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## Ryegrass staggers: genetics accounts for a six-fold difference in susceptibility between selection lines of lambs at Ruakura

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### ABSTRACT

Ryegrass staggers (RGS) is a neuromuscular syndrome affecting susceptible ruminants when they graze perennial ryegrass (*Lolium perenne* L.) containing the mycotoxin-producing endophytic fungus *Neotyphodium lolii*. Genetic selection for divergence in RGS susceptibility began in 1993 at Ruakura in Romney-cross sheep, with single-trait RGS selection lines for resistance (R) and susceptibility (S) being established. Stock from both lines are repeatedly challenged when grazing together on high-toxin endophyte pastures, and scored for RGS. Lambs and 'yearling' females (16 to 18 months of age) have been recorded each year, as well as breeding ewes and yearling rams in some years. Results up to the 2000 lamb crop are reported here, as well as preliminary records from the 2000 born yearling females. In the 2000 born lambs (n=61), 14% and 88% of the R and S lines showed clinical RGS, respectively (P<0.01). In the 1999 yearling ewes (n=48), corresponding percentages by line were 0 and 66% respectively (P<0.01), whilst for the 2000 yearling ewes (n=18) percentages were 0 and 63%, respectively (P<0.01). Line-crosses, born and reared together with the R- and S-line animals, had levels of clinical RGS between those of the corresponding R and S lines. From restricted-maximum-likelihood analyses, the single-record heritability for RGS in all stock classes was  $0.17 \pm 0.02$ , and the repeatability (between animals across years) was  $0.28 \pm 0.02$ . Breeding value estimates suggest that most of the divergence between lines has resulted from genetic change in the S line, but the genetic mechanism is not yet clear. Reasons for this asymmetry include: (1) greater genetic and phenotypic variation in the S line than in the R line, following a given challenge; (2) greater selection intensity applied in the S line; and (3) a major gene might be present, with an allele for resistance which is recessive or partially recessive.

**Keywords:** ryegrass staggers; endophyte; sheep; genetics; selection.

### INTRODUCTION

Ryegrass staggers (RGS) is a neuromuscular syndrome affecting susceptible ruminants when they graze perennial ryegrass (*Lolium perenne* L.) containing the mycotoxin-producing endophytic fungus *Neotyphodium lolii*. The endophyte produces the tremorgenic toxin, lolitrem B, which causes muscular incoordination and may result in severe distress to affected animals. RGS can also lead to management problems for farmers. Sheep may be difficult to drive or muster, and affected animals are prone to accidental injury. It is well known that the effects of the toxin in sheep are reversible, in that the symptoms are alleviated when the stress and the toxin are removed.

There are at least three different approaches to solving an RGS problem on a sheep farm: through sowing alternative strains of perennial ryegrass containing selected low toxin endophytes, avoiding the grazing of toxic high endophyte perennial ryegrass, or grazing using resistant sheep. Although new low toxin endophyte strains of perennial ryegrass are now available, few sheep farmers have the opportunity to re-sow enough pastures on extensive hill country. There may also be difficulty in preventing the germination of all high toxin endophyte infected seed from an old sward. Avoiding grazing toxic pastures tends to restrict the farmer's grazing flexibility at a time of year when high quality feed supplies are often limiting. An animal genetics solution may be feasible, but it is a long term solution. The present paper gives an update on progress in selecting two flocks at Ruakura for resistance or susceptibility to RGS, and it investigates the mechanisms that might help to explain the flock

divergence achieved in the last 8 years. Earlier progress reports have been given by Morris *et al.* (1995a, 1998, 1999).

### MATERIALS AND METHODS

#### Phase 1: progeny tests

Over the 5 years from 1988 to 1992, a total of 18 different rams were progeny tested by AgResearch (formerly the Ministry of Agriculture & Fisheries) in order to rank them for genetic resistance or susceptibility to RGS (Morris *et al.*, 1995a). The rams tested were predominantly Romneys from the facial eczema (FE) selection experiment at Ruakura (Morris *et al.*, 1995b), but they also included a Border Leicester x Corriedale crossbred ram that was highly susceptible to RGS (used for mating on a North Canterbury farm and later donated to Ruakura: Hewett, 1983). The ewes recorded at Ruakura for this work consisted of Coopworths, and some Romneys from the Ruakura FE-selection experiment.

