINGS AND FUTURE DIRECTIONS

Jane Quinn / Martin Combs / Leslie Weston / David Rendell / Kevin Reed / Wade Mace

- Clinical investigation in 2011 in the Hamilton area of 15 sheep from 7 properties experiencing severe neurological signs consistent with PRGT
  - 2 distinct gait abnormalities were identified (dysdiadokinesis & rhythmic myoclonus) which were related to severity or stage of disease and may allow improved classification of stage of disease
  - Signs of toxicity persisted as pasture toxin levels declined suggesting toxin accumulation in affected sheep (or long lasting effects)
  - A range of therapeutic options were explored with good success
  - Recommendations included:
    - On farm risk management strategy

- ▪ Pasture toxin analysis service
- ▪ Further study of lolitrem B PK in exposed animals
- ▪ Investigation of therapeutic interventions to establish evidence of efficacy

POTASSIUM BROMIDE: PHARMACOKINETICS & PRGT MOUSE MODEL: A FLEXIBLE TESTING PLATFORM FOR THERAPEUTIC TRIALS.

Martin Combs

- Potassium bromide, an antiepileptic, anxiolytic and sedative agent, was selected as a possible treatment for the neurological signs of PRGT
- The pharmacokinetic behaviour of KBr administered intravenously or orally was investigated and oral bioavailability found to be 100%. The agent had an elimination half life of around 14 days
- A mouse model for study of the neurological signs of PRGT was investigated and found to provide the following potential benefits:
  - o Testing of a range of therapeutic agents to screen for therapeutic candidates.
  - o Identification of early behavioural/ neurologicalchanges.
  - o Metabolomic studies using purified toxin.
  - o Improved diagnostic criteria on histopathological samples.

GRASS ENDOPHYTES FOR INSECT MANAGEMENT AND IMPROVED PASTURE PRODUCTIVITY

John Caradus

- The type of alkaloids produced depends on the strain of fungus present and the host plant has a major effect on the quantity of alkaloids
- Four main groups of endoyphte alkaloids identified
  - o Peramine
  - o Ergovaline
  - o Indole diterpenoids
    - ▪ Lolitrems
    - ▪ Epoxy janthitrems
  - o Lolines
- Endophytes alkaloids can have serious affects on ruminants but some alkaloids are beneficial and the best outcome is a case of balancing the upside of insect resistance and drought tolerance with the downside of animal health and welfare
- Since the link between endophytes and insect resistance and PRGT was established in 1980 a number of significant achievements have been made at Grasslanz including
  - o AR1 novel endophyte introduced in 2000 (no staggers, no heat stress, Argentine Stem Weevil resistance)
  - o AR37 novel endophyte introduced in 2007 (some staggers but not as severe as WT, better persistence than AR1)
- Ellinbank dairy trial comparing WT, AR1 and AR37 over three years found no differences between endophyte groups on milk production

THE VALIDITY OF SHORTER TERM CONTROL PRODUCTS WITHIN THE BROADER PRGT PREVENTION PROJECT

Neil Gannon/Ian Sawyer

- Both prevention and treatment have valid claims to focus on & support within this project
    - The aim overall is to offer end-users methods to control the negative impacts of PRGT
    - Both control and treatment tools are important
    - Controlling the nature of rye grass varieties is a prevention based approach only
    - People experiencing problems Today, need a response to those problems in immediate term
- Short term issues will inevitably continue for many years to come, so supporting both short and long term control strategies is both valid and important
    - Available novel endophyte varieties may retain ergovaline which has adverse health and economic impacts on sheep and cattle
    - Uptake of new varieties will be slow (pasture renovation rates are only 3-5% in beef/sheep)
    - Will new varieties persist?
- Challenges to commercialisation of additives for prevention and treatment:
    - APVMA registration requirements
    - Route of administration and effective dosing
    - Predicting the effects of PRGT
    - How big is the market and what is the return on investment (ROI)

DAIRY INDUSTRY IMPACT OF PRGT

John Evans

- PRGT widely considered of low importance throughout much of the dairy industry
    - This may relate to low level of awareness
    - Acceptance of some behavioural and production problems
    - Little or no reporting of adverse impacts
- Wild type endophyte pastures declining
    - Good uptake of safe endophytes
    - Higher renovation rate in dairy (10%) than in sheep/beef (2-5%)
- Impact of insects on Australian pastures requires further information

SW VICTORIA & TASMANIA PRGT PERSPECTIVES

Graham Lean

- Since the 2009 workshop:
    - More clinical impacts confirmed
    - Novel endophyte pastures showing promise, they are persisting and seem to reduce PRGT impacts
    - Concerned every year seems to be a PRGT year and economics are more significant
- Animal impacts include:
    - Cattle fertility
    - Effects of PRGT experienced every year and seem to be getting worse
    - Subclinical impacts confirmed
- Novel endophyte pastures and prevention of subclinical effects – evidence needed to support recommendations

- Economic impact may be even higher than outlined in Sackett and Francis report – in view of impacts every year and presence of subclinical effects
- Research priorities should include:
  - Clinical and subclinical losses from PRGT
  - Pasture renovation success with different species?
  - Pasture renovation success with novel endophytes?
  - Economic loss needs to be better determined
  - Welfare impact on humans of experiencing PRGT outbreaks needs work

