1-1-1986

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Annual ryegrass toxicity

research update

By A.G.P. Brown, Principal Plant Pathologist and P. Vogel, Biochemist, Animal Health Laboratories

Annual ryegrass toxicity (ARGT) is a complex disease that kills sheep and cattle grazing pastures which contain infected ryegrass. The disease results when a nematode (Anguina agrostis) and a bacterium (Corynebacterium sp.) invade annual ryegrass and cause the seed heads to become toxic as the grass dries off.

The bacterium produces a complex toxin of 18 glycolipid compounds or corynetoxins which have been found to be virtually identical to the antibiotic tunicamycin.

Signs of the disease are usually most obvious when a flock or mob of sheep is disturbed. The disease is characterised by staggering and convulsions, ranging from mild tremors to severe muscular spasms. Affected animals collapse, convulse for some minutes then may regain their feet and stagger away with a stiff-legged, unco-ordinated or 'rocking-horse' gait. After a few minutes they may regain the flock and become apparently normal.

The best strategies at present for minimising stock losses from ARGT are cultivation and the use of herbicides to control ryegrass in crops, thereby breaking the life cycle of the nematode in following pastures, the identification of toxic paddocks and the surveillance of stock at risk.

A wide range of possible therapeutic agents has been investigated. Some beneficial effects have been produced by drugs of the benzodiazepine group, particularly Librium®, a well known tranquilliser, in association with close care of affected animals.

The possible development of a vaccine to protect animals at risk has been studied. Immunisation trials with rabbits and sheep involving several strategies have been completed, but were unsuccessful. Studies on the fate of the corynetoxins after ingestion have started.

Distribution and spread

ARGT was first detected at Black Springs in the mid north of South Australia in 1956, and it progressively spread to a number of properties in this area. After a few years there were no further outbreaks, then in 1976 it appeared at Murray Bridge, in 1978 at Port Gawler and in 1979 at Butler Tanks on the Eyre Peninsula and later on the Yorke Peninsula. Since 1956, about 8500 sheep and 290 cattle deaths have been attributed to ARGT in South Australia.

Since the first outbreak of ARGT in Western Australia in 1968 the disease has spread from five properties in the Gnowangerup area to more than 580 properties in an area extending from Dalwallinu in the north to Borden in the south, and extending across the 350 to 500 millimetres average annual rainfall zone. Reported losses to December 1985 amounted to 40 000 sheep and 422 cattle. ARGT has recently been diagnosed at several localities in South Africa which receive up to 700 mm average annual rainfall.

The distribution of the disease corresponds with that of the nematode which is likely to have a greater climatic tolerance than its ryegrass host. Hence all the area where ryegrass has successfully adapted is potentially at risk. The bacterium may be more widely distributed than the nematode, but without its nematode vector or carrier it cannot infect ryegrass. Ryegrass is the only pasture grass known to support reproduction of the nematode. However, the nematode will attempt to colonise other grasses mixed with ryegrass. Although it fails to reproduce on these grasses the nematode can introduce the bacterium to the sward. Grasses that are not hosts of the nematode and which have been found toxic include Phalaris spp. and wild oats, however this is unimportant in the field.
Outbreaks of toxicity depend on the extent of pasture infection, which is strongly influenced by cropping practices. There is invariably a high build-up of nematodes on ryegrass in a crop where cultivation or herbicides have failed to give good grass control (less than 10 plants per square metre). The surviving nematodes colonise a ryegrass dominated pasture the following year, resulting in a high disease level.

Little is known about the time taken between when a paddock first becomes infected with the nematode and bacterium and when there is sufficient toxic material to cause stock losses. Some nematode diseases have typical ‘lag phases’ of 10 to 12 years. The ‘lag phase’ of ARGT is influenced by the amount of toxic material first introduced in the paddock, its original distribution or subsequent spread by cultivation or stock movement, and the density of ryegrass. Locally the disease is readily spread by wind and water.

The disease is probably spread over long distances by man in the movement of hay, seed, contaminated stock or machinery. The ease of disease spread precludes any possibility of developing an effective containment policy, although legislation is in force which prohibits sale of seed not declared free of nematode infection.

Disease cycle

Inflective larvae of the nematode colonise ryegrass plants during their vegetative growth phase and induce galls when flowers start to develop. Galls typically develop in place of ovules, less commonly in place of stamens and rarely on glumes (husks) or rachis (the central spine of the grass head). The flask-shaped galls have a thickish cell wall surrounding a central cavity where the nematodes develop and reproduce. Galls are shed as the plant dries off, just like normal seeds.

