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Perennial ryegrass staggers research—an overview

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ABSTRACT

Investigations into perennial ryegrass staggers, a neurotoxic disease affecting sheep, cattle, horses and deer in New Zealand, recently incriminated a fungus growing as an endophyte in perennial ryegrass in the occurrence of this disease. This *Lolium* endophyte (an *Acremonium* sp.) is most prevalent in the lower parts of infected ryegrass plants (leaf sheath) but invades the inflorescence and seed. Newly-emerging ryegrass seedlings are so infected.

The close association of the endophyte with the presence of lolitrems, potent neurotoxic tremorgens previously isolated from staggers-producing pasture samples, further incriminated the endophyte in the occurrence of ryegrass staggers.

Subsequent lamb grazing trials on plots sown with lines of either high- or low-endophyte ryegrass seed confirmed that adequate intakes of short-grazed ryegrass pasture material readily produced ryegrass staggers in all lambs grazing the endophyte-infected plots but there was little or no disease on low-endophyte plots identically grazed.

The trials also revealed that the spring-sown high-endophyte ryegrass plots had considerable drought resistance and persistence when compared with low-endophyte plots. The infected swards were found to be highly resistant to Argentine stem weevil attack and a significant insect-feeding deterrent factor has since been detected in endophyte-infected ryegrass.

A subsequent sheep grazing trial has indicated that plots sown in autumn with fungicide-treated seed to control endophyte and then conservatively grazed in the first year subsequently gave good production and also provided excellent protection from ryegrass staggers.

Keywords Ryegrass staggers; *Lolium* endophyte; lolitrems; Argentine stem weevil; mycotoxic disease; neurotoxins perennial ryegrass; insect resistance of pastures; tremorgens; pasture persistence

INTRODUCTION

Investigations into perennial ryegrass staggers (PRGS), a neurological disorder of grazing livestock in New Zealand (Cunningham and Hartley, 1959; Mortimer, 1983) and other countries (reviewed by Mantle and Penny, 1981) have recently provided convincing evidence on the cause of the disease. Applied research into PRGS with certain chance field observations has led to discoveries which, aside from PRGS, have importance for pasture management and animal production based on New Zealand grazing systems.

PRGS occurs only in summer and autumn and is often associated with close grazing of ryegrass-dominant swards and these factors have suggested a possible mycotoxic aetiology (Cunningham and Hartley, 1959; Mortimer, 1978; Mantle and Penny, 1981). Observations by Keogh (1973, 1978) and Byford (1978) indicated that noteworthy PRGS outbreaks occurred only when swards in which perennial ryegrass (Lolium perenne L.) was dominant were overgrazed.

The Disease of PRGS in Livestock

In New Zealand, sheep, cattle and horses are commonly affected with PRGS and it has recently appeared in farmed deer. The disorder is characterised by tremors together with incoordinated postural reflexes and movements. The severity of incoordination and overt clinical signs usually worsen, the longer livestock are grazed on toxic pasture. Forced exercise exacerbates the symptoms and is used to demonstrate the degree of incapacity present when scoring clinical severity in PRGS field trials (Keogh, 1973).

When removed from toxic pasture, stock usually make full recovery in 1 to 3 weeks. However when foraging and watering are restricted by the disorder stock losses can be considerable, especially in drought conditions. In a flock or herd there is a wide range of individual susceptibility to the disorder and Hewett (1983) presented evidence to show that there is a strong heritable basis to PRGS susceptibility.

Research into the Actiology of PRGS

Isolation of Neurotoxic Lolitrems from Toxic Pastures In 1979 tremor-producing fractions were isolated from extracts of 25 g samples of dried toxic ryegrass. Mouse bioassays suggested that these fractions differed from known mycotoxic tremorgens earlier examined as the possible PRGS toxins (Mortimer and White, 1980). Further purification of extracts of toxic ryegrass

yielded 2 new tremorgens which were named lolitrems A and B (Gallagher et al., 1981). Later these and other minor lolitrems were isolated from ryegrass seed and seed from this batch, pelleted and fed to sheep, caused 'severe PRGS-like symptoms' (Gallagher et al., 1982).