SEEDS OF THE FUTURE

Alan Newman

- Novel endophytes
  - Alkaloid profile is only part of the development process
  - Delivery issues are as important as the animal effects
  - Grazing management is critical to the success of novel endophyte pastures
- Persistence
  - Need to find the balance between persistence and productivity
  - No point in being present if not productive
  - Often hear that "new varieties don't persist like the old ones"
  - Linking productivity & persistence to profitability
- Grazing
  - New endophytes do persist as well under well managed situations
  - Poor grazing management (i.e. over grazing) will reduce persistence
  - Low toxicity endophytes pastures are exposed to overgrazing
- Adoption
  - Need the information to develop a convincing argument to promote increased pasture renewal
  - Illustrate benefits (not features) and profitability
  - Management packages to improve the success rates of moving to novel endophyte
- Looking ahead
  - We need safe and productive pastures
  - The future will involve novel endophytes – no question!
  - We need to convince people there is value in re-sowing new & improved varieties containing new & improved endophytes
  - We need to improve the grazing management systems to allow the value to be reliably extracted from the new varieties & endophytes

THE FUTURE OF PERENNIAL RYEGRASS ENDOPHYTES

Rob Salmon

- Why do we need new endophytes? Standard endophyte (Wild Type) has killed 1000s of sheep in Australia over this last decade – the tip of the iceberg
- Adoption is slow
  - Low rates of pasture renewal
  - High proportion of commons

- o Gaps in Knowledge (insects, agronomy, animals, endophytes)
- Future of endophytes
  - o Identify the market, its gaps & its failures
  - o Persistence
  - o Broader pests spectrum
  - o Safety
  - o Delivery challenges
  - o Thought Leadership

THE FINANCIAL IMPACT OF PRGT

John Webb Ware

- Methodology
  - o Base assumptions similar to 2006 MLA report (HSA)
  - o Enterprises
    - Self replacing merino flock
    - Dual Purpose
    - First cross ewes
    - Beef cattle breeding herd
  - o Three levels of impact
    - High (1 in 5 years 2 in 5 medium)
    - Medium (1 in 3 years)
    - Low (1 in ten years)
    - Beef (High 1 in 5 years)
  - o Regional impact across PRG regions of Australia
  - o Stock numbers
    - Sheep 66% of 2006 (more DP & PL)
    - Beef 106% of 2006
- Combined total losses to industry
  - o Self replacing merino flock $ 47,482,963
  - o Dual Purpose $ 12,787,990
  - o Prime lamb $ 30,175,324
  - o Beef cattle breeding herd $ 6,519,014
  - o TOTAL $ 96,965,290
- Conclusions
  - o Smaller sheep flock
    - Total loss higher due to high commodity prices and bigger impact in prime lamb production even with large reduction in flock size
  - o Beef herd
    - Loss increased due to higher commodity prices and bigger herd
  - o Subclinical losses potentially very large
  - o Next step decision support tool to aid management impact

CONSOLIDATED RECOMMENDATIONS

There are a number of important points to consider when interpreting the recommendations summarised in the chart below.

1. There was dissimilar distribution of expertise within each of the three working groups and therefore the rankings should be interpreted as being qualitative only.
2. There is inevitably a variety of working definitions of the timeframe necessary to implement an initiative, the likelihood of (technical) success and the impact of a particular initiative. Once again, this reinforces the qualitative nature of the recommendations.
3. While recommendations may be qualitative, they provide the basis of further and more detailed analysis

There was general agreement on four main areas of need:

1. **Animal Production based research**

   Areas for further study include production, reproduction, lifetime effects (mature stock and lambs, different breeds), development and validation of models (small and large animals), subclinical effects
   The justification for this work was the absolute need to develop the compelling case for change, development of tools and testing of solutions.

2. **Therapeutic Options**
   a. SHORT TERM OPTION - there is a case for a short term therapeutic agent that would assist in the rapid recovery of livestock from a staggers event – research has commenced and preliminary results look promising but further evidence of efficacy and safety is essential
   b. MEDIUM TERM OPTIONS - primarily focussed on feed additives. There has been some work done to date, however, there was some discussion regarding what further work needs to be done. The exact mode of action of these agents appears to be unknown. Before such approaches can become available commercially it is necessary that each product is approved by APVMA. Further work should be guided by the requirements of APVMA and a meeting with APVMA may be invaluable in order to define more precisely the data needs to increase the likelihood of a successful APVMA application.

3. **Awareness and Extension**
   a. Awareness and extension will need to be focussed on the key important areas of Gippsland, SW Victoria and Tasmania as these are the major areas for PRGT in Australia.
   b. A key issue raised was the continued use of perennial ryegrass containing WT endophyte which is the cause of PRGT – this needs to stop wherever animal health and welfare are likely to be compromised
   c. Awareness and extension will assist greatly in developing/presenting the compelling case for change and the current solutions as they are developed/available

d. Awareness and extension will operate as nodes for dissemination of information from other research activities such as in 1 and 2 above.