#### Phase 2: selection flocks

Before the start of mating in autumn 1993, breeding values (BVs) for RGS were calculated for lambs, their sires and the ewes repeatedly used in the progeny tests, as detailed later. These BVs were then used to classify ewes and 18-month females for the 1993 mating into two selection lines which were genetically resistant (R) or susceptible (S) to RGS. The 1992-born ewe lambs were also classified at this stage. Thereafter, R and S females remained in their allocated flocks for mating each year to elite R and S rams, respectively. BVs for rams of all ages were used in the same way to select rams on RGS

susceptibility for their respective selection lines, for mating in 1993 and subsequent years. Flocks were potentially open to the introduction of outside genes, with some selected rams for RGS matings also originating from the Ruakura FE flocks. Two private sources of rams were also used for mating (one Romney ram, which was thought to carry susceptibility genes, and three Borderdales from the North Canterbury breeder mentioned in Phase 1, and which were thought to be resistant). A total of 22 R rams and 16 S rams have been used in the selection lines in 1993-2000 (14 of these twice or more). In total, 678 R and 504 S lambs were available for scoring at 4-6 months of age from the 1993-2000 lamb crops. In Table 1, details are given of the RGS pure line animal numbers, as well as the line crosses. First cross animals between the R and S lines were generated in 1996 and 1997. Three of the 1997-born first cross ram lambs were then mated to R and S line ewes to generate back cross ( $\frac{3}{4}$ -R and  $\frac{3}{4}$ -S) lambs in 1999 and 2000, with further  $\frac{3}{4}$ -S back crosses being derived in the same years by mating S sires to first cross ewes.

**TABLE 1:** Percentage (%) of lambs experiencing clinical ryegrass staggers, classified by line (n = numbers of animals recorded; R = Resistant; S = Susceptible).

Year of birth	R line		$\frac{3}{4}$ R		$\frac{1}{2}$ R		$\frac{3}{4}$ S		S line	
	n	%	n	%	n	%	n	%	n	%
1993	78	5							55	9
1994	94	56							68	74
1995 <sup>1</sup>	101	0							74	0
1996	82	20			89	52			56	75
1997	110	3			95	10			53	32
1998	129	0							109	17
1999 <sup>1</sup>	49	0	48	0			41(26) <sup>2</sup>	0(0)	63	0
2000	35	14	109	44			99(35) <sup>2</sup>	53(74)	26	88
Total	678		157		184		140(61)		504	

<sup>1</sup> No clinical scores achieved in these years.

<sup>2</sup> Records in brackets were from lambs bred by using S sires over first cross ewes, whilst the remaining  $\frac{3}{4}$  S back crosses were bred by using first cross sires over S ewes.

### Data recorded

One score for RGS was recorded per lamb during each season of RGS-challenge (from December or January to March or April). The opportunity was also taken whenever possible to score 16- to 18-month ('yearling') animals and the breeding ewes.

With at least 600 animals to be scored each season (generally 100 to 300 animals per mob), the intensive six point RGS scoring system of Keogh (1973) was not used. Instead a simple score of 0 for no staggers, or 1 for a clinical case (i.e., an animal unable to walk, and susceptible to RGS) was assigned after mustering with sheep dogs. Clinically affected animals were removed from toxic pasture and, where possible, transferred onto safe pasture or onto crop to recover. All those scoring zero remained on toxic pasture and were challenged again at a later date so that, by the end of the season, each animal had accumulated just a single score of 0 or 1.

For direct RGS comparisons, the first cross and back cross lambs were scored when grazing together with the contemporary pure R line and S line lambs, and similarly (some years only) the RGS and FE flocks were scored

when grazing together. All scoring has been carried out at Ruakura, except in 1996/7 and 1997/8 when lambs were transferred to AgResearch's Poukawa property in Hawkes Bay for scoring.

