

## Studies into the dynamics of perennial ryegrass (*Lolium perenne* L.) seed mixtures

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The dynamic interactions of four perennial ryegrass seed mixtures sold in Northern Ireland were studied under simulated grazing and conservation managements. Mixture composition was determined as changes in phosphoglucoisomerase isozyme frequencies by calculation from known isozyme frequencies of the component varieties. Mixture productivity was measured over 4 growing seasons and compared with yields predicted from those of the components in monoculture, weighted for their actual proportion in the mixture. No significant differences were found between actual yields for mixtures and their predicted yields, but when these differences were regressed against the heading date range among the varieties in each mixture, a significant relationship was observed. A wide range in heading date among the components of the mixtures was associated with increased yield stability over years and with a declining yield advantage for the mixture compared to its components grown as monocultures. In this aspect, the mixtures showed a more rapid decline under conservation management than under simulated grazing. Mixtures also had a flatter seasonal yield-production profile than their component varieties. Tetraploid components were more aggressive than diploids, though a more open-growing diploid maintained its proportion in the sward better than a dense-growing type and manipulating the sowing ratios could be used to influence final sward composition after 2 years. It was concluded that the differences in heading date range within mixtures had a significant impact on mixture dynamics, with the tetraploid component being the most aggressive.

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### Introduction

In the UK and Ireland, where perennial ryegrass (*Lolium perenne* L.) is the dominant grass used in reseeded pastures, most seed is sold as variety mixtures (Culleton and Cullen 1992; Gilliland, Johnston and Connolly 2007). Initially this practice was driven by the pragmatic logic that reproducing the botanical composition of the most productive natural pastures would deliver the highest herbage yield. The earliest science-backed mixture recommendations came from Robert H. Elliot of Clifton Park, who first published his work under the title of 'Agricultural Changes' in 1898. This was subsequently reprinted several times, with the fifth and final edition in 1943 (Elliot 1943). This promoted complex compilations of grasses, legumes and deep rooted herbs. Furthermore, prior to the formation of the International Seed Testing Association in 1924 (Steiner Kruse and Leist 2008; Gilliland 2010), which formalised reliable standardised tests for seed quality in support of national statutory controls, sowing a mixture of several seed lots provided a degree of insurance against total seed failure.

Today the primary driving factors in the continued predominance of seeds mixtures are largely commercial. Compiling seeds mixtures affords merchants better price control and helps balance low supply and high demand for the newest varieties. Mixtures also facilitate the sale of residual seed of varieties that are becoming out-classed. As they are regarded as being more flexible and suitable for a wider range of conditions, mixtures have a potentially greater market size than any single variety and this can create 'merchant brands' that attract customer loyalty. In harmony with this is farmers' perception of an often loosely defined 'added value' in mixtures, including a belief that mixtures provide greater yield and adaptability under

differing farm enterprises and environments (Ingram 1997) than do monocultures. In the past, some authors have reported that mixtures never yield less than the mean of the components in monoculture, and sometimes can exceed that, or even exceed the best component variety in the mixture (Simmonds 1962; England 1968). However, others (Donald 1963; Woodford 1966; McBratney 1978; Culleton, Murphy and O'Keeffe 1986) have reported that mixtures did not yield more than the highest yielding component grown in monoculture and that most mixtures yield at a level within the range of the monoculture components. Little evidence of 'under yielding' has been reported. Trenbath (1974) and Culleton *et al.* (1986) found that mixtures did not yield significantly less than the components in monoculture. Recently, grass and legume mixtures have been reported to exhibit transgressive over-yielding, whereby the mixtures yielded more than any of the individual components (Kirwin *et al.* 2007; Connolly *et al.* 2009; Nyfeler *et al.* 2009).

Research and development into the compilation and performance of grass-seed mixtures began as early as 1898 with the multi-species mixtures of Clifton Park and Cockle Park (Elliot 1943). These proved to be well adapted to dry conditions (Greenaway and Budden 1958, 1959), but for more moist soils and with breeding efforts in the 1950s focusing on the more palatable and higher yielding ryegrasses (*Lolium* spp.), mixture formulae became dominated by perennial ryegrass (*Lolium perenne*). These often also included white clover (*Trifolium repens*) and for colder or heavy soil areas may include Timothy (*Phleum pratense*). Despite their widespread use, peer reviewed studies into mixtures of perennial ryegrass varieties are surprisingly rare and very few are recent. There is extensive published research on