4. **Longer term change in the feedbase**

   This included development of long term (agronomic) solutions that reduced toxicity to livestock but with better persistence and longer term productivity.

   a. Improved understanding of the insect (biotic) pest problems was clearly raised – and how it relates to persistence and long term productivity of pastures

   b. Important that there be developed ryegrass/endophyte combinations that are not only safe for livestock, but also persist and improve productivity – thereby basing the argument for renovation in terms of greater overall productivity and not just on animal health reasons

CONSOLIDATION OF RECOMMENDATIONS*

Summary of all groups: Areas of work and rating for impact and likelihood of success
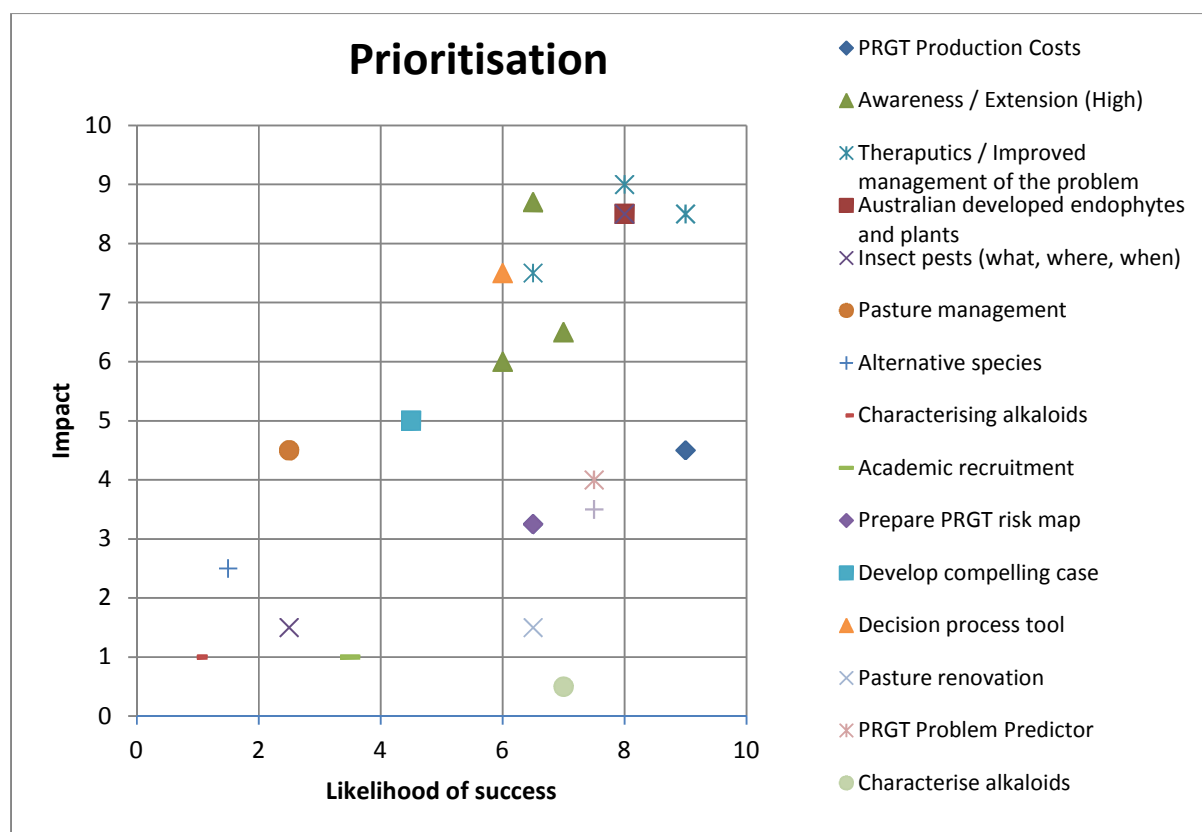
* For individual group recommendations refer to Appendix 3

APPENDIX 1 PROGRAMME

TOPIC	PRESENTER
Introduction	J Schröder
Review of recommendations, priorities and outcomes	S Page
Genomics, PRG and PRGT	F Driver
Working to make the feedbase safe	C Allan
Field study	K Reed
MLA project	K Reed/W Mace
MORNING TEA	
M Henry, MLA & Grasslanz project	B Leury/M Henry
CSU summary of current activities	J Quinn/M Combs
Summary of NZ research in Meat Wool and Dairy	J Caradus
Treatment and Prevention	N Gannon/I Sawyer
LUNCH	
Dairy industry Impact of PRGT	John Evans
Recent PRGT experiences – sheep	G Lean
PRG Seeds of the future	A Newman/R Salmon
Update of MLA economic analysis	J Webb Ware
AFTERNOON TEA	
WORKSHOP 2-3 groups, each to: • Review objectives and priorities • Identify and discuss gaps • Develop recommendations to fill gaps and provide outline of potential benefits if recommendations adopted / implemented	All
WORKSHOP REPORTS	All
OPEN DISCUSSION	All
WRAP UP	J Schröder

APPENDIX 2 PARTICIPANTS

PARTICIPANT	ORGANISATION
John Caradus	Grasslanz
Wade Mace	AgResearch
Johann Schroder	MLA
Felice Driver	MLA
Cameron Allan	MLA
Brian Leury	University of Melbourne
Michelle Henry	University of Melbourne
John Webb Ware	Mackinnon Project
Jane Quinn	Charles Sturt University
Martin Combs	Charles Sturt University
Leslie Weston	Charles Sturt University
Stuart Kemp	PastureWise
Kevin Reed	RPS
John Evans	Dairy Australia
Stephen Page	Advanced Veterinary Therapeutics
Dr Graham Lean	Sheep Vet
Dr Leah Tyrell	Macinnon Project
Dr Lisa Warn	Macinnon Project
Dr Neil Gannon	Biomin
Ian Sawyer	Feedworks
Rob Salmon	PGG Wrightson
Alan Newman	Heritage Seeds

APOLOGIES

David Hume   AgResearcn
Dr Peter de Garis   Dairy Vet W Gippsland
Dr Fergus Irving & Rosemary Irving   Veterinarian & farmer Woodside, VIC
James Sewell   PGG Wrightsons
Dr David Hucker   Para-Tech Veterinary Services