Frequently, the Corynebacterium sp. (presumably from the soil) adhere to the surface of the infective larvae of the nematode which subsequently colonise ryegrass. In some galls these bacteria multiply to fill the cavity of the galls, preventing the nematodes from reproducing. Consequently in infected pasture there is a mixture of galls, some containing nematodes and others colonised by bacteria. The latter are toxic to grazing animals.

In rainy periods or seasons bacteria multiply rapidly in the young ryegrass flower heads, causing copious development of yellow ‘slime’, the first visual indicator of potentially toxic ryegrass. The slime is only slightly toxic at first, but increases in toxicity with age. The bacterial ‘slime’ can usually be seen from mid-September when the ryegrass heads first appear.

Studies under the light and electron microscope have compared toxic and non-toxic galls and have followed the development of galls containing nematodes.

Life cycle of the nematode

The nematode goes through its reproductive cycle while it is parasitic on ryegrass, and survives the remainder of the year as larvae that are resistant to desiccation while protected within the gall. Larvae within galls are known to be viable after dry storage for 13 years. However, once exposed to water, larvae will not survive for more than a season. In the absence of a suitable host, they die.

Larvae move into the leaf sheaths of the vegetative ryegrass plant and eventually penetrate to the embryonic flower head. Within the flower the galls are formed and inside the galls the nematode molts three times, mates and the females lay eggs which hatch to second stage larvae. Galls initiated by four to five larvae can contain up to 1500 larvae by the end of the season.

The close-knit and balanced host-parasite relationship and ryegrass’ enormous potential for seed production make it an ideal host for the nematode. Sufficient sites are provided to carry over extremely high populations of nematodes, while the survival of the grass is never seriously threatened. Nevertheless, nematode populations can fluctuate considerably between seasons, depending on the proportion of galls that are colonised by bacteria. Frequently nematodes will disappear from infected pastures for reasons as yet not understood.

Nematode-bacterial association

The Corynebacterium sp. relies on the nematode to introduce it to the gall to complete the disease cycle. This is achieved by a specific recognition between the bacterium and the nematode which allows the bacterium to adhere to the nematode’s surface. Nematodes presumably contact the specific bacteria as they move through the soil and on to a host plant. The specificity of this recognition is being studied as a possible means of breaking the disease cycle.

Pathology of the disease

In cattle and sheep the liver may be enlarged and pale tan, and animals may be jaundiced. Other pathological changes include diffuse fat deposits in the liver, haemorrhages in various organs and vascular damage in the brain, particularly the cerebellum.
Toxin studies

Separated fractions of parasitised annual ryegrass fed to laboratory animals have shown that only galls colonised by bacteria are toxic, and that the toxin is concentrated in the walls of the galls. By testing extracts from plants collected at weekly intervals from infected paddocks, it has been shown that even though the galls are colonised by bacteria while the plants are still booting, some four to six weeks elapse before toxicity develops. The first sign of toxicity is evident when most of the heads have emerged and flowering is advanced. Toxicity increases rapidly during the milk and dough development stages, reaching a maximum at the same time as grain ripening. This indicates a close relationship between senescence (drying off) of the plant and the accumulation of toxin. Once a pasture reaches its most toxic stage there is an almost immediate decline in toxicity because the galls are shed. The rate and degree of shedding has also been studied. This supports field observations that once the critical period has passed, toxic pasture becomes relatively safer to graze.

Studies of the toxin have established that it is bacterial in origin, consisting of 18 glycolipid compounds. These corynetoxins closely resemble tunicamycin, an antibiotic produced by the soil-borne organism, *Streptomyces lysosuperficus.* The *Corynebacterium* sp. can easily be cultured in the laboratory, but it produces very low yields of toxin, with about 20 litres of cultured bacteria yielding the same amount of toxin as a single toxic gall. The first clinical signs of ARGT occurred in rats 36 to 48 hours after they were injected with the toxin. In sheep given oral doses of the toxin clinical signs have not been observed before six days. Preliminary studies have shown that corynetoxins are not detoxified in the laboratory by fermentation with ovine rumen fluid. Immunisation trials with rabbits and sheep involving many different strategies were unsuccessful. However, as new procedures become available, they will be investigated. Prophylactic treatment of ARGT-affected rats with agents capable of reversing some of the known laboratory effects of the analogous tunicamycin was also unsuccessful.

