ANNUAL RYEGRASS TOXICITY

Review of Current Management Options and Research Needs

Project number ARGT.001
Final Report prepared for MLA by:

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EXECUTIVE SUMMARY

Annual ryegrass toxicity (ARGT), resulting from the consumption of corynetoxins (CTs) produced by bacteria in the seedheads of annual ryegrass, is a significant cause of death in livestock in Western Australia and South Australia with more than 12 million sheep and cattle at risk. Since outbreaks were first documented in Australia in 1968, it has been estimated that more than 500,000 sheep and cattle have died. Non-lethal effects on production and reproduction have been poorly described but may exceed in value the losses attributed to mortality. A related syndrome, flood plain staggers which occurs sporadically in NSW and SA, contributes further deaths. While ARGT is usually manifested as a disease of Australian livestock, toxic annual ryegrass containing CTs can contaminate grain and hay, potentially rendering these products unsuitable for export and consumption. A variety of regulations and quality assurance programs continue to be implemented to safeguard animal and public health.

An intensive review of current knowledge related to the management of ARGT has been undertaken by consultation and discussion with key stakeholders and research organisations supplemented with critical appraisal of published literature and unpublished reports.

Sustained ARGT research into the biology, pathology, toxicology, chemistry, epidemiology, treatment and control of pastures and livestock has resulted in a wealth of available information and improved understanding of the syndrome. From 1969 until the current time there appears to have been continuous funding support by MLA, AWI (in their various forms) and other organisations of this broadspectrum research program. While a number of critical areas of knowledge remain to be investigated it is clear that optimal application of the existing ARGT solutions to the field and adoption by affected farmers has not yet been successful. It is believed that a significant and positive impact on the current status of ARGT can be achieved by enhancing the use of available information and tools. Furthermore, selective investment and investigation of additional areas with potential to make long lasting contributions to ARGT control are worthy of consideration.

Recommendations are presented that permit minimisation of the impact of ARGT on all affected parties, without compromise of animal productivity and welfare. Recommendations fall into four categories comprising COORDINATION AND MANAGEMENT, ENHANCING ADOPTION, FURTHER RESEARCH, and FOOD SAFETY.

<table>
<thead>
<tr>
<th>RECOMMENDATION</th>
<th>INDICATIVE COST</th>
<th>TIME</th>
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<tbody>
<tr>
<td><strong>COORDINATION &amp; MANAGEMENT</strong></td>
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<tr>
<td>1 National Coordination and Advisory Team</td>
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<td>Appoint a convenor and assemble a national team representing funding bodies (MLA, AWI, GRDC), research organisations (CSIRO, WADA, SARDI, UA), commercial partners and others as appropriate to provide advice on priorities and goals and to drive focused actions.</td>
<td>30,000</td>
<td>3m</td>
</tr>
<tr>
<td><strong>ENHANCING ADOPTION OF CURRENT SOLUTIONS</strong></td>
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<tr>
<td>2 Preparation and implementation of extension program that addresses past extension failures, seeks out and investigates successful on-farm approaches, undertakes field work to demonstrate best integrated use of available biocontrols (including twist fungus and SafeGuard) and other approaches, develops reliable predictive model, describes methods of ensuring maximum long term adoption, is able to be monitored and measured against current disease prevalence, and sets achievable targets for success. (WADA, SARDI, and commercial partners)</td>
<td>750,000</td>
<td>3y</td>
</tr>
<tr>
<td><strong>FURTHER RESEARCH</strong></td>
<td></td>
<td></td>
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<tr>
<td>3 Confirmation and implementation of research priorities.</td>
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</table>
National Coordination and Management Team to review critical research needs and develop recommendations and an implementation timetable. In addition to the prevention projects identified below research priorities include:

- Non-toxigenic Rathayibacter biocontrol studies (subject to AQIS approval)
- Relationship of bacteriophage and toxin production.

Design of a long term basic research program will provide further refinements to ARGT control.

<table>
<thead>
<tr>
<th></th>
<th>Vaccination against ARGT</th>
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<tr>
<td></td>
<td>Prepare business plan and identify and confirm potential commercial partners (Consultant)</td>
</tr>
<tr>
<td></td>
<td>Subject to successful outcome of business plan, develop and implement research proposal that defines and addresses critical issues and sets out ambitious but achievable objectives (CSIRO, commercial partner)</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th></th>
<th>Adsorption of CTs in rumen</th>
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<tbody>
<tr>
<td></td>
<td>Undertake systematic study of practical benefits of available food additives that may inactivate CTs after ingestion (WADA)</td>
</tr>
</tbody>
</table>

**FOOD SAFETY**

|   | Meet with GRDC, AFFA and FSANZ to establish current status of food safety research and determine data gaps. | 3m |
|---|--------------------------------------------------------|
|   | Develop and implement proposal to determine corynetoxin tissue residue concentrations in edible tissues of CT treated sheep. (CSIRO) | 150,000 | 15m |
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0. Current Research, Recommendations From Previous Assessments & food safety
1. List of consultations in preparation of ARGT Review December 2003
2. Bibliography of published literature related to ARGT and the prevention of intoxication by natural toxins.
3. Notes on key publications
4. Projects funded by MLA, AWI, GRDC, RIRDC and WADA
5. Publications describing work supported by MLA, AWI and RIRDC
7. 2002 ARGT Workshop Priorities
1. INTRODUCTION

There is currently a low level of satisfaction with the available approaches to managing annual ryegrass toxicity (ARGT) in Western Australia. It is apparent that ARGT remains a continuing (and possibly expanding) challenge to livestock production despite 35 years of investment in research and extension.

