RYEGRASS STAGGERS: FURTHER SUBSTANTIATION OF A **LOLIUM** ENDOPHYTE AETIOLOGY AND THE DISCOVERY OF WEEVIL RESISTANCE OF **RYEGRASS** PASTURES INFECTED WITH **LOLIUM** ENDOPHYTE

P.H.MORTIMER and MARGARET E. di MENNA
Ruakura Agricultural Research Centre, Hamilton.

Abstract

An experiment was planned and undertaken to obtain further evidence on the association of the *Lolium*-endophyte (a fungus parasitic in some perennial ryegrass) with the occurrence of ryegrass staggers in sheep (Fletcher & Harvey, 1981).

In the experiment, 39 sheep grazing four plots established with perennial ryegrass seed (*Lolium perenne*) highly (>90%) infected with the endophyte all developed clinical ryegrass staggers over a 26 day grazing period. The same number of sheep grazing plots established from seed found to have negligible (<3%) endophyte infection showed no evidence of ryegrass staggers.

The results clearly confirm that *Lolium*-endophyte infested ryegrass pastures are closely associated with the occurrence of ryegrass staggers in sheep.

Observations made during regrowth of the ryegrass plots at the end of the grazing period showed that there was a 3 to 4-fold greater production of dry matter from the high endophyte plots than from the low endophyte plots. The poor regrowth and low production recorded in low endophyte plots was found to be largely due to pasture predation by the Argentine stem weevil (*Listronotus bonariensis*). There is a firm indication that *Lolium*-endophyte infection of ryegrass confers resistance to stem weevil predations. This finding is discussed in relation to the field control of ryegrass staggers. It will clearly have implications in relation to plant selection and seed production, as well as to pasture production trials conducted over many years during which seed and pasture of unknown endophyte status was evaluated.

INTRODUCTION

The recently-proposed hypothesis (Fletcher & Harvey, 1981) that *Lolium*-endophyte (Le) fungus infection in perennial ryegrass was associated with the spontaneous occurrence of ryegrass staggers (RGS) in sheep during a ryegrass cultivar – hogget growth trial at Lincoln presented a new and exciting lead in the search for the causative agent of this troublesome disease of livestock.

Trials were immediately planned at Ruakura with the objective to gain more evidence and information relating to the proposed Le/RGS hypothesis,

TRIAL DESIGN AND METHODS

The trial plan was to establish replicated plots of pure stands of perennial ryegrass (*Lolium perenne*) both with and without endophyte infection. To eliminate the possibility of a relationship between RGS occurrence and different ryegrass cultivars only one cultivar was utilised. It will be recalled that multiple cultivars had to some extent confounded the interpretation of events in the Lincoln RGS outbreak (Fletcher & Harvey, 1981). Multiple batches of seed of the one cultivar were obtained from one source.

Seedlines with high and low rates of viable endophyte infection were identified by culture in glucose peptone broth of seedlings grown under sterile con-

Measurement of herbage-on-offer for the pre-clinical RGS period in both years showed no significant difference between pastures (Table 2). No herbage measurements were made later in summer, but living material was higher for OP and Nui than for Ruanui.

Fletcher and Harvey (1981) established a link between ryegrass staggers and infection of ryegrass plants with *Lolium* endophyte. More definitive testing of presence of *Lolium* endophyte has since been possible using an enzyme linked immunosorbent assay (Musgrave pers. comm.). The percentage of endophyte infected plants in 1982 was OP 95%, Nui 42%, and Ruanui 5%. In the four week pre-clinical staggers period the concentration of endophyte per unit of available pasture ranged from 89 $\mu\text{g}/\text{mg}$ to 206 $\mu\text{g}/\text{mg}$, whereas in the previous six weeks the range was 6 to 44 $\mu\text{g}/\text{mg}$ and in the succeeding 10 weeks it was 67 to 15 $\mu\text{g}/\text{mg}$ (Table 3).

Table 3: CONCENTRATION ($\mu\text{g}/\text{mg}$) OF FUNGUS IN 'OP' RYEGRASS

19 October	5.7
2 November	20.1
16 November	43.8
30 November	88.6
14 December	50.9
30 December	205.8
11 January	66.9
25 January	14.7
8 February	28.2
22 February	17.6
9 March	26.3

The use of the immunosorbent assay for *Lolium* endophyte has reinforced the link between RGS and endophyte infected plants suggested by Fletcher and Harvey (1981). The data suggest this association may also have an effect on animal weight change. Significance in weight change could only be established for the extreme high level of endophyte in OP pastures compared to the negligible endophyte in Ruanui.

Factors other than the presence of the fungus may be involved, for example, quantity and composition of herbage of the three pastures, but no likely effect was detected (Table 2). The earlier effects of pasture type on weight gains tending to be reversed in the period post peak RGS, could reflect higher herbage growth of OP and Nui pastures in that period.

In finishing lambs for slaughter, the presence of *Lolium* endophyte in ryegrass pastures may be of considerable significance.