### Data analyses

The original allocation of females to the R and S flocks to begin Phase 2 relied on BV estimates from a computer package providing repeated-record animal-model best linear unbiased prediction (Groenvelde *et al.*, 1990). Preliminary repeatability and heritability values of 0.20 and 0.10 respectively were adopted from Phase 1 binomial RGS data.

In the current analyses, Phase 1 and Phase 2 data were restricted to records of animals in contemporary groups in which the mean RGS incidence was at least 3%. A contemporary group was defined to include animals grazing together, and from the same stock class (lambs, yearlings, or ewes). The heritabilities and across-year repeatabilities reported here were obtained using animal-model restricted maximum-likelihood (REML) procedures (Gilmour, 1997), with a full relationship matrix, a repeated-animal term (for records across years), and a fixed effect for contemporary group. There were 4995 animals recorded, with 7138 RGS scores (i.e., 1.43 RGS records per animal). These animals were by 276 sires (including sires of ewes in all flocks, as well as sires of lambs); of these sires, 163 had ten or more progeny with RGS records.

For the Phase 2 data, beginning with the 1993-born lambs, selection responses in the R and S lines of the RGS experiment were tested using chi-squared analyses. Responses were also estimated from mean BVs for each year-of-birth x selection-line combination, obtained from solution files from the REML analyses.

Before 2000, analyses and interpretations of RGS scores had been restricted by having only 0/1 data for each animal. Additional information, recorded from the 2000 born crop onwards, first as lambs then as yearlings, has consisted of the date when each animal first experienced RGS in the season. The method of combining this 0/1 and date information (as a description of severity) was to compute a 0 to 10 score for each lamb in 2000/01, and a 0 to 4 score for each yearling female in 2001/02. Scores were effectively coded dates, since using actual dates failed to reflect the non linear trends in pasture toxicity over time. Lower scores were given to animals experiencing clinical RGS later in the season, and scores of '0' represented those most resistant to the season's cumulative challenge.

## RESULTS

### Line means

Table 1 shows the annual incidence of clinical RGS in the R and S lines of lambs and their crosses, indicating considerable year to year variation in the degree of challenge experienced. Annual incidences of RGS in each contemporary group during 5 of the 8 years of lambs in Phase 2 were low to moderate ranging from 0 to 16% (similar to the Phase 1 years; data not presented), while incidences in the other three years of Phase 2 (1994, 1996

and 2000-birth years) were considerably higher (between 42 and 64%). The first cross lambs were intermediate between the R and S lines in 1996 and 1997, and both back crosses were intermediate between the R and S lines in 2000. Figure 1 shows evidence from the BV data that divergence was achieved in the foundation lines established in 1993, and it also demonstrates the genetic trend in the selection lines over the 8 birth years, 1993 to 2000. Most of the response occurred in the S line, amounting to an average of about a 5% increase in the percentage of clinical cases *per annum*. In contrast, little genetic progress appears to have been made in the R line, until at least the 1999 lamb crop. The divergence between the lines was greatest in BV units for animals born in 2000 (Figure 1), with the BVs in that year representing a regressed version of the phenotypic means for clinical cases, 14 and 88% in the R and S lines of lambs, respectively (s.e.d. 9%,  $P < 0.01$ ).

For the R and S selection line yearling females remaining from the 1999 crop ( $n=48$ ), percentages with clinical RGS were 0 and 66%, respectively ( $P < 0.01$ ), whilst for those remaining from the 2000 crop ( $n=18$ ) preliminary means midway through the season were 0 and 63%, respectively ( $P < 0.01$ ).

From the 0 to 10 scores in 2000/01 (2000 born lambs;  $n=304$ ), and from their corresponding 0 to 4 scores as yearling ewes in 2001/02 ( $n=106$ ), line differences were significant in the first year ( $P < 0.001$ ) and in the second ( $P < 0.05$ ). The correlation between line means in the two years was 0.97, and the correlation among individual scores from animals recorded in both years was 0.46.