APPENDIX 3

PRGT WORKSHOP 2012 RECOMMENDATIONS BY GROUP

GROUP 1 RECOMMENDATIONS

Review Existing Priorities (we now know more about solutions)	Recommended Wish List (what do we still need)	Priorities This is not a precise ranking – should be driven by the chart below.
1 Less characterisation of alkaloids as we know most of the challenge components in wild endophytes 2 Maintain efforts on commercial cost of clinical and subclinical impacts to show importance of acting now. Include life time impacts across generations 3 Improve awareness of PRGT costs – map focus not crucial. Highlight that there are many answers. Include fodder conservation issues 4 Less academic recruitment focus – expect this to occur as a result raising awareness and development of existing research work at universities 5 Management of animals at risk or effected in immediate area go up in priority – therapeutic applications. (1) Bromide today, (2) binders seasonally 6 Pasture management – all in awareness, not in research. The answers are known.	7 Aussie focused endophytes + ryegrass for sheep and beef 8 Registered therapeutic products that are legal (bromide, binders) 9 Greater appreciation of biotic/abiotic stresses leading to PRGT as Aussie focused approach 10 Consideration of alternative species (fescue, phalaris) ie breed a ryegrass replacement	#2 What PRGT costs (High) #3 Awareness (High) #5 Managing current hassles (incl #8) (High) #7 Australian developed endophytes and plants (incorporating #9) (High) #6 Pasture management (Low) #10 Alternative species (Low) #1 Characterising alkaloids (Low) #4 Academic recruitment (Low)
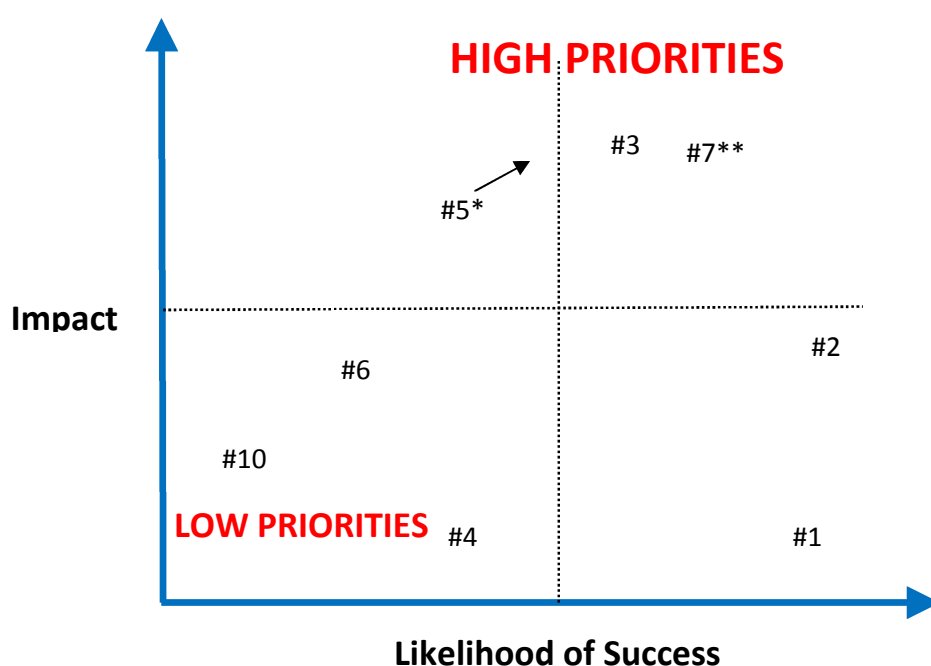
*#5 will go up and right with APVMA approval

