Six years of selection responses for resistance or susceptibility to ryegrass staggers in sheep

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Abstract

Ryegrass staggers (RGS) is a neurotoxic disease in ruminants. It is caused by the mycotoxin lolitrem B, found in endophyte-infected perennial ryegrass (*Lolium perenne* L.). This paper reviews genetic studies on the resistance of sheep to RGS at Ruakura. Genetic differences in susceptibility among sire groups were evaluated in lambs born in 1988–92 (Phase 1). Selection lines were then formed and responses to selection for resistance or susceptibility are described for the 1993–98 birth years (Phase 2). The incidence of RGS was recorded on a presence or absence basis from January to March or April each year, in experimental flocks with animals of known pedigree. For estimating genetic parameters (Phase 1), there were 3587 RGS records available from 2307 animals representing 232 sires. Clinical cases of RGS occurred in 17 of 25 contemporary groups, with incidences per group up to 16%. Estimates of heritability and across-year repeatability for RGS incidence were 0.068 ± 0.028 and 0.24 ± 0.05 respectively. Breeding value data were used at the beginning of Phase 2 to screen ewes, two-tooths and ewe lambs into resistant and susceptible selection flocks (lines). Breeding values were also used to select rams that were elite for RGS, and to allocate these to each line for mating. At least some natural RGS challenge was experienced by the resulting selection-line stock born in five of the six years of Phase 2. Susceptible-line lambs had a mean incidence of 39% clinical RGS cases over these five years, whilst the resistant-line lambs grazing the same paddocks had a much lower mean clinical RGS incidence ($P<0.001$) at 15%, a difference of 24%. Breeding ewes from the resistant RGS line also experienced significantly less clinical RGS than those in the susceptible RGS line ($P<0.001$). Evidence is presented from another experiment at Ruakura, in which selection pressure was applied for increased or decreased resistance to facial eczema. The facial eczema-resistant line was also more resistant to RGS than the facial eczema-susceptible line, suggesting that there is a common biochemical pathway explaining some of the resistance to both sporidesmin and lolitrem. Studies of the RGS selection flocks are being continued in order to investigate the underlying genetic mechanisms. The selection response so far indicates that genetic differences in RGS susceptibility can be achieved by using records collected under extensive conditions.

Keywords: *Lolium perenne*, resistance, ryegrass staggers, selection, sheep

Introduction

Ryegrass staggers (RGS) is a neurotoxic disease in ruminants caused by the mycotoxin lolitrem B, found in endophyte-infected perennial ryegrass (*Lolium perenne* L.). RGS can cause severe distress to animals and it can also lead to management problems for farmers, generally under summer/autumn grazing conditions. It causes muscular incoordination in animals, and in sheep it is most obvious when they are under the stress of being moved, mustered, or being driven by sheep dogs. It is well known that the effects of the toxin are reversible, in that the symptoms are alleviated when the stress and the toxin are removed. In this paper, the genetic parameters for susceptibility of sheep to RGS are summarised, and the 6-year selection responses at Ruakura are described. Earlier progress reports have been published by Morris *et al.* (1995a, 1998).

Materials and methods

Phase 1: progeny tests

In 1988 and 1989, six Romney rams were progeny tested over Romney-cross ewes at Wairakei Station (Taupo). This followed the mating to Romney ewes at Wairakei in 1987 of one highly susceptible ram (described later), although no useful RGS results were recorded there. Twenty-five individual ram-mating groups were set up at Ruakura for progeny testing over the 1988–92 years. The ewes at Ruakura consisted of Coopworths, and some Romneys remaining from the Ruakura Facial Eczema (FE) Performance-Test selection flock (Morris *et al.* 1995b). The Ruakura ewes were progressively supplemented with daughters bred at Wairakei (1987 crop), then from both sites (1988 crop)

and from Ruakura only (1989 crop onwards). This progeny testing phase involved 17 different Romney rams (most being repeated across years, and two rams linking Ruakura and Wairakei data) plus the original highly susceptible ram, a Border Leicester x Corriedale cross animal (ID 79/143) used by a North Canterbury breeder in 1981 (Hewett 1983). Other progeny testing details were described by Morris *et al.* (1995a).