This report provides an assessment undertaken in December 2003 of the current knowledge related to ARGT and its prevention, control and management. In addition, recommendations are provided which aim to ensure that the accumulated experience and expertise is harnessed to the benefit of livestock production and food safety.

To gain an appreciation of the key issues facing effective control and an understanding of the knowledge that had been acquired two concurrent avenues of enquiry were followed. A number of consultations with key stakeholders and scientists actively researching ARGT were conducted. A list of those involved is presented in Appendix 1. Complementing the personal interviews a comprehensive and systematic literature review was undertaken. Searches of key databases, including Pubmed, Agricola and CAB were made and relevant literature identified and retrieved. The reference lists of all retrieved publications were also searched and further relevant publications identified and acquired. A database was developed and is presented in Appendix 2 and notes on many of the key publications are included in Appendix 3. It became apparent that most of the research into ARGT had been continuously supported by MLA and AWI in their various forms. A list of supported projects and publications that arose from these projects is presented in Appendix 4 and Appendix 5.

Supplementary information, not included in this database includes the meeting notes of a workshop on ARGT held in WA in November 2002 and the report of the SCARM Working Group on issues associated with ARGT (February 2000) (details presented in Appendices 0, 7 and 8).

A summary of the key finding from these sources is set out in this report followed by an overall assessment and key conclusions. Recommendations to improve the current status are presented in the Executive Summary. In addition, an interim report on basic research needs has been prepared by Dr Bob Hannam on behalf of MLA and this document is presented in Appendix 6.

A difficult and complex topic has been reviewed in a short time. This has been made possible by the valuable, generous and willing assistance of those identified in the list of consultations. While the content of this report has been heavily reliant on the valuable contributions of many, any omissions and errors are purely the responsibility of the author.
2. OVERVIEW OF THE SYNDROME OF ARGT

(Allen, ARGT Workshop 2002)

Annual ryegrass toxicity (ARGT) is generally described as an acute and often fatal neurological disease of livestock caused by consumption of the seed heads of annual ryegrass (ARG) (*Lolium rigidum*) infected and replaced by corynetoxin (CT) producing bacteria, *Rathayibacter toxicus*. *R toxicus*, while present in the soil, gains access to ARG in winter by adhering to receptors on the surface of its principal vector, the parasitic seed-gall nematode *Anguina funesta*. Nematode galls forming in the seeds of ARG are colonised and overtaken by *R toxicus* frequently producing a grossly visible yellow slime in the inflorescence. As the grass matures increasing concentrations of CT are produced, possibly promoted by the presence in *R toxicus* of a specific bacteriophage. Infected ARG and galls remain toxic throughout the following summer and autumn and can remain toxic for many years if kept dry and protected from the weather.

Livestock develop ARGT when grazing toxic ARG, either in pastures or crop stubbles or when provided with feed (grain, hay, crop fines) contaminated with toxic ryegrass. ARGT occurs every year in the agricultural areas of Western Australia predominantly, although ARGT continues to affect livestock in South Australia. A related intoxication of livestock, flood plain staggers (FPS), caused by CT accumulation in blown grass (*Agrostis avenacea*) or annual beard grass (*Polypogon monspeliensis*), occurs infrequently in association with prolonged floods and has been reported in both NSW and the SE of South Australia. The only other manifestation of CT intoxication reported in Australia occurred in pigs consuming mouldy wheat in NSW. The source of CT production was not identified though *R toxicus* was eliminated as a suspect. A syndrome similar to ARGT affecting cattle and sheep was described in Oregon in the 1940s and was attributed to consumption of Chewings fescue infected with nematodes. No further reports of this syndrome followed the introduction of repeated annual thermal sanitation (burning) of fescue pastures which would have effectively eliminated nematodes. Sporadic ARGT-like syndromes have been described in South Africa (from 1979), Russia (1980) and California (2002).

Key Points for ARGT in Western Australia

- Outbreaks of ARGT have been described across an area of 10 million ha.
- From beginnings in the south around Gnowangerup in 1968, ARGT has spread to the central and northern districts (as far as Perenjori) with extended eastern and western boundaries. The distribution of *R toxicus* extends beyond the current boundaries of clinical ARGT.