Evidence for an Association Between PRGS, the Lolitrems and *Lolium* Endophyte

A major advance into the cause of PRGS was the occurrence of a severe PRGS outbreak which disrupted a multi-replicated lamb grazing trial comparing ryegrass cultivars at Lincoln during summer drought (Fletcher, 1982). The 100% incidence of severe PRGS in lambs on all 6 replicate plots of 1 cultivar was in total contrast to the absence of PRGS in lambs grazing a second cultivar. Examining the possibility that a seed-borne infection was responsible for the difference in PRGS incidence, a search was made for Lolium endophyte, a fungus previously examined by Neill (1940) in New Zealand in relation to the cause of both facial eczema and ryegrass staggers. A firm association was found between the occurrence of PRGS in the Lincoln trial plots and the presence of endophyte in the ryegrass plants. In addition both were correlated to amounts of lolitrems extracted from ryegrass leaf samples from the plots (Fletcher and Harvey, 1981; Fletcher, 1982; Mortimer *et al.*, 1982).

Investigations into Lolium Endophyte

Neill (1940) showed that an endophytic fungus was normally present in the aerial parts of perennial, but not Italian, ryegrass in New Zealand. It was concentrated in the leaf sheaths, flowering stems and seeds and did not appear to spread from plant to plant but to be entirely seed-borne, the fungus dying however in stored seed. Its presence in the plant could be shown by microscopic examination of stained leaf sheaths in which it appeared as septate, rarely branched, parallel strands running the length of the sheath. A method of measuring the comparative amounts of fungus within infected plants uses a count of the number of strands seen /mm breadth of sheath. This count varies with season, reaching a peak in February and a low in August but does not appear to be affected by cultivar or by sites within the North Island at least (Fig. 1).

Neill (1940) isolated the endophyte by broth culture of seedlings grown from surface-sterilised endophyte-infected seed and this method was used to determine the percentage of viable infection in seedlines used in grazing trials.

PRGS Grazing Trials

A series of grazing trials was established on the Ruakura, Rukuhia and Takapau Research Areas, first to confirm the association of *Lolium* endophyte with PRGS and then to enlarge upon observations made in previous trials.

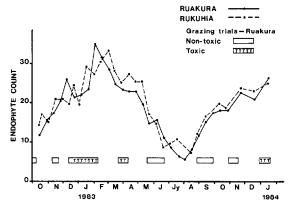


FIG. 1 Mean counts of *Lolium* endophyte hyphae in leaf sheaths of perennial ryegrass samples (10 infected plants per plot × 4 plots) from 2 PRGS trial sites (Ruakura and Rukuhia) October 1982 to January 1984. Periods of PRGS toxicity, demonstrated by sheep intermittently grazed on the Ruakura plots, are also indicated.

In the first trial (1982 Ruakura trial) 8 plots were spring sown with 4 lines of ryegrass seed of the same cultivar, 2 plots/seedline. Two freshly-harvested lines had high viable endophyte infections (>95%) and 2 lines stored for 2 and for 4 years had low infections (<3%). After the plots had been grazed for 24 days in January 1982 there was 100% incidence of PRGS in sheep on the high endophyte plots but none in sheep on the low (Table 1; Mortimer et al., 1982).

In autumn 1982 (1983 Rukuhia trial) a single line of freshly harvested seed of the same cultivar used in the Ruakura trial was used to sow 8 plots. Untreated seed was used for 4 plots and the other 4 were sown with seed treated with 1 g prochloraz (BFC Chemicals 'Sportak')/kg to control endophyte infection (Harvey et al., 1982; Latch and Christensen, 1982). All plots were identically set-stock grazed during January to March 1983 and all 40 sheep on high endophyte plots developed clinical PRGS while only 3 mild clinical cases occurred on the low endophyte plots (Table 2).

The same trial protocol was used in the same year at the Takapau Research Area, Hawkes Bay. There also, incidence of PRGS was 100% in plots sown with untreated seed while very mild PRGS occurred in 6 of the 40 lambs grazing plots sown with prochloraztreated seed.

The plots established for the Rukuhia 1983 trials were stocked with sheep for the second year on 14 December 1983, when all plots were covered with long dense swards. Available feed was in excess of 3500 kg DM/ha. On high endophyte plots, PRGS first appeared after 9 days grazing, and after 37 days all 40 sheep showed marked clinical PRGS. Of these, 30 collapsed while running the length of the plot. Only 1 mild clinical case was detected in 40 sheep grazing low endophyte plots established with prochloraz-

TABLE 1 Ruakura 1982 PRGS trial results. PRGS clinical incidence (%; N = 39), adult stem weevil counts ($/m^2$) and subsequent pasture production (kg DM/ha) in relation to *Lolium* endophyte status of plots (% of plants infected).

