Paddock sampling for management of annual ryegrass toxicity

Ian Riley

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An estimated 200,000 sheep and 600 cattle have been killed by ARGT in Western Australia since 1969. Typical annual livestock losses in the State are about 20,000 sheep and 20 cattle. Other animals, including goats, horses and pigs have, on occasions, been killed.

By Ian Riley, Research Officer, Plant Pathology Branch, South Perth

Annual ryegrass (Lolium rigidum) seedheads containing corynetoxins, a group of bacterially produced antibiotics, are poisonous to livestock. Animals that eat affected ryegrass develop annual ryegrass toxicity (ARGT), suffer convulsive staggers and die within a few days of eating a lethal dose. ARGT is characterised by high death rates, especially where pastures are not known to be affected and where stock-owners are not expecting the problem.

Fortunately, there are commercial tests that determine the levels of ARGT organisms in the paddock and the risk of getting the disease. These tests will help farmers plan their cropping and pasture programs for the coming growing season. The results can also be used immediately to plan grazing over summer and autumn.

About the disease

ARGT was first recorded in the mid-1950s in South Australia. In Western Australia, the first reported outbreak was near Gnowangerup in 1969. Since then, ARGT has occurred over much of the wheatbelt between Moora and Jerramungup, with more than 1000 farms affected.

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FROM LEFT: Nematode gall, bacterially colonised gall and a normal seed.
Two tests are available: pre-flowering test and mature ryegrass test. The pre-flowering test detects and quantifies the ARGT bacterium in ryegrass collected mid-season. This provides information on bacterial infection before toxin is produced. It will indicate if control measures should be applied in the year of testing. Timely sampling is strongly recommended. Collect the sample after the main flush of seedheads have emerged from the boot but before flowering (pollen shedding). The correct development stage for sampling is shown in Figure 1.

Paddock sampling

Kits and tests

In Western Australia, SBS Rural IAMA markets sampling kits for the ARGT Testing Service. The cost ($50 in 1992) covers priority paid postage to the laboratory in South Australia, processing of the ryegrass sample, determination of ARGT organism levels, notification by telephone and mail and advice on the risk of ARGT.

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Management

Management of ARGT affects many aspects of the cropping and grazing programs. Stock grazing in infested paddocks must be monitored regularly during high risk periods. Paddocks with no or low risk of toxicity should be identified to allow stock to be moved to safe pasture when signs of ARGT are seen.

Controlling annual ryegrass reduces nematode and bacterial multiplication. In pasture, the main flush of ryegrass seedheads should be killed or removed by herbicides, grazing or mowing. Ryegrass levels in crops need to be kept as low as possible.

Management to prevent ARGT should be directed toward infested paddocks. Although livestock deaths can be an indicator of such paddocks, it is best to know the status of a paddock before animals are affected.

Efficiency in managing the risk of ARGT can only be achieved when stock-owners know the distribution and levels of causal organisms on their farms. Unfortunately, the nematode cannot easily be detected or counted in the field. Although finding seedheads with yellow slime indicates the bacterium is present, it is an unreliable guide of the level of bacteria present; high levels bacteria may be present without slime being evident.

The cause

A yellow slime bacterium (Clavibacter toxicus) produces the corynetoxins in ryegrass seedheads. This bacterium is carried into the grass by a parasitic nematode (Anguina funesta). The nematode invades the developing seedhead, replacing seed with galls (see photo on page 51). The nematode life cycle is completed in the gall, with about 2000 juveniles per gall being produced by the end of the season. In the next winter, juveniles move from the gall to invade a new generation of ryegrass plants.

The bacterium may either colonise the nematode galls, mostly killing the nematode in the process, or infect the whole seedhead. The bacterium contains a virus that appears to be necessary for corynetoxin production. Toxin levels increase dramatically as the grass matures. The bacterium survives over summer to adhere to the nematode juveniles moving on the soil surface in the next season.