Outbreaks have also been described in the Esperance area and in pockets along the west coast.
3. DISTRIBUTION OF ANNUAL RYEGRASS TOXICITY & FLOOD PLAIN STAGGERS*

* Indicative only. Consult bibliography for accurate and detailed distribution.
4. IMPACTS OF ARGT

4.1. Summary of Elements of Impact

- Mortality
  - Subclinical effects on wool production, meat production, feed conversion efficiency, reproduction etc
    - This aspect of production loss is poorly understood but one study suggested that ARGT reduced annual WA wool production by 3%.
  - Stocking rates reduced, toxic paddocks unavailable
  - Cost of fodder purchased to replace feed lost to ARGT
  - Daily monitoring costs
  - Disincentive to restock
  - Costs of control (eg Twist fungus at $6/ha or $30/kg)
  - Feed-lot production delayed
  - Animal welfare
    - In the absence of effective treatments, animal welfare in compromised by ARGT.
  - Human welfare
    - ARGT outbreaks exert a powerful adverse psychological effect on graziers and their families.
  - Public health and food safety
    - Cost of QA programs to assure edible tissues safe for consumption (no current QA program for livestock, but future QA expected).

- Hay exports
  - Hay exports from WA were valued at $80 million in 2001. Exported hay is prescribed by AQIS and must be shown to contain < 1 bacterial gall/kg.

- Interstate movement of hay
  - Vic, NSW and Tas do not permit entry of contaminated hay.

- Grain exports
  - Cost of QA programs to assure absence of contamination with \textit{R. toxicus} or CTs.
4.2. Annual Costs of ARGT: Only four of the impact elements have been costed

- **1988** (Roberts and Baxter 1991)
  - $16.5 million pa (1988 A$)
  - 5% 0.8 million livestock deaths
  - 40% 6.6 million chemicals to control ARG
  - 40% 6.7 million loss of grazing potential
  - 15% 2.4 million livestock inspection costs

  Additional costs include those attributed to sub-clinical losses and reproductive inefficiency.

- **2002** (Allen, ARGT Workshop 2002), annual costs in WA based on 1988 assumptions total approximately $26 million (2002 A$)

- **2003**, The ARGT Action Committee estimated the costs in WA at approximately $35 million.
5. MORTALITY ATTRIBUTED TO ARGT

A summary of mortality data collected in WA from 1968 until 1992 is set out in the following table. If it is assumed that a normal ARGT season results in 20,000 deaths and if it is accepted that 2000 was an exceptional season with 88,000 deaths, then the cumulative mortality amongst sheep in WA from 1968 to the present time exceeds 500,000. The Australian total for ARGT deaths will also include deaths in SA, and those arising from FPS in SA and NSW. It should be noted that records of losses due to ARGT have not been analysed by the Western Australia Department of Agriculture since 1994.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of farms reporting outbreaks</th>
<th>Sheep deaths</th>
<th>Cattle deaths</th>
<th>Average sheep death rate per outbreak</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1968-71</td>
<td>5</td>
<td>550</td>
<td>0</td>
<td>110</td>
<td>Stynes &amp; Wise 1980</td>
</tr>
<tr>
<td>1971-72</td>
<td>9</td>
<td>1,247</td>
<td>0</td>
<td>139</td>
<td></td>
</tr>
<tr>
<td>1972-73</td>
<td>12</td>
<td>889</td>
<td>45</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>1973-74</td>
<td>16</td>
<td>681</td>
<td>0</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>1974-75</td>
<td>24</td>
<td>1,419</td>
<td>123</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>1975-76</td>
<td>17</td>
<td>1,772</td>
<td>5</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>1976-77</td>
<td>49</td>
<td>3,753</td>
<td>15</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>1977-78</td>
<td>51</td>
<td>864</td>
<td>56</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>1978-79</td>
<td>62</td>
<td>2,305</td>
<td>55</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>1979-86</td>
<td>239</td>
<td>65,230</td>
<td>50</td>
<td>273</td>
<td>Derived from Pink 1989</td>
</tr>
<tr>
<td>1986</td>
<td>414</td>
<td>27,502</td>
<td>65</td>
<td>66</td>
<td>Pink 1989</td>
</tr>
<tr>
<td>1987</td>
<td>457</td>
<td>28,854</td>
<td>122</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>1988</td>
<td>307</td>
<td>11,571</td>
<td>31</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>1989</td>
<td>520</td>
<td>20,079</td>
<td></td>
<td>39</td>
<td>ABS, quoted by Roberts &amp; Bucat 1992 (Table 13)</td>
</tr>
<tr>
<td>1990-91</td>
<td>165</td>
<td>13,844</td>
<td>23</td>
<td>84</td>
<td>Roberts &amp; Baxter 1991</td>
</tr>
<tr>
<td>1991-92</td>
<td>782</td>
<td>88,186</td>
<td></td>
<td>113</td>
<td>ABS, quoted by Roberts &amp; Bucat 1992 (Table 13)</td>
</tr>
<tr>
<td>TOTAL 1968-1992</td>
<td>3,129</td>
<td>268,746</td>
<td>590</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>1992-99</td>
<td>160,000</td>
<td></td>
<td></td>
<td></td>
<td>NO SOURCES FOUND</td>
</tr>
<tr>
<td>2000</td>
<td>88,000</td>
<td></td>
<td></td>
<td></td>
<td>Estimate by Allen, ARGT Workshop 2002</td>
</tr>
<tr>
<td>GRAND TOTAL 1968-2000</td>
<td></td>
<td>516,746</td>
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### 6. MAJOR EVENTS IN THE HISTORY OF ARGT RESEARCH