Phase 2: selection flocks

Breeding values (BVs) for RGS were calculated for lambs, their sires and the ewes repeatedly used in the progeny tests (further details of the calculations are described later). BVs were then used to classify ewes and 18-month females for the 1993 mating into two flocks (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 birth flocks for mating each year to elite R and S rams. BVs for rams of all ages were also calculated, in order to select rams on RGS for their respective selection lines, for mating in 1993 and subsequent years. Selected rams for RGS matings were derived not only from the R and S RGS flocks, but also from the Ruakura FE flocks which were grazed together with the RGS animals until the 1995-born lamb crop inclusive, and in the 1998/99 season. There were also two private sources of rams used for mating (one Romney ram which was thought to be carrying susceptibility genes, and three further Borderdale rams from Mr Hewett which were said to be resistant; their results are given below). A total of 18 R rams and 14 S rams have been used in the selection lines in 1993–98 (11 of these twice or more). In total, 593 R and 415 S lambs were available for scoring at 4 months of age, and selection results are reported here for the 1993–98 lamb crops.

Data recorded

One score for RGS was obtained on each lamb during the season of RGS-challenge (generally January to March or April). The opportunity was also taken whenever possible to score 16- to 18-month animals and breeding ewes. When the RGS and FE flocks were grazed together, both sets of flocks were scored.

With up to 600 animals to be scored each season (generally 100 to 300 animals per mob), a score of 0 (no staggers) or 1 (susceptible to RGS) was allocated, after mustering with sheep dogs. Susceptible animals were removed immediately from toxic pasture and, where possible, transferred onto a clover-dominant paddock to recover. Knowing the identities of all animals grazing together in each contemporary group, the susceptible animals (which were defined as clinical cases, i.e., those unable to walk) were noted and given a score of 1, whilst the remainder received a zero score by default. All those scoring zero remained on toxic pasture and were challenged again later so that, by the end of the season, each animal had accumulated just a single score of 0 or 1. For the current paper, proportions between 0 and 1 were converted to percentages.

Heritabilities (described below) were estimated using data in the five seasons up to the 1992 year of birth (autumn 1993 scoring season), and over this period clinical cases were recorded in 17 of 25 contemporary groups. A contemporary group was defined to include animals which were grazed together, of the same sex, from the same stock class (lambs, 18-month animals, ewes), and born in the same genetic group (one of four groups, defined below). Annual incidences of RGS in each contemporary group in Phase 1 and subsequently in Phase 2 (4 of the 6 years) ranged from 0 to 16%, while incidences in R and S lambs scored from the 1994 and 1996 birth-years were considerably higher, being 64% and 42% respectively. In Phase 2 all the scoring work has been carried out at Ruakura, except in the autumns of 1997 and 1998 when lambs were transferred to AgResearch's Poukawa property in Hawke's Bay for scoring.

Data analyses

The original allocation of females to the R and S flocks in Phase 2 relied on results from a computer package providing repeated-trait animal-model best linear unbiased prediction (Groenveld *et al.* 1990). Parameters for these predictions were based on preliminary estimates from binomial RGS data including the records from all contemporary groups, and adopting a repeatability and heritability of 0.20 and 0.10 respectively.

The Phase 1 data before the selection lines began were later analysed only from records of animals in the 20 contemporary groups where the mean was at least 4%. Heritabilities and across-flock repeatabilities reported here were obtained from these 20 contemporary groups using animal-model restricted maximum likelihood (REML) procedures (Johnson & Thompson 1995) with a full relationship matrix and a repeated-animal term (for records across years). Fixed effects were contemporary groups, and genetic-group effects for flocks (n=4) were as follows: RGS selection lines (both represented as one flock, because of common foundation pedigrees for both lines), and FE-resistant, FE-susceptible and FE-control flocks. There were 232 sires (including sires of ewes of all flocks, as well as sires of lambs), 2307 animals with scores and 3587 animal records (i.e., 1.55 records per animal).