Bioassays for the toxin depended at first on giving measured extracts to laboratory animals, but a much more sensitive method has been developed using the growth of *Corynebacterium tritici* as determined by optical density measurements. This technique can detect extremely low levels of toxin. The concentration of the toxin in the gall wall and the low yields of toxin in the laboratory suggest that these bacterial toxins are produced in the plant in response to a plant stimulus. Efforts to identify this stimulus continue.

Studies on the absorption, distribution, metabolism and excretion of corynetoxins may lead to prophylactic or therapeutic treatment of animals. Although the use of Librium® for affected animals in a pen was beneficial, its use in the field was not effective.

Control methods

Chemical methods of controlling the nematode have been evaluated in field trials. The nematicide, Nemacur®, used at rates as low as two litres per hectare, will control the nematode if applied during the brief period when the infective larvae are in the soil, between the time they emerge from the galls and when they colonise ryegrass. However,
the high cost of chemical and the difficulty in predicting the period when larvae will emerge suggest the use of Nemacur® is not practical.

Chemical control of the *Corynebacterium* sp. is not feasible; there are no suitable bactericides and even if there were the same problems of cost and effective timing would apply.

Control of ARGT at present is based on using herbicides and other agronomic practices to control the nematode and the growth of ryegrass, combined with close supervision of stock. These practices are unlikely to eradicate the nematode or prevent spread of ARGT between farms. All the methods to some extent destroy ryegrass pasture, thereby breaking the nematode’s life cycle and reducing or preventing the production of toxin. The relationship shown between cropping practices and outbreaks of toxicity has influenced the development of control strategies. See ‘Control strategies for annual ryegrass toxicity’ on page 7.

The development of toxicity in infected pastures can be controlled if the right herbicide is applied early enough to stop the development of galls, followed by intensive grazing to control regrowth. This ‘spraytop-graze’ treatment however, substantially reduces pasture content. Under favourable conditions the nematode can virtually be eliminated by in-crop and in-pasture application of selective and non-selective herbicides. Burning-off pastures in autumn also reduces nematode survival.

More research is needed to determine the period and degree of ryegrass control necessary to achieve an effective break in the nematode’s life cycle, and to determine the time required for nematode populations to build up to the point where pasture becomes dangerously toxic once more. It should be possible to devise satisfactory strategies to offer a number of options retaining ryegrass as a pasture species. Selective herbicides would be used in-crop and perhaps in-pasture and legume components of the pasture encouraged to assume dominance.

Another and perhaps more desirable means of control is to find an alternative pasture species to replace ryegrass, particularly for land unsuited to cropping. A research programme has started at Katanning to investigate a range of legumes and grasses. The burr medic *Medicago polymorpha*, particularly the late-maturing cultivar Circle Valley and the early-maturing cultivar Serena, appear to offer an outstanding alternative to ryegrass. In 1984 ungrazed pastures of these two medics yielded almost six tonnes per hectare of dry matter. A mid-season burr medic variety and the development of acid-tolerant soil Rhizobia which will allow medics to nodulate and thrive on acid soils are primary research objectives.

### A fungal invader?

A fungus which appears to interrupt the reproductive cycle of the ARGT nematode *Anguina agrostis* has been found on annual ryegrass.

The fungus *Dilophospora alopecuri* is a minor pathogen causing ‘twist disease’ of many species of grasses and cereals. It can infect its hosts by (presumably) wind-blown spores, but much greater infection levels occur when spores are picked up by a suitable nematode vector. The spores attach themselves to the nematode’s skin and are taken into the plant’s growing point. This occurs with *Anguina agrostis* and ryegrass. Once in the developing nematode gall, the fungus spreads through all the nematodes within, killing them. It can spread out to colonise and kill most or all other galls on the same flower head.

*Dilophospora* is already present in paddocks in South Australia and Western Australia. The fungus appears to partly control reproduction of the ARGT nematode, but its infection rate can vary from season to season. It seems unlikely that the fungus will naturally maintain itself at a sufficient level to solve the ARGT problem, even if it were introduced to every affected farm.

An inoculum suspension of the fungus, which would be sprayed on to a nematode-infested pasture, could possibly control ARGT in the season of application. We now need to discover how effective this control method would be compared with herbicides, and if production of inoculum would be economical.

One other possible drawback is that the fungus is known to attack wheat and other cereals.

### Acknowledgement

This research programme is supported by the Wool Research Trust Fund, the Australian Meat Research Committee and Toxend, comprising Elders Pastoral, Town and Country Building Society and CSBP and Farmers.