<table>
<thead>
<tr>
<th>ARGT TIMELINE</th>
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<tbody>
<tr>
<td><strong>1940’s</strong></td>
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<tr>
<td><strong>1950’s</strong></td>
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<tr>
<td><strong>1960’s</strong></td>
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<td><strong>1970’s</strong></td>
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<td><strong>1990’s</strong></td>
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<td></td>
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<tr>
<td><strong>2000’s</strong></td>
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7. BIOLOGY OF NEMATODE AND BACTERIUM OF ARGT

(McKay, ARGT Workshop 2002)

- Nematode and bacteria survive the dry summer / autumn months dormant inside seed-galls on the soil surface or attached to ryegrass seedheads. Bacteria can also survive as dried slime.
- Nematodes become active when moist and emerge during rain events when galls are decomposing.
- Bacteria adhere to nematodes as the latter seek out ARG plants.
- Nematode numbers peak in ryegrass tillers about 11-12 weeks after opening rains.
- Nematodes do not feed until they have produced a gall in the developing ovary.
- Bacteria colonise galls soon after gall initiation, displacing nematodes. Toxin production starts around flowering and continues until plants hay-off.
- While it is easy to reduce large nematode populations to low levels by controlling ARG, the nematode is difficult to eradicate, as low numbers can persist and quickly increase as ARG reappears.
- Seasonal conditions have a significant effect on nematode multiplication rates and pasture composition. High nematode numbers are favoured by short wet growing seasons and late seasons with adequate rainfall. Drought conditions until early spring may prevent nematodes invading ARG in time to produce galls. Long growing seasons that begin early (eg mid April) allow nematode numbers to peak in ARG well before ovary initiation. Most nematodes have insufficient energy to survive and produce galls.
- Vulnerable features of *A funesta* include the need to reproduce each year, the need for developing grass ovaries in which to reproduce, and the inability to reproduce in galls colonised by bacteria or twist fungus.
- *R toxicus* appears to be the sole or principal producer of CTs in grasses
- While *Anguina funesta* is the preferred vector of *R toxicus*, other *Anguina* species can act as vectors and infect other species of grass. This is observed with FPS in NSW and SA where blown grass and annual beard grass are infected with *R toxicus*.

*A funesta* prefers *Lolium rigidum* but can parasitise other grasses, including other *Lolium* spp, *Phalaris*, *Danthonia*, *Avena* and *Vulpia*.

9. CHEMISTRY OF THE TOXIN OF ARGT

The toxins produced by Rathayibacter toxicus and isolated from galls colonised by bacteria in infected annual ryegrass are named corynetoxins (CTs) and comprise a characteristic glycolipid complex of 16 structurally similar nucleoside antibiotics, closely related to the tunicamycins (TM) produced by Streptomyces lycosuperificus. Both the CTs and TMs share a common core (illustrated in the following diagram) containing the nucleoside uracil to which is attached the unique 11 carbon dialdose aminosugar tunicamine to which in turn is attached an N-acetyl-glucosamine. The various glycolipids that comprise the toxin complexes of the CTs and TMs differ from each other only in the nature of the fatty-acid chain linked to the amino group at C-10’ of tunicamine.
10. MODE OF ACTION OF CORYNETOXIN

The biochemical mode of action in vertebrates of the corynetoxins is shared with the tunicamycins and arises from the specific inhibition of uridine diphospho-N-acetylglucosamine:dolichol-phosphate N-acetylglucosamine-1-phosphate transferase (GPT), the first enzyme in the dolichol pathway of N-glycosylation of proteins, as illustrated in the following figure. Normal cell function and survival is absolutely dependent on proteins with appropriate structure and conformation. Inhibition of N-glycosylation impedes innumerable cell activities and all clinical manifestations of toxicity and subclinical affects in livestock exposed to CTs can be attributed directly or indirectly to inhibition of GPT.

The dolichol pathway has been observed in every eukaryotic organism and glycoprotein-synthesizing tissue in which it has been examined. Analogous processes of glycosylation are inhibited by CTs and TMs in invertebrates, fungi, yeasts, viruses, bacteria, protozoa and plants.