The realised selection intensities applied to the selected R and S line rams which were used for mating (Figure 1), averaged over the eight years 1993-2000, were  $-0.013$  (s.e. 0.0063) and  $+0.101$  (s.e. 0.0245), respectively. These estimates are genetic (regressed) values based on the BVs for the selected sires and their resulting pure line lamb crops. Thus, it is expected that genetic change in susceptibility in the S line should increase at a rate of  $0.5 \times 0.101$ , equalling approx. 0.05 or 5% more clinical cases *per annum* as described above. The selection intensity in the S line was greatest from the 1996 born animals onwards, averaging  $+0.139$  *per annum* in the five years

to 2000, compared with 0.101 for all years together. However, the BV of rams used in the S line now appears to be plateauing, and this may be evidence that recent sires are carrying a major gene that is now fixed for the susceptible allele.

### Genetic parameters for RGS score as a binomial

The heritability and repeatability estimates, for animals born in the RGS S line only, were  $0.27 \pm 0.06$  and  $0.35 \pm 0.04$ , respectively, with a phenotypic standard deviation of 0.406. Corresponding values from all RGS and FE selection lines combined, for comparison, were respectively  $0.17 \pm 0.02$ ,  $0.28 \pm 0.02$  and 0.297. It was not possible to analyse data from the RGS R line alone because of the very high percentage of zero values, but the S values appeared to be higher than the combined ones.

## DISCUSSION

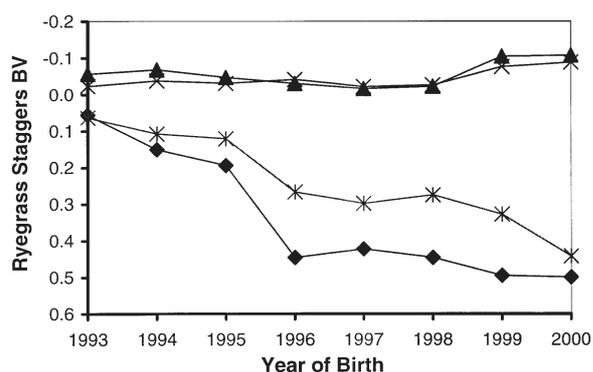
Considerable genetic progress has been achieved over eight years (Figure 1), mainly in the S line, in less than three generations of selection. By the 2000 lamb crop, the relative risk of clinical cases in lambs between the S line (88%) and the R line (14%) was a factor of 6.2 (95% confidence intervals for the relative risk being 2.7 to 14.1). Reasons for the greater response in the S than the R line include: (1) the greater genetic and phenotypic variation in the S line, following a given challenge; (2) the greater selection intensity applied in the S line; and (3) the possible presence of a single gene with large effect, comprising an allele for resistance (*s*) which is recessive or partially recessive, and an allele for susceptibility (*S*) which is dominant. In this single gene hypothesis, let us assume that both lines in 1993 consisted of large numbers of *Ss* heterozygotes with smaller numbers of resistant *ss* (R line) or susceptible *SS* homozygotes (S line). Then it is likely that:

- The  $\frac{3}{4}$ -S line means (with a mixture of *SS* and *Ss* genotypes) would be close to the S line mean because of dominance,
- The  $\frac{3}{4}$ -R line means (with a mixture of *ss* and *Ss* genotypes) would be in between the R and first cross line means, and
- The selection response would be faster in the S than the R line, but it would then plateau in the S line sooner, when the S allele became fixed.

## CONCLUSIONS

From the Phase 1 screening, followed by single trait selection over eight years in Phase 2 for or against RGS resistance, the R and S selection lines have been developed with 14% vs. 88% clinical staggers incidence in lambs, and with a 0% vs. at least 63% clinical staggers incidence in the R and S lines as yearling ewes (values of 66 and 63%, respectively, for the 1999 and 2000 birth groups). The scientific challenge now is to identify the gene(s) that explains the line differences in susceptibility. This could lead to the development of a DNA test, eliminating the need to challenge potential breeding stock in order to rank them, and enabling farmers to identify susceptible animals on commercial properties.

**FIGURE 1.** Breeding values (BV) for ryegrass staggers incidence, obtained from scored animals of all ages, in the selection lines by year of birth, and in sires by year when mated (▲ Resistant sires; × Resistant line; ◆ Susceptible sires; \* Susceptible line).



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