**#7 high based on awareness and medium/long term
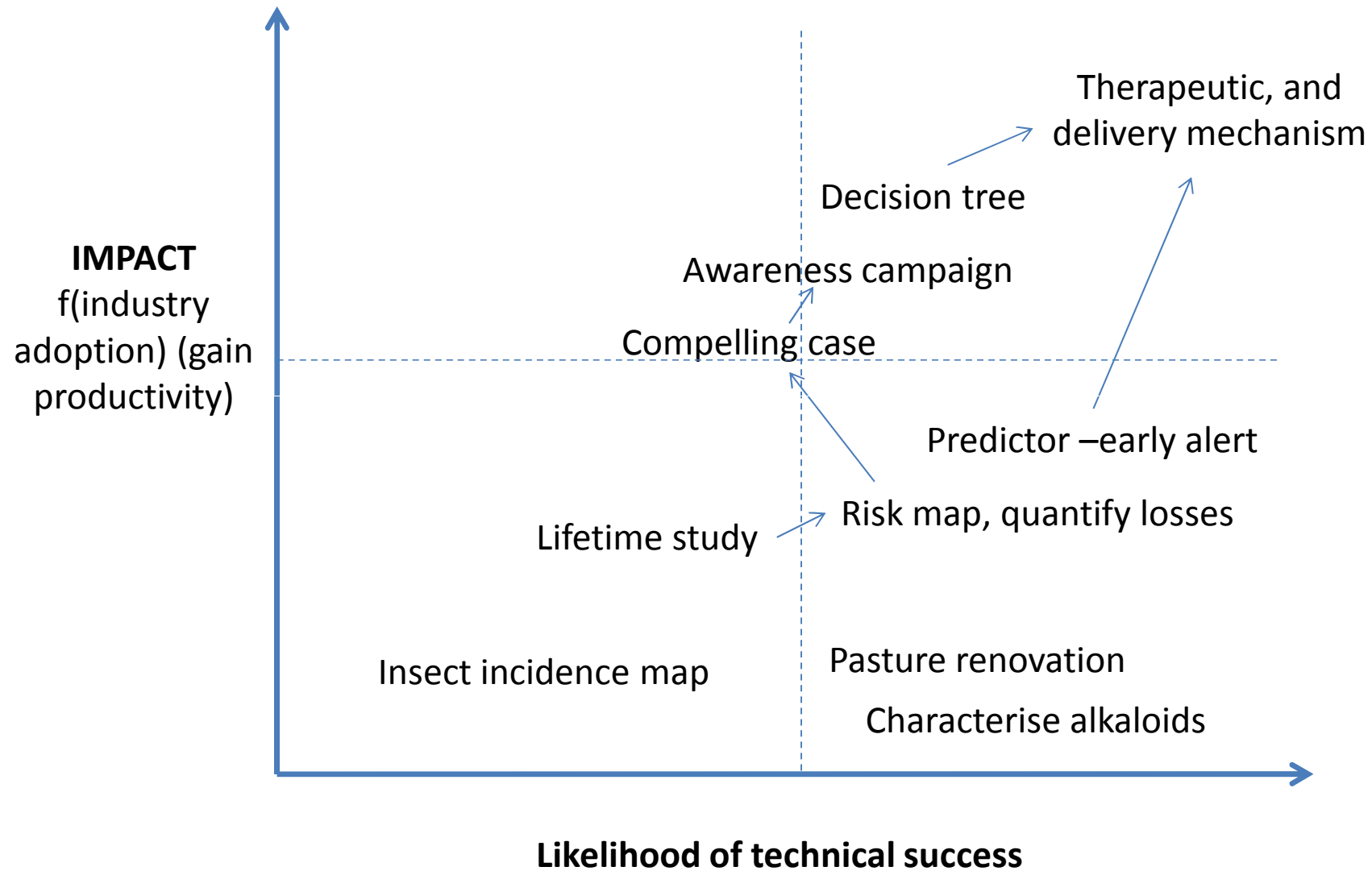
GROUP 2 RECOMMENDATIONS

Recommendations Group 2- "Biggest bang for buck" (i)

Recommendation	Justification	1-5	+5
Animal Production Studies (WT v Safe). Major PRG regions	- Production data and reasons for losses inform intervention strategies. Research (incl agronomic) to measure gross margins. - Compelling case needed; Farmers and advisors; data to get loans; Make issue a production issue not health - Value add to existing trials for small cost - Package with best technologies (best plants, mgt, estab methods, utilisation)		XX
Prepare PRGT risk map; Quantify losses	- Case studies need to support a compelling case for change, what, where how much pain	XX	
Develop compelling case	Current motivations insufficient, so no change	XX	
Insect incidence map	Need to understand pest & what endophyte required. Differs from NZ context	X	
Decision process tool	Too many options, decision tree - situation - reaction. Identify gaps (thus R&D needs). Define thresholds for action (R&D)	XX	

Recommendations Group 2- "Biggest bang for buck" (ii)-

Recommendation	Justification	1-5 years	+5 year
Lifetime study – fertility impacts; quantify sub clinical losses	- Support compelling case; subclinical impacts		XX
Pasture renovation & mgt – for persistence	Good business case for improvement anyway; use best plants		XX
Therapeutics / preventives	Prevention or treatment needed; higher adoption likely (compared with resowing)		XX
Predictor of when problems will occur	Lead indicators, early warning; Producers can be responsive, and have an immediate solution	XX	
Awareness campaign	Critical to underpin change and options	XX	
Characterise alkaloids	Base info to inform the issue, what seed companies test for		XX

Prioritisation

IMPACT
f(industry
adoption) (gain
productivity)

Therapeutic, and
delivery mechanism

Decision tree

Awareness campaign

Compelling case

Predictor –early alert

Risk map, quantify losses

Lifetime study

Insect incidence map

Pasture renovation

Characterise alkaloids

Likelihood of technical success

GROUP 3 RECOMMENDATIONS

CURRENT PRIORITIES

RANK	RESEARCH SUBJECT	CURRENT STATUS / GAPS IN KNOWLEDGE
1	Quantification of production / reproduction losses of sheep exposed to PRG with wild type endophyte	Work in progress, GAPS: reproduction, long term effects on offspring
2	Characterisation of endophyte alkaloids associated with production losses, morbidity and mortality	Work in progress Large animal model developed GAPS: Small animal model to do broader screening of compounds &mixtures and identify novel metabolites
3	Management of animals affected or at risk	GAPS: Tips & Tools needs updating to include current knowledge More detailed risk management strategies for producers
3	Pasture management to reduce likelihood of PRGT	Work in progress GAPS: Financial efficacy of reseeding strategies, monitoring of reseeded farms for better understanding of outcomes/financial benefits
4	Improve awareness of PRGT throughout the PRG risk map	Not really relevant as an outcome. GAPS: Tips and tools and risk management strategies as identified above Monitoring to alert risk Risk matrix
5	Academic recruitment	Implementation plan

GAPS IN KNOWLEDGE (not in order of priority)

1. Long and short term reproductive effects of toxin exposure, clinical and subclinical levels. Maternal effects, fetal effects, paternal reproductive effects e.g. sperm viability, motility etc.

Small animal models utilised for short timeframe to guide evidence based approach for larger animal studies.