11. BIOSYNTHESIS OF THE LIPID-LINKED OLIGOSACCHARIDE INTERMEDIATE

Corynetoxins inhibit the activity of GPT which catalyses the first reaction illustrated below
12. DIAGNOSIS OF ARGT

- Clinical signs can be strongly suggestive, especially intermittent convulsive episodes separated by normal interictal periods.
- Exposure to infected grass (usually but not exclusively ARG), hay or grain.
- Confirmation of presence of R toxicus (ELISA) in pasture or feed.
- Confirmation of presence of CTs (ELISA) in pasture or feed.
- Identification of R toxicus Ag (ELISA) in rumen contents or faeces.
- Inhibition of hepatic GPT
- Pathology is not conclusive nor pathognomonic, however, plasma leakage from cerebellar meningeal vasculature is strongly suggestive.

13. ARGT DETECTION (McKay 2002)

To implement an effective management program it is essential to identify infected paddocks. Supplementing field assessment of galls and bacterial slime are a number of rapid laboratory tests. Available tests include:

- **Mature Ryegrass Test**: Two tests: (i) Visual examination of seed for nematode and bacterial galls (SARDI). ARGT risk categories based on number of nematode and bacterial galls have been validated. (ii) Bacterial ELISA for presence of *R toxicus* (WADA and SARDI). This test is run regularly in WA but adoption on-farm to guide grazing practices is very low with only 100’s of tests run each year.

- **Preflowering Test** (WADA and SARDI): ELISA test for *R toxicus*. In theory results can identify safe paddocks and prioritise those to be managed to reduce toxin production. However, practical experience suggests predictive value is unacceptably low with many false positive and negative results.

- **Hay Test** (WADA and SARDI): ELISA test for *R toxicus* can detect 1 bacterial gall/kg of hay. Used in prescribed QA of export hay. Around 15,000 tests are run each season in WA and 6,000 tests in SA. The cost varies according to number of tests per submission from $12 - $50/assay. Sampling programs are designed to ensure hay is acceptable for export, with composite sampling at one extreme and individual bale sampling to identify acceptable bales from batches found positive at the other.

- **Toxin Test** (CSIRO, WADA): ELISA test for CTs with LOD of 25 ppb (µg/kg) in grain and LOD under investigation in fodder. In practice the CT test has a more complicated extraction process than the bacterial ELISA, is more expensive, the LOD may be higher than 25ppb, and the sensitivity is less acceptable than the bacterial ELISA which can detect the equivalent of 2ppb.
14. TOXICOLOGY

- Sheep, cattle and pigs appear equally susceptible to acute exposure with an oral lethal dose in the order of 1 to 6 mg CT/kg bodyweight.

- A latent period of at least 4 days precedes clinical signs of intoxication, though specific liver enzyme (GPT) inhibition can be detected within one hour of SC administration. The latent period may complicate the management of intoxicated livestock as exposure may have occurred prior to current feeding practices.

- The lethal subcutaneous dose is approximately 100 fold less in sheep (0.02-0.04 mg CT/kg bodyweight).

- Guinea pigs, rats, mice, chickens and horses are all susceptible.

- CTs are cumulative toxins. The total lethal dose is unchanged if the dose is divided and administered even at an interval of 9 weeks in sheep. This means that the second exposure to CTs may have a greater impact than the first exposure.

- Inhibition of sheep hepatic GPT activity by a single SC dose of TM was sustained and recovery to normal took 16-22 weeks.

15. RISK FACTORS (ARGT Workshop 2002: Allen; Roberts; Mckay)

- Most cases of ARGT in grazing livestock occur between mid October and mid December after seed heads have developed maximum toxicity and while pastures are undergoing senescence.

- Increased incidence in January may be associated with introduction of stock to stubbles.

- Feeding of contaminated hay or grain can lead to ARGT at any time.

- Cases in autumn are usually associated with reduced feed availability

- Many farmers report an association between rainfall and outbreaks of ARGT, particularly in summer.

- Mustering, shearing and transport may precipitate ARGT.

- Monitoring of 73 paddocks in endemic area for 4 years revealed:
  
  - 80% of ARGT outbreaks occurred in high risk paddocks.
  
  - After pasture sampling, deaths reported in 1/6 high risk areas and 1/25 medium risk areas.
  
  - Concentration of bacterial galls is poor predictor of ARGT

- Short wet growing seasons

- ARG dominant pasture

- Poor ryegrass control in previous year, commonly associated with cropping, low stocking rates during spring, both permitting expansion of nematode populations.
16. TREATMENT

A number of compounds have been evaluated by observation of effects on livestock with natural ARGT (xylazine, acetylpromazine, chloral hydrate) or by laboratory assessment of artificially exposed animals (chlordiazepoxide, MgSO₄). While some interventions have resulted in significant improvement in outcome, no agent has been found to offer any practical advantage in field use.

17. PREVENTION AND CONTROL

Notwithstanding the imperative to reduce the area affected by ARGT, if a reliable means of protecting livestock from the affects of CTs could be found then not only would large tracts of pasture become immediately available for grazing but contaminated hay and grain could be safely consumed. Three approaches have been described in the literature: cobalt, vaccination and cyclodextrins.

Cobalt administration had a positive effect in increasing the toxic threshold of CTs by approximately 30%, even in cobalt sufficient animals. However, given continuous field exposure to CTs, Co has not found an application.