Sampling the paddock for ARGT organisms is essential for proficient ARGT risk management. Management decisions made without adequate information may be ill-directed. Unnecessary or overly severe ryegrass control can result in selection of herbicide-resistant ryegrass, a decline in pasture productivity and poor pasture composition. On the other hand, a lack of ryegrass control because slime is not evident can lead to heavy stock losses.

Figure 1. For the pre-flowering test, collect ryegrass samples when the heads are a quarter to three-quarters emerged from the boot.
The mature ryegrass test detects and quantifies both the nematode and bacterium in ryegrass collected after the grass has matured. It is usually possible to detect the nematode well before there is enough bacteria for livestock to be poisoned. This information will help farmers plan their cropping and pasture programs for the coming growing season and can be applied immediately to grazing over summer and autumn.

**Sampling the paddock**

The usefulness of the information is directly related to the care taken in sampling. Collect three to five ryegrass heads at each of about 100 points well dispersed throughout the paddock. Do not specifically select heads that appear diseased or the results will be biased.

As a guide, two sampling schemes are shown in Figure 2. Sampling done on foot gives you time to observe the condition and composition of the pasture, but by vehicle is quicker.

It is important to cover most of the paddock and not to miss areas where ryegrass tends to persist or where it is not readily controlled; such as headlands, contour banks, floodways, near dams, fence lines, rock piles and tree belts.

Do not combine samples from different paddocks. The information obtained is more useful if it relates to a single area which can either contain or exclude stock. In large paddocks, there may be advantages in sampling the paddock in several sections, however, a single sample taken carefully over the whole area will give useful information.

**In the laboratory**

The pre-flowering test samples are crushed under high pressure to extract the juice and any bacteria. The exudate is diluted and tested using enzyme-linked immunosorbent assay. This assay uses antibodies raised in rabbits that are specific to the ARGT bacterium. The bacteria, if present, bind to the antibodies. The number of bound bacteria is assessed with an enzyme reaction.

For the mature ryegrass test the grass is threshed and the seed cleaned, ensuring that the galls remain in the seed fraction. The seed is then examined over a light box and number of galls in 10 g of seed determined. A standardised procedure is used to give reliable estimates of ARGT risks.

**Understanding the results**

The tests determine the number of galls in a standard weight of grass or seed. This lets us compare infection levels between paddocks and seasons. Gall levels are used to calculate a risk of toxicity to grazing animals.

Figure 2. Two patterns for collecting ryegrass for ARGT testing.
Table 1. Estimation of ARGT risk from gall levels in ryegrass samples

<table>
<thead>
<tr>
<th>Gall levels</th>
<th>Risk</th>
<th>Advice</th>
<th>Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>No galls</td>
<td>None</td>
<td>Safe to graze</td>
<td>ARGT in these paddocks is unlikely. On rare occasions, poor sampling may miss galls in toxic paddocks.</td>
</tr>
<tr>
<td>Nematode galls only</td>
<td>Low</td>
<td>Graze with care</td>
<td>There appears to be no bacteria to produce toxin, so these paddocks should be safe. However, the presence of nematode galls indicates that bacteria may be in an unsampled part of the paddock. Grazing is recommended for control of the nematode, but stock should be monitored regularly.</td>
</tr>
<tr>
<td>Low bacterial galls</td>
<td>Medium</td>
<td>Graze with extreme care</td>
<td>The presence of some bacterial galls indicates a low level of toxin is present. Grazing helps to control the ARGT organisms, but stock must be monitored daily.</td>
</tr>
<tr>
<td>Medium to high bacterial galls</td>
<td>High</td>
<td>Do not graze</td>
<td>The medium to high level of bacterial galls indicates that high levels of toxin are likely. Grazing is not generally recommended, but depending on the value of the stock and availability of other feed, grazing is occasionally attempted. Stock should be monitored twice daily and at the first signs of toxicity moved to a safe paddock.</td>
</tr>
</tbody>
</table>

Figure 3. Diagrammatic representation of the relationship between nematode and bacterial galls in ryegrass samples and the incidence of annual ryegrass toxicity in samples farmers provided to the ARGT testing service between 1983 and 1988.