Seedline ^a	Seed harvested	Endophyte present	PRGS clinical incidence	Weevil population	Pasture regrowth after	
					4 wk	10 wk
A	1979	< 3	0	184	113	677
В	1977			229	321	1021
С	1981	>90	100	35	526	2196
D	1981			40	885	2853

^a2 replicate plots of each line-all same cultivar.

TABLE 2 Rukuhia 1983 and 1984 PRGS grazing trial on plots sown in autumn 1982 showing that prochloraz fungicide treatment of seed reduced *Lolium* endophyte in emergent swards and controlled PRGS in sheep during the first and second year of grazing.

Seed treatment ^a	% Endophyte in sward	Ryegrass staggers scores				% Sheep affected	
		Nil	Mild	Mod.	Sev.		
1983							
None	>93	0	9	11	20	100	
Prochloraz	< 25	37	3	0	0	8	
1984							
None	94	0	5	5	30	100	
Prochloraz	14	39	1	0	0	3	

^a4 replicates each – 40 sheep/treatment.

treated seed. Over the whole period temperatures were below seasonal averages, there was no moisture stress in swards and adequate rainfall maintained good regrowth, so feed intakes were not restricted by availability.

Endophyte-Infected Ryegrass Resists Stem Weevil Attack

Another previously unsuspected biological association, which has aroused considerable agronomic interest, was revealed during the 1982 PRGS trial. During regrowth after excessive grazing, all the highendophyte plots made good recovery. On lowendophyte plots recovery was slow and incomplete, with widespread loss of plants due to severe predation by Argentine stem weevil resulting in excessive tiller and plant deaths and in extremely low DM production (Table 1; Mortimer et al., 1982; Prestidge et al., 1982). For re-establishment of the low endophyte plots complete autumn reseeding was necessary.

DISCUSSION AND CONCLUSIONS

The role of the *Lolium* endophyte and of the lolitrems in the causation of PRGS is now established, but many facets of the complex plant-fungus-toxin-animal disease interrelationships are now open for detailed investigation.

In the sheep grazing trials briefly reviewed here, the facility with which PRGS was induced in sheep, with

100% involvement, in successive years and at various sites, is very remarkable. The trials illustrate the consequences of summer grazing of monoculture or highly-dominant perennial ryegrass swards containing a high percentage of endophyte-infected plants. This information on the basic requirements needed to produce, probably in any summer, the natural severe disease of PRGS will greatly facilitate studies of the disease itself and of its field control.

Events in the 1984 Rukuhia trial require comment, for commonly held beliefs that drought and the grazing of the base of the sward are requirements for PRGS occurrence were dispelled. In this trial the severity of PRGS produced in sheep was the highest we have so far encountered. This suggests that in our previous trials the election to graze short swards to induce PRGS probably restricted the intake of ryegrass material and endophyte toxin, so obtaining a lesser PRGS response. The results of this trial also demonstrated, in 2 successive summers, the effective control of PRGS when pastures which were sown with prochloraz fungicide-treated seed were grazed.

The year-round endophyte count data (Fig. 1) require the complement of parallel data on amounts of lolitrems present in pasture at intervals throughout the year. We do not know if amounts of lolitrems present in pasture are a reflection of the amount of endophyte present, or are more closely associated to the activity of endophyte growth or to pasture growth activity. The endophyte is present in lesser amount in

the cooler seasons, but it is not known if lolitrems are also present during these seasons but in amounts inadequate to produce even mild PRGS symptoms in grazing animals.

Discovery of the association of Lolium endophyte with the resistance of ryegrass to stem weevil feeding came from observations on the Ruakura PRGS trials (Mortimer et al., 1982; Prestidge et al., 1982). Insect resistance and the higher pasture productivity previously noted with certain ryegrass seedlines (Kain et al., 1982) is now largely explained by the presence of endophyte in those lines. For reasons unrelated to insect feeding, further information is needed on possible direct effects of endophyte on ryegrass pasture production, on animal grazing preferences and animal weight gains.

The difficult choice between high-endophyte status in ryegrass pastures to give better pasture production and persistence with attendant risks of PRGS outbreaks, and low-endophyte status possibly with lesser production and weaker persistence, but with freedom from PRGS, will now frequently face farmers and they will require soundly-based advice.

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