all aspects of perennial ryegrass/white clover mixtures (e.g., Anon 2009), but review books, intended as definitive resources on all aspects of grassland science, frequently omit the topic of grass-seed mixtures, for example those of Pearson and Ison (1997) and Barnes *et al.* (2007). Even when grass-seed mixtures are considered, the content has been limited to reporting current practice supplemented by some general observations on productivity of different compilations. The overview by Ingram (1997) and the study by McBratney (1978) are typical examples. This has been because most previous investigations of perennial ryegrass mixtures have not concerned the competitive ability of the components, but were restricted to comparing the overall performance of mixed swards, such as those by Humphreys and O'Kiely (2006, 2007). The problem is that there is no simple morphological method for identifying the component varieties once mixed. Camlin (1981) attempted to resolve this by complex and arduous taxonomic examination of excised tillers from mixed swards, but not until techniques based on isozyme frequencies were developed (Kennedy *et al.* 1985; Gilliland and Watson 1987) was it possible to accurately ascertain the composition of a perennial ryegrass mixture in the growing sward. Since then a number of useful studies have been conducted using this approach (Quaite and Camlin 1986; Gilliland 1995; Hazard and Ghesquière 1995), but these still comprise a relatively limited body of work.

### Materials and Methods

The current paper is based on new data on perennial ryegrass mixture dynamics derived from examination of mixtures sold in Northern Ireland. Some previously published data have also been reanalysed to elucidate aspects of the

aggressivity relationships between certain variety types.

#### *Estimating mixture composition using allozyme frequencies*

Starch gel electrophoresis, by the standardised method of Gilliland (1983), performed on individual leaves of perennial ryegrass was used to determine the genotype frequencies of the phosphoglucosomerase locus PGI/2. Leaves for electrophoresis examination were selected by point quadrat of the cut herbage, used on a first-hit basis, to reproduce the contribution of each component to the canopy, as performed by Kennedy *et al.* (1985). These proportions were used to calculate the 'predicted yield' for each mixture based on the yield of each component in monoculture multiplied by its proportion in the canopy. The number of leaves used to examine each mixture differed depending on the magnitude of the chi-squared differences in isozyme frequencies of the component varieties, and was calculated using the formula of Kennedy *et al.* (1985). Mixture composition was estimated using maximum likelihood analysis as described by Gilliland and Watson (1987) to relate the genotype frequencies of the sampled mixture to those of the components in monoculture.

In this study, the identity of any of the component varieties and commercial mixtures could not be declared due to a confidentiality agreement with commercial funders, including where modifications were made to the proprietary compilation to study ploidy effects. All component varieties were on the current DARD recommended list and so representative of the seed being used on farm (e.g., Gilliland 2009). The heading date, or mean date of ear emergence of perennial ryegrasses, has been defined by Green, Carroll and Terry (1971) as being when 50% of tillers

show ear emergence, and the dates used in the current study were those published on the DARD Recommended List.

#### *Mixture studies*

The results presented in this study are a compilation of two different mixture studies, one designed to examine the effects of variety maturity (Table 1) and one concerning the competitive interactions between diploid and tetraploid components. The comparison between varieties of different ploidy involved two experiments. One involved mixtures comprising 5%, 10% and 80% of a tetraploid variety sown with either a dense-growing diploid (density score of 7.1) or an open-growing, less-dense diploid variety (density score of 6.0). The other experiment involved a replacement series involving 10%, 30%, 50%, 70% and 90% of the tetraploid with the complement being an open-growing diploid variety. All experiments were conducted, on a medium loam soil (pH 6.0), at the Plant Testing Station, Crossnacreevy, Belfast, Northern Ireland (54°33'N, 5°52'W). Swards were established by broadcasting seed on plots (1.5 m × 6.5 m) and all experiments involved a randomised block design with 3 replicates. In order to account for the differences in seed size between diploid and tetraploid ryegrass, seed rates of 22 and

33 kg/ha were used for diploid and tetraploid varieties or mixtures, respectively, and a rate of 27.5 kg/ha for diploid/tetraploid mixtures. In all mixtures the diploid and tetraploid components were represented by both intermediate and late heading varieties, thus eliminating any maturity effect in comparisons between ploidies.

All plots were sown in July and evaluation commenced the following spring. Plots were harvested using a Haldrup plot harvester at a cutting height of 5 cm for simulated grazing management and at 8 cm for conservation management. A compound fertilizer was applied to deliver 360 and 370 kg N per hectare for simulated grazing and conservation managements, respectively, plus adequate P and K. The grazing and conservation managements were the standard UK National List procedures, as described by Weddell, Gilliland and McVittie (1997) and FERA (2011). All component varieties were fully recommended on the recommended list for Northern Ireland (e.g., Gilliland 2009).