2. Individual toxin / compound toxicity vs physiological / clinical effects

3. Life time impact

- Reproduction (for those exposed in utero or as neonates) as described above.
- Subclinical effects
- Toxin accumulation

4. Small animal model – metabolite effects, identification of novel active metabolites of toxins, assessment of activity of mixtures of compounds/metabolites, identification of specific neurological makers to aid diagnosis/ surveillance. Use of metabolomics/proteomics may improve understanding of specific activity of toxins in livestock and indicate therapeutics. May also be useful for understanding impact of climate change/stress on plant metabolism.

5. Mode of action/ clinical application of therapeutics (prevention and treatment)

6. Social impacts: human and animal, social and economic

7. Improved extension planning

- Animal
- Pasture (monitor success of programs to date to eliminate wild type PRG)
- Full economic model

8. Human food chain effects and impacts on produce quality: milk, meat, wool.

RECOMMENDATIONS AND JUSTIFICATIONS (all equal top priority areas)

1. Integrate/Validate small and large animal models
   - Justified by cost of approach for investigating physiological effects of toxin, particularly relevant for long term studies.
   - High likelihood of success
   - Less than 5 year horizon
2. Therapeutics
   - Mitigate ongoing impact of PRGT, reduce production losses, improve animal welfare outcomes
   - Treatment of outbreak
   - Lead to basic knowledge of mode of action, evidence – based approach for current / new treatment strategies
   - High likelihood of success
   - Less than 5 year horizon
3. Extension
   - Moderate likelihood of success but absolutely required for best- knowledge to be available to production communities,
   - Success dependent on implementation strategies and producer uptake
   - Critically required to include new knowledge and best practise for farmers and improve accessibility to information available to producers.
   - Consider web/ app based information with live risk update cf. facial eczema scheme.
   - Less than 5 years (<1 year with immediate implementation) but with constant review as new information is available
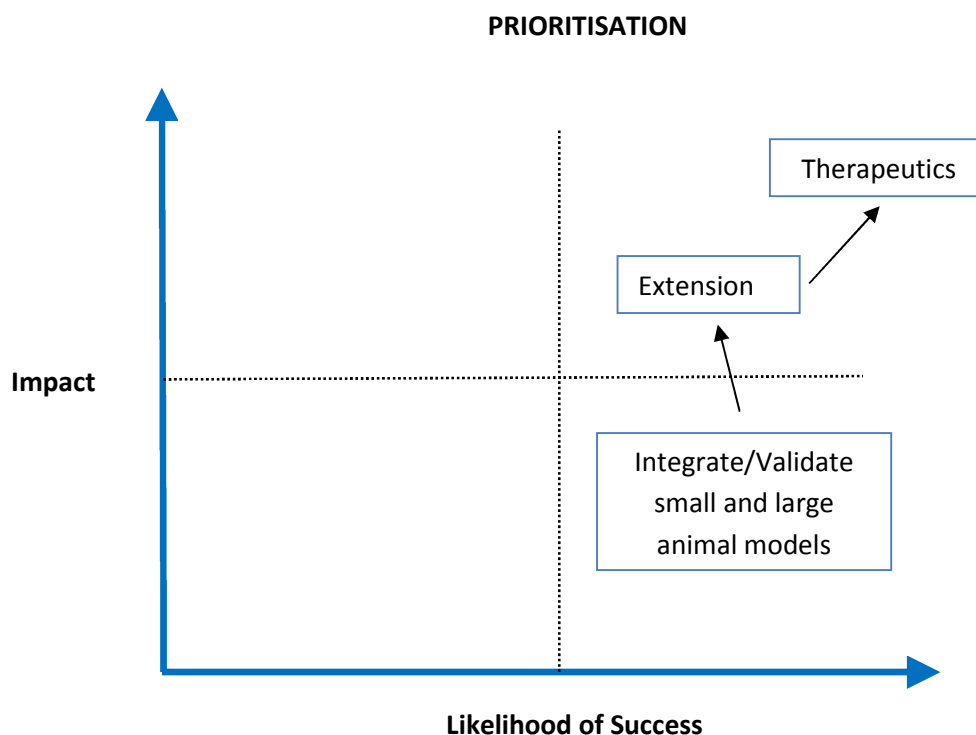
PRIORITISATION

APPENDIX 4 PERENNIAL RYEGRASS TOXICOSIS WORKSHOP
30 October 2009
University of Melbourne Veterinary Clinic & Hospital
250 Princes Hwy, Werribee VIC 3030

RECOMMENDATIONS
CONSOLIDATION OF PRIORITIES

PRIORITY	RESEARCH SUBJECT
1	Quantification of production / reproduction losses of sheep exposed to PRG with wild type endophyte Considerations: ○ Controlled pen and field studies ○ Adequate replication in time, season, sheep / PRG / endophyte variation ○ Comparison with safe endophyte or no endophyte ○ Duration of study (given need for lifetime information and capturing of delayed impacts)
2	Characterisation of endophyte alkaloids associated with production losses, morbidity and mortality Considerations: ○ Individual alkaloids and various combinations ○ Changes in alkaloid profile over time, season, location ○ Development of a reproducible experimental model in sheep or cattle ○ New knowledge of alkaloid profiles likely to guide development of more specific diagnostic tests (clinical and pathological)
3	**Management of animals** affected or at risk Considerations: ○ Document and critically review current management practices and strategies that appear to reduce impact (see also pasture management practices) ○ Development of crisis management 'best practice' guidance
3	**Pasture management** to reduce likelihood of PRGT Considerations: ○ Document and critically review current management practices and strategies that appear to reduce impact ○ Seed bank management (renovation, pasture persistence, recolonisation)
4	Improve awareness of PRGT throughout the PRG risk map Considerations: ○ Effective communication strategy that changes behaviour (for example, development and dissemination of 'best practice management guidelines') ○ Monitor and review process ○ Abattoir monitoring (noting its value and limitations) and feedback to property owners
5	Academic recruitment Captivate ongoing interest within: ○ Agronomy departments ○ Veterinary faculties ○ Chemistry schools