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ditions from surface sterilised seed samples of the lines (Neill, 1940). Four seedlines were selected. No endophyte could be grown (<3% infection) from two of the lines used, one harvested in 1977 and one in 1979. In the two other lines, both harvested in 1980-81, endophyte was grown from >90% of the seedlings cultured.

Plots were cultivated, prepared and broadcast-seeded in October/November, 1981. The incidence of endophyte in the plots was monitored by microscopic examination of stained leaf sheaths (Neill, 1940); material from four plants from each plot was examined at monthly intervals from 25 November 1981. This indicated that endophyte status of ryegrass in all plots was in accordance with the trial design. Plots were uniformly treated and grazed during the establishment period.

GRAZING TRIAL RESULTS

In late January 1982, ten sheep were placed on each of the eight plots. The first clinical signs of RGS were recognised in 15 sheep after 12 days of grazing. The incidence and severity of RGS in the sheep increased day by day. By the 26th day the incidence of RGS was 100% on all four high-endophyte plots. Sheep on the four lowendophyte plots remained apparently free of clinical RGS (Table 1).

Table 1: STAGGERS TRIAL - RUAKURA - 1982. PLOTS ESTABLISHED EITHER ENDDPHYTE FREE OR INFECTED - ONE RYEGRASS CULTI-VAR.

Line*	Seed Year harvested	Viable endophyte present %	Day	Sheep (n = 39) Clinical ryegrass stagers (%)		
				12	19	26
A.	1979	< 3		0	0	0
B	1977					
C	1981	>90		15	61	100
D	1981					

* two replicates each

REGROWTH OF THE TRIAL PLOTS

The trial plots, which were closely grazed in order to promote maximal clinical RGS in the sheep, were spelled after 26 days of set-stocked grazing. In the final week of this grazing period and in the first week subsequent to spelling there was frequent and abundant rainfall (total >100 mm) evenly dispersed over this period. Conditions for pasture regrowth in these respects could therefore be regarded as near optimal.

Within one week of spelling it was observed that the high endophyte ryegrass plots were uniformly producing good regrowth while all the low endophyte plots were showing uniformly poor regrowth. Over the first weeks of spelling

these between-treatment differences appeared to increase day by day. The differences appeared to be maintained even after 10 weeks. Mean dry matter (DM) determinations of pasture regrowth at four and 10 weeks (non-grazed) (Table 2) showed that total DM production of the endophyte-infected plots was more than four times greater than on the endophyte-free plots at four weeks and was still approximately three times greater at 10 weeks post-grazing.

Table 2: STAGGERS TRIAL — RUAKURA — 1982. ENDOPHYTE INFECTION, WEEVIL POPULATIONS, HERBAGE YIELDS AFTER 1st GRAZING.

Line*	Seed Year harvested	endophyte present (%)	Weevil population adults/m ²	Pasture (DM kg/ha) @ 4 Weeks	Regrowth kg/ha 10 Weeks
A	1979	< 3	184	113	677
B	1977		229	321	1021
C	1981	>90	35	526	2196
D			40	885	2853

* two replicates of 4 seed lines ~ all same cultivar

Investigations were made into the reasons for the remarkable and unexpected differences in post-grazing regrowth and DM production. cursory examination of the pastures revealed that many ryegrass plants had died and others showed widespread damage to vegetative tillers. This took the form of external brown discolouration and tillers with central burrows with larval exit holes. Plant mortality and injury was considerably more extensive in low endophyte plots. Heavy predations by Argentine stem weevil were suspected. This was confirmed and the stem weevil populations on the plots were quantified (Table 2). The low endophyte plots were found to be harbouring five times more adult stem weevils than were found on the high endophyte plots (Mortimer et al. 1982). Further evidence has subsequently been obtained to indicate that endophyte infections in ryegrass confer plant resistance to Argentine stem weevil predations (Prestidge et al. 1982).

DISCUSSION

The results of these trials gave strong confirmation of the initial findings and conclusions of Fletcher & Harvey (1981). It is clear that endophyte-infected ryegrass is in some way closely associated with potential pasture toxicity which may manifest itself as RGS in grazing animals. How the mechanisms operate, and under what conditions, is currently not clear.

Discovery of the association of Le with the occurrence of RGS immediately suggested means of controlling this disease in grazing stock. Several investigations had already been mounted using, for example, fungicides with a view to controlling Le in pastures and for the production of endophyte free ryegrass seed (Harvey et al. 1982). It now seems likely that the elimination or the reduction of Le infections in ryegrass pastures as a means of controlling RGS may have

serious implications in lowered pasture production through repeated Argentine stem weevil predations of ryegrass swards. In particular, the loss of valuable post-drought autumn pasture production would be most serious.

Retrospective consideration of the results of many perennial ryegrass cultivar comparisons in pasture and animal production trials may now need to be made since endophyte status of the seed or established pastures would be unknown. A variable component of any differences found in such pasture production comparisons could well be ascribable to endophyte status.

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