#### *Examination of productivity*

This study involved a comparison between four commercial seed mixtures sold in Northern Ireland, which contained different combinations of maturity and ploidy (Table 1), and their components as

**Table 1. Classification of components in four commercial seeds mixtures**

Mixture code <sup>†</sup>	Maturity group contribution		Ploidy group contribution		No. of varieties	Heading date range	
	Dates	Days					
Commercial 1	Intermediate:	50%	Diploid:	52%	4	18 May to 8 June	21
	Late:	50%	Tetraploid	48%			
Commercial 2	Intermediate:	50%	Diploid:	63%	2	24 May to 6 June	13
	Late:	50%	Tetraploid	37%			
Commercial 3	Early:	50%	Diploid:	71%	3	12 May to 19 May	7
	Intermediate:	50%	Tetraploid	29%			
Commercial 4	Intermediate:	100%	Diploid:	61%	2	24 May to 27 May	3
			Tetraploid	39%			

<sup>†</sup> Mixture and variety names not provided due to confidentiality restriction.

monocultures. The four mixtures and their components, in monoculture plots, were managed under both the simulated grazing and conservation management systems over 4 full growing seasons following the single autumn sowing. The yields under simulated grazing were available as total annual yield and also as yield in each of four seasonal periods. The final harvest dates for these seasonal periods were: 30 April (spring), 30 June (early summer), 31 August (late summer) and 31 October (autumn), as used on the DARD recommended list (Gilliland 2009). Similarly, for the conservation management the yields were analysed as total annual yield and as the total yield from three silage cuts (3-cut yield), as in the management protocol described by Weddell *et al.* (1997).

#### *Examination of ploidy and density effects on composition*

This study involved two parallel experiments, in which mixture reconstructions similar to Commercial 1 (Table 1) were compiled to examine the effects of higher and lower density of plants of the diploid component in the sward, and of changing the sown proportions of the components, on canopy composition. The first experiment involved a comparison between a binary mixture of a tetraploid variety and either an open-growing or a dense-growing diploid variety, all with the same heading date. Density was defined as percentage ground cover of the sown species, converted to a 10-point scale (0 to 9) as described by Weddell *et al.* (1997) and FERA (2011). In this classification a value of 7.0 represents a very dense, usually prostrate, growth habit and 6.0 is an open erect growth habit (tetraploids are all open and erect growing and 5.0 is a typical density score for varieties in commerce; Gilliland 2009). Three different proportions of the tetraploid to each

diploid were constructed on the basis of seed weight, giving six different mixtures in a randomised block trial under simulated grazing management. The composition of the swards, at each of the four seasonal periods (as defined above), was determined during the second full growing season following sowing in July. Composition of these swards was determined on the basis of plants present, and not the canopy, in order to better examine effects of density on plant competition. This was achieved by sampling tillers, using a point quadrat to determine the nearest tiller to the point quadrat position on the ground, and analysing a leaf of each selected tiller by the isozyme electrophoresis method. By sampling in the second year it was hoped that all the mixtures would have reached a stable end point. The second experiment involved establishing a four-step replacement series between the ratios 90:10 and 10:90 of the open diploid to the tetraploid in the mixture. The experiment involved a randomised block design, managed under the simulated grazing regime. The final canopy proportions were sampled by point quadrat at the September harvest of the second full growing season (28 months after a July sowing).

Statistical analysis involved one-way ANOVA of differences between mixtures within grazing and conservation managements, and for differences among mixtures across seasonal periods (Genstat 8; VSN International Ltd., Hemel Hempstead, UK).

### **Results**

The average yields of dry matter from 4 harvest years for the 4 commercial mixtures are given in Table 2. Under the simulated grazing management, there were significant differences between the commercial mixtures for both total annual and

**Table 2. Four-year mean dry matter yields of commercial mixtures and corresponding relative yields under simulated-grazing and conservation management systems**

Mixture code <sup>†</sup>	Annual yield		Spring yield <sup>‡</sup>	
	Actual (t/ha)	Relative <sup>§</sup>	Actual (t/ha)	Relative <sup>§</sup>
<i>Simulated-grazing management</i>				
Commercial 1	14.65	99	2.69	97
Commercial 2	14.13	101	2.72	103
Commercial 3	14.41	99	3.02	98
Commercial 4	14.27	100	2.78	100
<i>Mean</i>	14.37	99.5	2.80	99.5
<i>s.e.</i>	0.157		0.111	
<i>Significance</i>	*		*	
<i>Conservation management</i>				
Commercial 1	17.70	101	14.08	101
Commercial 2	18.41	103	14.67	103
Commercial 3	19.02	101	15.33	101
Commercial 4	17.75	95	14.06	94
<i>Mean</i>	18.83	100	14.54	99.5
<i>s.e.</i>	0.314		0.372	
<i>Significance</i>	***		*	

<sup>†</sup> Mixture names not provided due to confidentiality restriction.