APPENDIX 5

Perennial ryegrass toxicosis in Australia –
Recommendations for coordinated research, development and technology transfer

Comments by Kevin Reed, 17.7.2012

I thought it might be helpful to embed my comments within relevant text from the PRGT Steering Committee's report (in italics) which summarized their discussions held during 2005-07. (The SC: K Reed, L Cummins, B Leury, I Caple, I Aberdeen, C Bell, D Champness, J Evans, D Hucker, S Kemp, S Page, A Sheridan and B Watson). Recommendations included considerable detail re justification and research methodology. The scope of the SC's 20-page report is indicated in the table of contents, below.

TABLE OF CONTENTS

Quotes from the SC's EXECUTIVE SUMMARY
Production losses are associated with life-long exposure to …..alkaloids……….. common in the vast majority of PRG pastures in southern Australia. The use of wild endophyte-infected seed remains common and the rate of eliminating toxic pasture is not rapid. Still the case.

The SC emphasise the role of technology transfer programs to increase the awareness of the animal welfare risk and economic significance of lost production from perennial ryegrass toxicosis. A cross-industry coordinated program of education, research and development is proposed. MLA provided some tools but cross-industry coordination (aka Pastures Australia) has not been sustained? Little improvement in awareness of many professionals. Eg Badly affected lamb producers unaware of relevant tips 'n tools. A concerted effort required.

Training of specialist research scientists is advocated. Nine research projects are outlined and set in priority. Research is needed to investigate endophyte chemistry and the unique clinical and sub-clinical production losses observed in the Australian environment so that risk-alert systems can assist producers to avoid/manage potential epidemics. Animal production studies are required to define the magnitude of sub-clinical lossess and help producers to consider objectively the need for investment strategies to detoxify the feedbase for our pasture-based industries. Some sig. progress: AgResearch NZ are carrying out chemical

research in Australian PRG; some MU-AgR data is emerging on impact within sheep systems at Dookie; Michelle Hunt emerges as a trained research scientist. Biomin R&D confirmed some sub-clinical losses and some benefits from Mycofix® Plus.

Aside from problem definition, appropriate research is also proposed for both eliminating toxin from pasture and for utilizing toxic pasture so as to minimize production losses in the dairy, meat and wool and other pasture/fodder-based industries. The SC is willing to assist in the transfer of technology, in the formation of sponsored research teams, net-working with appropriate international research agencies and the full development of research projects of most interest to the R&D corporations. In addition to MU, interest has grown at CSU - but elsewhere, eg DPIs, limited human resources are locked into big RIRC-CRC programs.

Project topics that address these priorities and which we recommend for coordinated support are tabled below with suggested major sponsors.

Research Projects (nos. indicate steering committee's priority)	Main industries	Possible major sponsor	Suggested co-investors (TBC)
Understanding/defining PRGT problems			
1. Perennial ryegrass endophyte chemistry (refer AHW.089 research rec. 4.6.2.3)	*Dairy, meat, wool*	PA	GT
2. Effect of PRG wild endophyte on heifer growth, development, behaviour & milk yield potential (refer AHW.089 4.6.2.3)	*Dairy, meat*	DA	GT
5. Effect of PRG wild endophyte on thermoregulation & hormone production (refer AHW.089 4.6.2.5)	*Meat, wool*	MLA	AWI, GT
9. Investigate 'heat stress' pre-condition in exported live sheep ex Portland, Vic. (refer AHW.089 4.6.2.6)	*Meat*	MLA	
Elimination/replacement of toxic pasture			
4. Benefits of toxin-free perennial ryegrass for prime lamb (refer AHW.089 4.6.2.3 & 4)	*Meat, wool*	MLA	AWI, NLP, GT
7. Management of the seed bank to avoid contamination of non-toxic pasture (refer AHW.089 4.6.2.1 & 2)	*Meat, wool, dairy*	PA	Seed companies
Management/utilisation of toxic pasture			
3. Evaluation of detoxifying agents for improving animal welfare and reducing animal production losses	*Dairy, alpaca, equine*	GF	RIRDC, Manufact-urers
6. Integrated animal research studies	*Meat, wool*	MLA	AWI
8. Breeding sheep resistant to mycotoxins	*Wool, meat*	AWI, MLA	CRC Sheep

Update on the 8 numbered 'projects' in the above Table:
No. 1. There is some limited evidence that unique alkaloids maybe important in the Aust environment. This is a pertinent question and is under investigation.
   2. An important question for the outpaddocks but no researchers interested.

3. Biomin and Feedworks have financially supported interested researchers. Numerous other products are advertised but lack credible testimonials. Some evidence of efficacy with Mycofix/Biofix and Elitox but research is needed on dosage, timing and admin of the therapeutics. This would best be addressed by use of pen rations spiked with AgR seeds that supply either ergovaline or lolitrem B (and their mixes) rather than be at the mercy of seasonal conditions.