Vaccination is considered separately below.

Cyclodextrins (CDs) have been evaluated in a number of laboratory and field studies by both CSIRO and a commercial partner Virbac. Unfortunately CDs did not prove acceptable as an antidotal intervention.
18. VACCINATION AGAINST ARGT

(Andrew et al, ARGT Workshop 2002; Appendix 9)

The Plant Toxins Research Group of CSIRO Livestock Industries has developed a protein conjugate of a hapten derived from TMs that after SC administration has successfully induced antibody production and protected sheep from acute and chronic challenge with TMs or CTs under controlled laboratory conditions.

Benefits

- Prevention of clinical ARGT in livestock
- Potential prevention of sub-clinical losses
- Ability to improve stocking rates
- Ability to feed contaminated hay, grain and other feeds.
- Possible reduction of tissue concentration of CTs

Disadvantages

- Reduction in imperative to control infected ARG by other methods
- Possibility that not all sheep will respond to vaccination
- Possible increase in tissue concentrations of CTs

Critical Factors

- Attractiveness of commercial manufacture
- Market assessment
- Cost and source of immunogen
  
  (eg Tsvetanova et al 2002: *Streptomyces chartreusis* NRRL 3882)
- Immunogen dose, frequency and duration of protection
- Field performance: efficacy and safety
- Vaccine stability
19. PASTURE AND CROP MANAGEMENT FOR THE CONTROL OF ARGT (McKay, ARGT Workshop 2002)

Pasture and cropping strategies to prevent ARGT are usually designed to exploit vulnerable features of the nematode life cycle. It is important to ensure that ARG refugia on fence lines, contour drains and other inaccessible places is controlled.

Autumn/Winter. Options to manipulate ARG populations at the start of the growing season include the use of selective herbicides in crop and pasture and the sowing of nematode resistant ARG (SafeGuard).

Spring. ARG seedheads begin to emerge in spring and control in crops requires use of seed catchers during harvesting to collect and destroy as much ARG seed as possible. In pastures, options include crash grazing, mowing and spraytopping.

Late spring, summer, autumn. The nematode will have completed its life cycle and toxin levels will have reached maximum levels. High risk paddocks must not be grazed. Burning is an option when permitted.
20. BIOLOGICAL CONTROL (Riley, ARGT Workshop 2002)

Biological control can be defined as use of one organism to control another. It is important to emphasise that control does not equate to eradication and the objective of control will vary with circumstances.

A single biological control agent may not reduce the target population as much as required in all circumstances. When used within an integrated pest management system, that may incorporate both biological control and agronomic methods, biocontrol may contribute to a robust and environmentally sustainable solution.

Classical biocontrol is exemplified by the introduction of an antagonist organism obtained from the centre of diversity of the target pest in an attempt to establish a new equilibrium where the pest is reduced to an acceptable level. Alternatively, inundative biocontrol takes an already established antagonist that can be mass cultured and applied at high rates to achieve an enhanced benefit that may be short term. Finally, breeding and genetic modification to increase tolerance and resistance of an affected plant or animal can be considered a form of biocontrol.

Biological control strategies (interrupt nematode life cycle)

NB: Control ≠ eradication

RYEGRASS (Lolium rigidum)
- Pathogens of ryegrass
- Other grasses and legumes

NEMATODE (Anguina funesta)
- Trapping plants (Vulpia, Fescue, other Lolium spp)
- Verticillium spp
- Dilophospora alopecuri: TWIST FUNGUS

SAFEGUARD

BACTERIUM (Rathayibacter toxicus)
- Deprivation of vector (nematode)
- Non-adherent nematodes
- Engineered non-toxic bacteria with adhesins to compete with R toxicus
- Non-toxigenic Rathayibacter spp

BACTERIOPHAGE
- Mutant phage that does not increase toxin production but competes successfully with natural phage.

CORYNETOXIN
- Rumen microorganisms that degrade CT

After reviewing the possible targets for biological control of ARGT, those considered most likely to yield practical results include SafeGuard, twist fungus (Dilophospora alopecuri), and non-toxigenic Rathayibacter.
21. SAFEGUARD

Description

- SafeGuard is a variety of ARG bred for resistance to infection with *A funesta* and early flowering. Other features include herbicide susceptibility, resistance to cereal root diseases and increased herbage production.

Advantages

- SafeGuard will provide increased herbage for grazing in winter while presenting a significantly reduced risk of ARGT later in the season.

- Resistance to nematode infection is a dominant trait and will be introduced to naturalised ARG varieties as interbreeding takes place.

Challenges

- Finding the ideal program for use of SafeGuard in cropping systems where establishment of SafeGuard may require reduced herbicide use and initial crop quality may be jeopardised by the increased seed production of SafeGuard.

- Ensuring flowering times are appropriate in each area

Availability

- SafeGuard is commercially available but supplies have been scarce and use in WA is low.