For the Phase 2 data, beginning with the 1993-born lambs, selection responses were tested using chi-square analyses. Genetic progress was also estimated

from mean BVs for each year-of-birth x selection-flock combination, obtained from solution files from the REML analyses.

Results

Genetic parameters

The estimates of between-year repeatability and heritability for single-record RGS score in Phase 1 (all stock classes) were 0.24 ± 0.05 and 0.068 ± 0.028 (Morris *et al*. 1995a). The interpretation of the repeatability was that about a quarter of animals with RGS in one year also experienced RGS in any other year. The mean RGS score from this data set was 6.1%, with a phenotypic standard deviation of 27.4%. The heritability of RGS score in lambs and 18-month animals alone, comprising two-thirds of all the data, was higher at 0.128 ± 0.048. The corresponding mean was 7.1%, with a phenotypic standard deviation of 29.2%.

Selection line differences

Table 1 shows the selection line differences resulting from the first six years of selection. The line differences were significant in four of these years (P<0.01), i.e., when moderate or serious natural RGS challenge was experienced.

Table 1 Percentage of lambs experiencing clinical ryegrass staggers, classified by flock.

Year of birth	Resistant flock[a]		Susceptible flock		Significance[b]
	N	%	N	%	
1993	77	5	55	9	n.s.
1994	94	56[c]	68	74[c]	**
1995	101	-[d]	74	-[d]	-
1996	82	20	56	75	***
1997	110	3	53	32	***
1998	129	0	109	17	***
Total (all except 1995)	492	15	341	39	***

[a] Number of animals (N) exposed to ryegrass staggers challenge.
[b] Significance test for a difference between flocks.
[c] Percentages staggering by mid-autumn only were 4% and 15% respectively.
[d] No clinicals observed.

Progeny tests, of the industry rams up to the 1998 birth year, showed that the original susceptible animal 79/143 had a BV of +3%. The susceptible Romney had a BV of +11%, and the three resistant Hewett rams had an average BV of -13%, compared with the Ruakura-bred rams used for mating in 1996 and 1997, whose BVs averaged -14% (Resistant) and +24% (Susceptible).

Success with progeny testing resistant rams for RGS depended on the severity of the natural challenge. In low challenge years, where the R-line mean was close to zero, there was little opportunity to find progeny-group differences among R-line sires, whereas the opportunity was greatest in years of serious challenge. Data from the two years of most serious challenge so far (1994- and 1996-born lambs recorded in the autumns of 1995 and 1997), show that there were differences among the five R-line sire groups born in those years (Table 2). Sire means ranged from 45 to 68% in 1994 and from 13 to 27% in 1996. Use of a common sire across years has allowed us to rank all five sires together. As a result of identifying such R-line genetic differences and using only selected sires for matings with R-line ewes, genetic progress has been made in the R line over six years (and similarly in the S line). Our best estimates of genetic progress, from the assessment of all relatives together, are shown in Figure 1. The clearest evidence of R-line progress comes from the BVs of the best available rams, which are becoming more extreme with time. The mean genetic difference of the S line over the R line in the last two birth years (1997 and 1998) was 23% (Table 1), indicating that 23% more S-line than R-line animals became susceptible to RGS when the two lines grazed together.

Table 2 Progeny-test results (% animals susceptible) of the Resistant-line sires used in the two birth-years with most serious challenge; the Susceptible-line means are shown for comparison.

Sire No.	1994		1996
	Mid	Late	
Resistant sires			
1	3	45	
2			13
3	5	61	25
4			27
5	4	68	
All	4	57	19
Susceptible sires	15	76	75

The mean RGS susceptibilities (phenotypic incidences) in S- and R-line RGS ewes in the 1998/99 season were 14 vs. 0%, respectively (P<0.001).