<sup>‡</sup> Includes all growth to 30 April.

<sup>§</sup> Relative (%) to predicted yield based on component yields in monoculture plots.

spring yield. Surprisingly, Commercial 3 was only significantly better than one of the other three mixtures, despite being the only one with early maturing components. If predicted yield was calculated on the basis of proportions of each variety at sowing, there was an apparent small gain ( $P < 0.05$ ) for the mixtures in total annual yield. However, when the actual composition, based on isozyme frequencies, was used to calculate the predicted yield there were no significant differences. This apparent discrepancy was due to changes in the component variety proportions since sowing and confirms the inaccuracy associated with using sowing proportion rather than actual proportion to determine predicted yield.

A similar result was found for the results under conservation management (Table 2). Here again there were significant yield differences between the mixtures in both yield categories. However, while there

was an apparent yield advantage for the mixtures when the yields of the monocultures were combined on a sown proportion basis, predicted yields based on the actual proportions showed no significant differences between any mixture and its predicted yield.

Although the differences between the actual and predicted yields for the individual mixtures were not significant, when these differences were regressed, using a second order polynomial equation, on heading date range a clear and significant relationship was observed (Figure 1); there was a very high correlation ( $R^2 > 90\%$ ) for both total and spring yields. Although this curvilinear relationship was only based on 4 data points, it indicates that in this experiment there was possibly a yield gain associated with a heading date span of up to 15 days, beyond which a deficit occurred. Performing this analysis for the conservation management showed

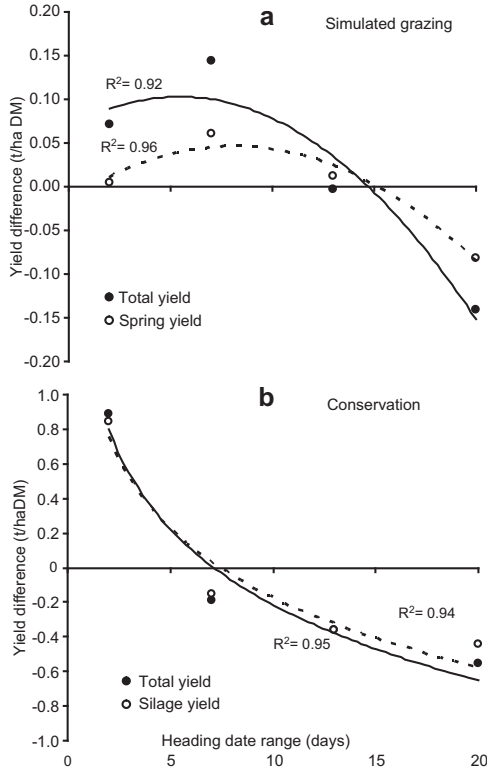


Figure 1. The difference, in dry matter yield, between mixtures and that predicted from performance of their components in mono-culture as a function of the range in heading date among the components under: a) simulated grazing, and b) conservation management. Lines are from a 2<sup>nd</sup> degree polynomial regression analysis.

a similarly strong and significant relationship with heading date range. In this case, however, differences in yield between that observed and that predicted, based on the components, showed a much more rapid decline as the range in heading date increased. The break-even point appeared to fall between 5 and 10 days for both total yield and the 3-cut silage yield.

When the annual variation in yield under simulated grazing was examined (Table 3), there were significant

differences between mixtures within years. There was, however, a relatively consistent ranking of the mixtures in each of the 4 years; Commercial 1 was generally highest and Commercial 2 lowest for total annual yield and Commercial 3 was highest for spring yield. It was not unexpected, therefore, that there was no correlation between heading date range and the variability of each mixture (calculated as the yield range across the 4 years as a