It is produced by Valley Seeds in Victoria (Don Coles ph (03) 9696-7784, mb 0408 333 028, email: dcoles@valleyseeds.com) and distributed in WA by Irwin-Hunter and Co (08 9383 4708).
22. TWIST FUNGUS

Mode of action

- Competes with *R toxicus* for its nematode vector and plant host.
- Fungal spores are larger than bacteria and may impede nematode movement and invasion to a greater degree than bacteria.
- Within the plant the fungus grows more rapidly with a greater inhibitory effect on nematode gall production.
- Its pathological effect on the plant appears to be mechanical rather than toxic.
- Twist fungus reduces nematode reproduction very effectively and in so doing reduces bacterial colonisation.
- The nematode population density at which the nematode is in stable equilibrium with the fungus is an order of magnitude or more below that between the nematode and *R toxicus*. At this low level, it may be difficult to detect the presence of bacteria.
- Therefore twist fungus provides biocontrol of both nematode and bacteria.

Advantages

- Under natural circumstances, twist fungus has controlled ARGT in the vicinity of Gnowangerup.
- Once established, twist fungus will persist and spread without further management efforts.

Challenges

- Achieving rapid establishment especially in dry seasons.
- Demonstrating that failure of ARGT control may be due to poor site selection or timing of application rather than directly attributable to twist fungus
- Determining situations where twist fungus is most or least likely to offer benefits
- Defining the potential for inundative biocontrol

Availability

- Twist fungus is readily available in WA and can be ordered on-line via [http://www.ARGT.com.au](http://www.ARGT.com.au).
23. NON-TOXIGENIC *RATHAYIBACTER* (Riley, ARGT Workshop 2002)

**Mode of Action**

- A number of species of *Rathayibacter* other than *R toxicus* are able to adhere to *A funesta* and colonize nematode galls
- None of these species produce CT
- In culture, these species grow more rapidly than *R toxicus*
- The potential for biocontrol is direct competition and displacement of *R toxicus* and possible reduction in nematode populations.

**Advantages**

- Controlled laboratory studies have shown *R toxicus* can be displaced to various degrees. However, repeated field studies will be required to demonstrate utility.
- Production of bacteria and application methods should be more efficient that with twist fungus.
- Bacteria may persist and spread allowing long term control
- Phytosanitary controls on *A funesta* and *R toxicus* may encourage the use of methods of control, reduction or elimination

**Challenges**

- Satisfying AQIS requirements for field use
- Development of methods of mass production
- Attracting commercial interest
24. INTEGRATED WEED MANAGEMENT FOR ARG

(Hashem, ARGT Workshop 2002)

Although ARG is a productive pasture species, it is the most important weed of the farming systems within the WA wheatbelt. It is highly competitive to crops because it is twice as efficient as wheat in converting absorbed nitrogen to biomass. While competition from ARG can reduce wheat yields by more than 50%, ARG can be an important host for cereal root diseases (such as take-all) and can reduce the quality of crops by seed and CT contamination.

It is difficult to control ARG because of widespread herbicide resistance, long seed dormancy, staggered germination, and high seed production (up to 50,000 seeds/m²). To sustain the productivity of wheat production, it is essential to reduce the seed bank of ARG and maintain a low level by integrated weed management practices.

25. PASTURE OPTIONS FOR THE WHEATBELT OF SOUTHERN AUSTRALIA (Revell, ARGT Workshop 2002)

ARG is a highly nutritious feed source at the break of winter and more accessible at this time than many of the annual legumes and herbs. ARG has been widely sown throughout pastures in southern Australia because it is easily established, frost resistant, productive and highly palatable to livestock. It is a winter growing annual of Mediterranean origin well adapted to regions with hot, dry summers and mild wet winters.

ARG is capable of growing on a wide range of soils from acidic sands to alkaline clay loams. It is also regarded as useful in the reclamation of salt-affected areas.
26. ASSESSMENT KEY RECOMMENDATIONS

26.1. Assessment

There is probably more known about ARGT than most other intoxications of livestock. It is ironic therefore that little impact on the expansion of ARGT in Western Australia appears to have been made. Recent reviews of the principal issues concerning food safety, pasture and animal management have identified most of the key areas for further work. However, what is missing is a coordinated national approach to the important issues and a committed and motivated team effort in implementation of the priorities and monitoring of outcomes.

A number of areas for additional focus are identified below followed by a list of key conclusions.

26.2. Current Status of ARGT

Records of livestock losses from ARGT were kept in WA from 1968 until 1992. For around 10 years no data on the number of deaths and the areas affected by outbreaks of ARGT have been reported. The consequence of this dearth of information is the fact that is not possible to determine whether ARGT is expanding or contracting in WA. In the absence of reliable data it will not be possible to determine if any new interventions are successful and to quantify responses.