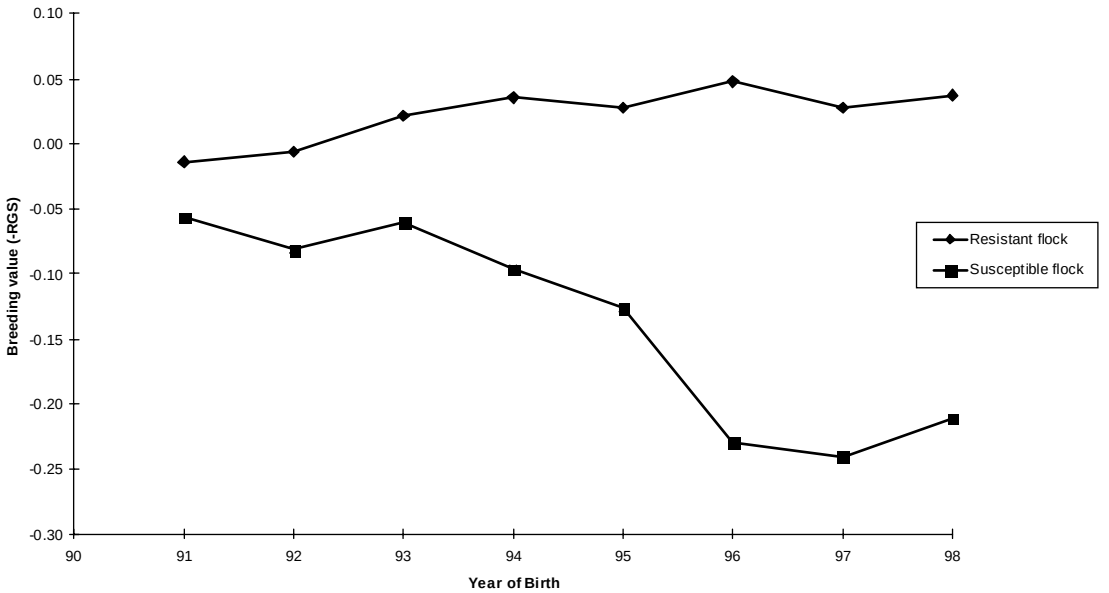
In the Ruakura FE-selection experiment in 1998/99, the mean phenotypic incidences of RGS in the S- and R-line lambs were 8.8% and 0%, respectively (P<0.01), and in the S- and R-line ewes 10.2% and 0%, respectively (P<0.01).

Discussion

Selection line differences

Over a 6-year period, this Ruakura selection experiment has demonstrated that genetic progress for differences in susceptibility to RGS can be achieved in sheep. The selection line differences in 1994 and 1996 were large,

Figure 1 Genetic divergence between the Resistant and Susceptible selection lines in breeding value (BV) for ryegrass staggers (RGS). The vertical scale is shown as *minus* BV(RGS), so that larger positive numbers on the 0 to 1 scale indicate more resistance; 1993 was the first year of selective matings.



approximately 0.37 and 1.11 phenotypic standard deviations respectively. Most of the progress has resulted from progeny-testing rams and then using the elite ones again. Although extensive sire-group differences have been demonstrated, it is unlikely that faster progress would have been achieved with a more refined scoring system, unless the heritability could have been increased. Greater progress would more likely have been achieved with more rams for progeny testing, a tester flock kept genetically separate from the elite flocks, and larger flock sizes of elite R and S sheep. Future prospects in selecting for increased resistance are discussed later.

The asymmetric response could be the result of a less effective challenge to R-line animals, or a lower heritability in the R-line flock. There have been insufficient numbers of sires used so far, to compare the two lines for heritability differences. An alternative explanation could be that there is a major gene segregating, which is dominant for susceptibility.