4. Brian Leury MU is interested, and is driving this research.

5. Brian Leury MU is interested, and is driving this research.

6. This is disappointing. Coordination needed. Lifetime wool and Evergraze ignored suggestions to compare WT and safe endophyte in terms of animal production. Suggestions for use of the Information Nucleus Flocks in some collaboration with AgResearch was not taken up. It would have been quite inexpensive for some of the above programs to do the obvious: ie monitor endophyte frequency and alkaloid levels in the PRG pastures from which they collected animal data - but those suggestions appear not to have been followed and this suggests low interest/awareness by animal scientists of the cultivar x endophyte options. The SC was thanked for alerting the Editors of the CSIRO Aust Sci Journals to the need for describing endophyte characteristics of PRG pastures from which persistence and animal data is being reported but in most cases such vital background information is just not collected.

7. No proposals. Ditto re entomology. State Departments (esp Vic) are increasingly relying on 'the market' (ie Seed Companies) for such R&D.

8. No proposals. LiveCorp showed some interest but did not follow through. Livesheep export R&D is traditionally concentrated in WA where old PRG sheep pasture is unimportant. Sheep CRC showed no interest in the 2005 PRGT Symposium - nor since.

SUMMING UP
Recent experience shows (1) research capability is decreasing, (2) Endophyte science is poorly represented in post-grad studies & (3) there are pertinent subjects that are not addressed and irrespective of our "priorities", lack of researchers means there will be no proposals. Graduate students seem the best hope?

We suggested that the limitations of WT endophyte pastures be discussed within best wool, best lamb and best beef groups; coordinators of those programs have not taken it up?

Animal Health and Production Research
Given its suspected importance, there's a dearth of data, esp re fertility and meat and milk production and the associated physiology and endocrinology. Univ-DPI coordinated research inclu modest-sized lamb production experiments in SW Vic and Tasmania would also *quantify the economics* of some sub-clinical losses of production and so rapidly *raise the awareness* of producers in the two most affected regions. This would facilitate some piggy-back agronomic research and the training of young post-grads. This should not be thought of as a "grazing demo" [which term embraces all manner of unreplicated paddock work conducted by the untrained].

Impact. Dramatic increases in pasture renovation were associated with animal production data reflecting cultivar improvement (eg. Trikkala vs Mt Barker sub clover, Kybybolite; Ellett vs Vic PRG, Hamilton) and impacted far more than the preceding extension based on numerous small-plot studies by agronomists.

At the request of SC member, Mr A Sheridan, the SC completed an application for GSSA seeking Federal funds (NLP-NRIG) to demonstrate agronomy options for wild endophyte control and pasture renewal, and to measure effects on animal production/reproduction associated with exposure to tremorgens and ergot alkaloids. NLP decided it was a RIRC matter. Although considerable co-investment was promised from the private sector, <u>a lack of trained researchers</u> saw the proposal lapse.

While some may emphasise awareness/adoption, this does not mean that proposed animal production studies are not also necessary to progress the <u>underlying science</u> and our understanding of the PRGT problem.

SC R&D reccs. & priority no., 2007	Progress at 2012	Interested workers available 2012	Apparent priority at 2012 workshop by Group 2?	Suggested priority
AWARENESS OF SIG OF PRGT				
By Professionals	*	**	**	***
By Students	*	**	*	**
By Producers	*	*	***	**
UNDERSTANDING PRGT				
1. PRG endo chem	**	*	*	**
2. Heifer dev & milk potential	0	0	Not discussed	***
5. Eff on thermoreg & hormones	**	***	**	**
9. Heat stress pre-condition in live export sheep	0	0	Not discussed	*
ELIMINATE TOXIC PASTURE				
3. Benefits of safe PRG for lamb (inclu agronomy)#	*	***	***	***
7. Manage seed bank	0	*	***	**
MANAGE TOXIC PASTURE				
3. Evaluate toxin deactivators	*	***	***	***
4. Record background, in existing studies on PRG	0	*	*	***
8. Sheep genetics	0	0	0	*

Endophyte related entomology considerations were regarded as important but a concern for plant breeding and cultivar development – not PRGT R&D

APPENDIX 6

PERENNIAL RYEGRASS TOXICOSIS

Meat & Livestock Australia

WORKSHOP TO REVIEW CURRENT STATUS & RECOMMENDATIONS

11 July 2012

PRESENTATIONS

PRESENTATION TITLE	PRESENTER / AUTHOR
Introduction	Johann Schröder
Review of recommendations, priorities and outcomes	Stephen Page
Molecular genetic technologies in plant breeding and pre-competitive facilitated adoption in rye grass	Felice Driver
Pasture mycotoxin research by Biomin	Kevin Reed
B.AHE.0040. Identification of metabolites associated with severe perennial ryegrass toxicosis	Kevin Reed/Wade Mace
Effects of Perennial Ryegrass Endophyte on Sheep: PhD and MLA/Grasslandz Project	Michelle Henry / Stuart Kemp / John Webb Ware / Brian Leury
PRGT Clinical Investigations: Current findings and future directions	Jane Quinn / Martin Combs / Leslie Weston / David Rendell / Kevin Reed / Wade Mace
Potassium Bromide: Pharmacokinetics & PRGT Mouse Model: A flexible testing platform for therapeutic trials.	Martin Combs
Grass endophytes for insect management and improved pasture productivity	John Caradus
The validity of shorter term control products within the broader PRGT prevention project	Neil Gannon/Ian Sawyer
SW Victoria & Tasmania PRGT perspectives	Graham Lean
Seeds of the future	Alan Newman
The Future of Perennial Ryegrass Endophytes	Rob Salmon
The financial impact of PRGT	John Webb Ware