When records were maintained of stock losses there were at times two annual surveys. The Australian Bureau of Statistics sent questionnaires to all farmers within the area of risk for ARGT. This amounted to 5,445 farmers according to the 1987-88 Agricultural survey. Response rates were in generally in excess of 90%. The other survey of the annual losses due to ARGT was undertaken by the Western Australian Department of Agriculture and relied on spontaneous reports by farmers. As is apparent in the following table, the difference in mortalities between the two approaches could be up to 16 fold.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>ABS</th>
<th>WADA</th>
<th>PERCENTAGE CAPTURED BY WADA (100*WADA/ABS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>27,502</td>
<td>2,376</td>
<td>9%</td>
</tr>
<tr>
<td>1987</td>
<td>28,854</td>
<td>5,226</td>
<td>18%</td>
</tr>
<tr>
<td>1988</td>
<td>11,571</td>
<td>2,204</td>
<td>19%</td>
</tr>
<tr>
<td>1989</td>
<td>20,079</td>
<td>5,096</td>
<td>25%</td>
</tr>
<tr>
<td>1991</td>
<td>88,186</td>
<td>5,512</td>
<td>6%</td>
</tr>
</tbody>
</table>

From Roberts and Bucat (1992)

There is a need to obtain contemporary data that is as reliable and complete as possible. It may be expeditious to have the ABS distribute another survey and generate current prevalence data.

26.3. Prevention and Control

Areas of study that appear to have been neglected but may prove fruitful include:

- Elucidation of basis of individual animal differences in susceptibility to ARGT.
- Influence of diet on susceptibility.
Investigation of approaches applied to reduce toxic risk of feed contaminated with mycotoxins.

- Adsorption, sequestration, binding of toxins
- Rumen flora manipulation to increase detoxification.

### 26.4. Treatment

There is currently insufficient information to develop an individual animal or flock approach to treatment. In most cases, removal of livestock from the source of toxin exposure, provision of alternative safe feed and minimisation of handling and stress is the preferred approach. However, in specific cases where individual animal treatment is warranted it is possible that specific drug intervention may be practical and effective. For example, the number of parenteral benzodiazepines has increased markedly since the studies of chlordiazepoxide (Richards et al 1979, Norris et al 1981). In addition novel anticonvulsants such as gabapentin are available. Amongst the multitude of CNS active drugs now available, it may be possible to develop an effective treatment protocol. This project is not one that should divert financial support from other priorities, but may be an area suitable for study by a Master's or PhD student.

### 26.5. Key Conclusions

- There is considerable experience, expertise and data available concerning the management options for the prevention and control of ARGT.

- The 2002 Workshop on ARGT provides an excellent summary of the syndrome and animal and agronomic approaches to control. While meeting papers are available, the proceedings of this meeting have not been generated and distributed. Given the importance of ARGT and the breadth of discussion at the workshop, the proceedings should be finalised as a matter of priority.

- Applying this knowledge to the benefit of sheep and cattle producers appears to be less than ideal.

- Despite extension efforts in WA, adoption, adaptation and implementation of best practices can be improved.

- Better use of existing information should result in significant progress in reducing mortality and increasing productivity and measurable improvements should be readily achievable.

- Gaps in knowledge do exist and their prioritisation for further investigation should be considered.

- Further work includes:
  - Survey of on-farm practices that have worked and those that have not succeeded. For example, there is abundant data in South Australia on changes in R toxicus status on individual farms over many years. It should be possible to match elimination of R toxicus with on-farm interventions and the resulting information may guide improved practices elsewhere.
  - Development of improved ARGT forecasting and prediction system.
  - Determination of best practices for use of SafeGuard
  - Determination of best practices for use of Twist Fungus
  - Cause of inter-sheep variation in susceptibility
  - Corynetoxin adsorption and inactivation (esp bentonite, zeolites, glucomannan polymers, lucerne etc). Significant experience has accumulated in reducing bioavailability of mycotoxins in the diet of ruminants, pigs and chickens. This data should be assessed for its applicability to CT inactivation in sheep and cattle. It is already known that CTs bind
tenaciously to a variety of substrates, including cell walls. Such products are available commercially as feed additives and require no registration or development and could be widely used if shown to offer any benefits.

- Determination of nature and magnitude of subclinical losses. Quantification of subclinical losses has never been determined. It is possible that subclinical losses may exceed the costs of mortalities. If continuous subclinical losses are experienced then this recognition should motivate greater action by farmers to introduce effective ARGT controls. The value of vaccination and other preventative measures would also increase commensurately and may justify a greater cost.

- Improved feeding systems for meat sheep and cattle that rely less on ARG and more on alternative feeds (for example grain).

  - Vaccine development: facilitate development of business plan, identify commercial partners, determine likelihood of commercial manufacture; development needs and approval timetable.

  - The recommendations presented in the Executive Summary address the priority research needs identified by both the ARGT Workshop and SCARM.

26.6. Food Safety

  - Review current GRDC risk assessment research program and determine impact on food derived from livestock

  - Assess safe CT levels in meat and need for meat assay method development

  - Determination of residue levels in edible tissues of sheep could be added to current GRDC funded chronic toxicity study

  - Prepare food safety strategy in collaboration with AFFA and FSANZ