The level of natural challenge required for separating R sires has only been severe enough in some of the years. The experiment has highlighted the need to develop and use an artificial RGS challenge system to sheep, but it is not yet possible to provide such an artificial challenge by using lolitrem or its mimics. This would also allow us to confirm that the current difference between flocks was the result of animal-genetic effects controlling tolerance of animals to the toxin, and not the result of genetically controlled grazing (diet-selection) differences. However, the fact that the Ruakura FE-resistant and FE-susceptible flocks, which have been developed using artificial challenge with sporidesmin, also show genetic differences in RGS susceptibility strongly suggests that diet-selection has not contributed much, if any, to the RGS flock differences in RGS susceptibility.

The next steps in the research process are:

1. to find whether the fast response to genetic selection can be explained (fully or in part) by a major gene for resistance/susceptibility;
2. to find the biochemical pathway(s) used by the animal to detoxify lolitrem, and
3. to find a genetic marker(s) for animal resistance, so that testing for resistance on commercial farms does not need to involve challenging animals with RGS, artificially or in the field.

One method of trying to achieve (3) is to develop markers for any candidate genes identified in (2). Another method is to screen the whole genome from a sire's progeny group for marker differences correlated with RGS phenotype. The sires for these genetic-marker searches must be heterozygotes (generally R-flock x S-flock cross-line animals), and these sires in turn are used to generate backcross progeny. Our studies have not yet progressed far enough to test definitively for a

major gene, nor to determine the dominance/recessivity relationship of such a gene.

Plans to improve the R line must take account of our previous experience, and will rely in the short term on:

- finding more serious natural challenge,
- finding a method of administering an artificial challenge (using lolitrem or a cheaper mimic of lolitrem), and
- ultimately, utilising a genetic marker solution.

Genetic parameters

Although the heritability of single records of RGS score was low, the opportunity to use repeated records on each animal and to use family data via the REML analysis has led to a large realised response between lines. This was significant in the 4 years when there was moderate or serious RGS challenge. The effect of analysing this binomial trait in a continuous-trait model is not known, but the corresponding heritability on a transformed liability scale was four times as great, at 0.27 (Morris *et al*. 1995a). Incorporating time of season when an animal first became susceptible would provide a more continuous scale of measurement. The effects of different means from season to season will also influence underlying heritability. Further matings, including the use of first-cross ewes, will be required to determine whether dominance or maternal effects may be important for RGS, when the selection lines are crossed.

Work by private breeders

It is clear that the two breeders who supplied us with outlier rams from their flocks have been able to characterise heritable differences in RGS. The prospects for making further progress for RGS resistance seem encouraging, and the RGS selection lines are being continued at Ruakura in order to investigate the underlying genetic mechanisms.

It is relevant to ask how much could be achieved on a private farm by natural and artificial selection. Some indications can be derived from the original split of 1991-born 18-month-old ewes into R and S lines in autumn 1993, and the splitting of the 1992-born lambs into R and S lines at the same time. As shown in Figure 1, the real (i.e., lasting) difference in the 1991 ewes was 4.3% in % animals susceptible, and in the 1992 lambs the difference was 7.5%. It is important to remember that the screening of animals into high and low groups was done by us using not only their phenotypes but, in this case, their sire and dam data as well. Our progress from screening was thus relatively small compared with the use of progeny tested rams as sires in 1993 and subsequent years, as shown in Figure 1. Therefore, for purchasing a line of young ewes, ensuring that they have never had RGS is not good enough to guarantee minimal RGS in future, because of the low heritability of RGS. Likewise, for purchasing rams, ensuring that candidate rams have never experienced RGS is not good enough. Identifying resistant sires from a properly conducted progeny test is necessary, in order to have confidence that they are resistant to RGS. At present, even this insurance is only relative; some seasons of challenge will be enough to cause some progeny by 'Resistant' rams to show signs of RGS. As time progresses, we expect that fewer and fewer animals from the Ruakura Resistant line will however experience this fate.

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