If the result indicates 100 bacterial galls per 10 g seed and 100 nematode galls per 10 g seed (see orange dot), the paddock is in the high risk class.

The risk categories, the gall levels, advice given and some background information is given in Table 1.

Criteria for rating the risk of ARGT were developed by examining more than 500 samples provided by stock-owners to the ARGT Testing Service between 1983 and 1988. The study was led by Dr Alan McKay, South Australia Department of Agriculture. Paddocks were grazed, but not otherwise treated to control the ARGT organisms.

The nematode and bacterial gall levels were determined in the laboratory and stock-owners asked to provided details of stock deaths. As a result, three risk categories — low, medium and high — were established. The proportion of toxic paddocks in each were about 10, 40 and 80 per cent respectivley. Stock deaths in uninfested paddocks (where no galls were found in the sample) were occasionally reported, however, these could not be confirmed to be caused by ARGT. Figure 3 shows the relationship between gall levels and toxicity found in the study.

The risk rating is generalised to cover the variability in sampling and ryegrass levels in paddocks.

When interpreting the results for a specific paddock, total ryegrass, ryegrass as a proportion of available feed and the likelihood of stock selectively grazing ryegrass must be considered. If it was difficult to find enough ryegrass for a good sample, the risk may be lower. Conversely, where ryegrass is prolific the risk may be slightly higher.

Reliability

ARGT organisms cannot be assumed to be uniformly distributed over an area to be sampled. Some stock-owners are concerned that data obtained from 300 to 500 ryegrass heads, collected over 40 ha or more, will be unreliable. To help alleviate these concerns and to learn more of the distribution of the nematode and bacterium within paddocks, the Department of Agriculture's Plant Pathology Branch conducted further experiments in 1988 and 1989.

Samples of two, five and 20 ryegrass heads were taken from infested paddocks in 1988 and assessed separately. There was no advantage in taking large samples at each sampling point. Two to five heads per point is suitable, provided an adequate number of points is sampled. The ARGT Testing Service recommends 100 points per 40 ha.

In the 1989 experiment, stock-owners sampled more than 70 paddocks in ten separate sections. Gall levels in each section and the risk of toxicity were determined. Figure 4 shows the average infested and uninfested portions of paddocks grouped according to the highest risk section in the paddock. In paddocks with at least one high risk section, 80 per cent of the sections were infested. Any reasonable attempt to sample such
RATE TOXICITY RISK

Although stock-owners may be aware that certain paddocks are infested, there is no way of estimating the risk of ARGT without using a test. Without a test, grazing decisions may be inappropriate and may result in stock losses or unused feed.

FIND SAFE PADDOCKS

Where ARGT has occurred, or is likely to occur, it is essential that stock-owners identify safe paddocks. Death rates are often the worst when affected stock are moved from one toxic paddock into another, assuming it to be safe.

AID DIAGNOSIS OF LIVESTOCK DEATHS

Occasionally animals show symptoms similar to those of ARGT but due to other causes. A direct diagnostic test for ARGT has not been discovered. In doubtful cases, to confirm ARGT as the cause of death, the feed must be examined for galls.

MONITOR CONTROL OF ARGT

The success of an ARGT management program can only be monitored by using the testing service. Ryegrass control beyond that required to control the nematode may result in degraded pastures or development of herbicide resistant ryegrass.

Program planning

ARGT organisms generally increase during cropping and decline under pasture (especially from the second year of pasture). Ryegrass control through heavy use of herbicide in crops causes rapid development of herbicide resistance. Paddock testing allows planning of an appropriate cropping program to avoid increasing the ARGT problem and developing herbicide resistance.

MARKETING PRODUCE

Hay and feed grains can have enough contamination with bacterial galls to be toxic. Similarly, contaminated hay, feed grains and seed can spread the ARGT organism to new areas. Testing the paddock or the produce will help you market farm produce or avoid the risk of litigation if toxic produce is sold.

Table 2. Benefits of using the ARGT tests

<table>
<thead>
<tr>
<th>Benefits</th>
<th>None</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early detection</td>
<td>Infested paddocks can be detected years before they pose a risk to livestock. Changes to cropping and pasture management may be made to avoid ARGT problems. In contrast, if stock losses are the first indicator of an infested farm, major disruption to programs can result.</td>
<td></td>
<td></td>
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</tr>
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Figure 4. Proportion of paddocks found to be infested with the ARGT nematode and bacterium for paddocks sampled in 10 sections. Paddocks are grouped according to the highest ARGT risk section in the paddock.

Figure 5. An area of a farm sampled in 50 sections showing ARGT risk in each section. Where some high risk sections are found, much of the area is infested at lower levels.

Table 2. Benefits of using the ARGT tests
paddocks each year, to reduce costs and sampling time. Well considered selection of paddocks for testing will improve the value of the tests.

Paddocks can be placed in four groups: infested paddocks; paddocks not known to be infested on infested farms; paddocks on farms not known to be infested but in or near ARGT areas; paddocks well outside the current distribution of ARGT. The impetus and strategy for sampling will differ for each group.

Paddocks known to be infested should be sampled to determine the likelihood of toxicity and the success of control measures. If livestock have been affected, sampling for the following two years would be advisable and thereafter less frequently, if ARGT is being controlled.

If tests indicate the presence of ARGT organisms but stock have not been affected, future sampling frequency will depend on the gall levels detected. Treat high risk paddocks as if stock had died. For lower level infestations, assuming efforts are made to control the nematode, test the paddock again after two years.

Results of the pre-flowering test can be used to decide in which order to graze infested paddocks. Graze paddocks with the highest level of bacteria grazed first; there is seldom enough stock to heavily graze all infested paddocks before toxin builds up towards the end of the season.

Test paddocks of unknown status on infested farms after several years of cropping (particularly in the year before returning to pasture) or if they are to be grazed in summer. Safe paddocks identified by testing are a valuable resource and should be grazed during harvest, when there is little time for monitoring stock, or conserved for affected stock. Use safe paddocks for the production of seed, hay or feed grains to be used elsewhere on the farm.

Owners of apparently uninfested farms in ARGT areas should not be complacent, but should check one or two paddocks each year. Paddocks with a history of high levels of ryegrass, which have been weedy when cropped, would be a good first choice.

Farms in areas well outside the current distribution of ARGT should not need testing. However, if potentially contaminated materials (seed, hay or feed grains) or equipment from ARGT areas have been used on the farm, testing should be considered. Cropping paddocks with a history of high levels of ryegrass and in which off-farm materials were used, could be tested first.

Farmers should follow reports of the spread of ARGT towards their district and avoid bringing potentially contaminated material onto their property. ARGT should be considered when stock losses cannot be attributed to another cause.

In areas where the incidence of ARGT has declined, paddocks are likely to have a low level of nematode galls and few if any bacterial galls. Test paddocks that were resown to ryegrass or where ryegrass has been allowed to build up. A reduction in ryegrass levels over several years will see a decline in ARGT. A return to high ryegrass levels may allow the ARGT organisms to proliferate.

A ryegrass variety resistant to the ARGT nematode will be released soon and should be considered if ryegrass will be resown.

Using advice and information services

Department of Agriculture district offices and the ARGT testing service can help you plan sampling and interpret test results. The Department has several publications that will help you understand ARGT and its control.

When seeking advice on managing infested paddocks, provide details of livestock deaths, test results, sampling methods, pasture composition and cropping history, as this will improve the quality and usefulness of the advice given.

Further reading


ARGT Testing Service Information Guide, IAMA.