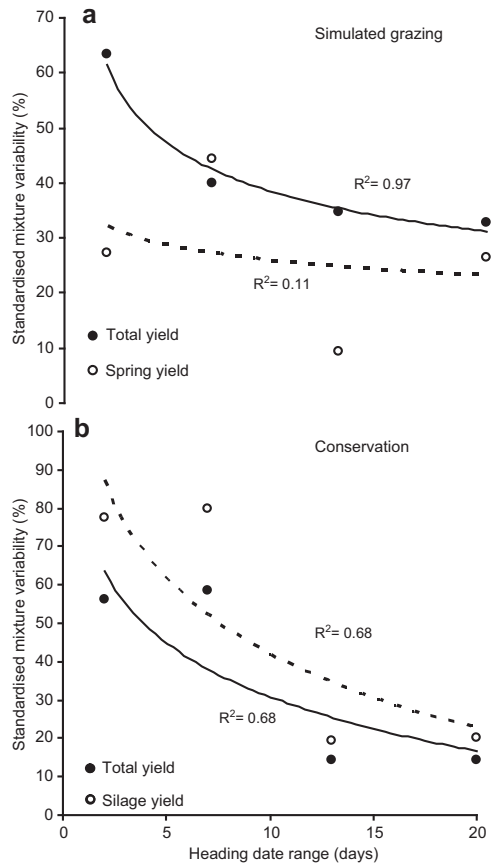


Figure 2. Relationship between the heading date range of commercial mixtures and the annual variation in yield, after adjusting for overall year effects, under: a) simulated grazing and b) conservation management. Lines are from a logarithmic regression analysis.

**Table 3. Variability in dry matter yield (t/ha) of mixtures, over 4 years, under simulated grazing management**

Mixture code <sup>†</sup>	Harvest year				Variability <sup>‡</sup> (% of Mean)
	First	Second	Third	Fourth	
	<i>Total annual yield</i>				
Commercial 1	15.00	15.15	14.35	14.11	7.10
Commercial 2	14.60	14.86	13.57	13.47	9.84
Commercial 3	14.82	15.24	13.60	13.98	11.38
Commercial 4	14.03	15.13	13.88	14.04	8.76
<i>Mean</i>	14.61	15.10	13.85	13.90	9.27
<i>s.e.</i>	0.218	0.182	0.237	0.354	
<i>Significance</i>	*	***	**	***	
	<i>Spring yield<sup>§</sup></i>				
Commercial 1	2.61	3.05	3.40	2.94	26.3
Commercial 2	2.51	2.85	3.14	2.90	21.9
Commercial 3	2.89	3.28	3.33	2.91	14.2
Commercial 4	2.50	2.88	3.38	2.79	30.6
<i>Mean</i>	2.63	3.02	3.31	2.93	23.3
<i>s.e.</i>	0.082	0.074	0.047	0.075	
<i>Significance</i>	*	***	**	***	

<sup>†</sup> Mixture names not provided due to confidentiality restriction.

<sup>‡</sup> Range in yield across years as a percentage of mean yield.

<sup>§</sup> Includes all growth to 30 April.

percentage of the average yield). The  $R^2$  values were only 0.00 and 0.17 for total and spring yields, respectively, and the associated regression coefficients were not significant. However, the year-to-year variation due to climatic effects needs to be removed to obtain a reliable evaluation of inherent variability. This was achieved by adjusting the mean yield each year to match the 4-year mean yield across all 4 mixtures. The same adjustment was then made to each mixture in each year. The variability for each mixture was then the range in yield of the mixture across the 4 years, corrected for annual growth effects and expressed as a percentage of its 4-year mean yield. This removal of the climatic variation revealed a significant relationship between the yield variation of a mixture and the range of heading date among the components in the mixture. Figure 2 shows a curvilinear relationship and, although only based on 4 data points (which gave a wide confidence

interval on the correlation), around 97% of the variation in total simulated-grazing yield was associated with heading date range. This relationship indicated that a greater heading date range was possibly associated with a lower annual variation in mixture yield. The greatest response occurred below 10 days, with a much lower response thereafter. Variation in spring yield was not associated with the heading date range of the mixtures.

When the annual yield variability of the mixtures was examined under conservation management, similar responses were found (Table 4). In this case Commercial 3 tended to be highest ranked for both total annual yield and 3-cut yield with Commercial 1 and 4 being lowest for total annual yield and 3-cut yield, respectively. The relationship between mixture variability for the total annual conservation yield and heading range was stronger than observed in the simulated grazing management ( $R^2$  0.26) but was not statistically



**Table 4. Variability in dry matter yield (t/ha) of mixtures, over 4 years, under conservation management**

Mixture code <sup>†</sup>	Harvest Year				Variability <sup>‡</sup> (% of Mean)
	First	Second	Third	Fourth	
	<i>Total annual yield</i>				
Commercial 1	19.48	17.45	16.61	17.24	12.66
Commercial 2	19.97	18.51	17.81	17.35	14.23
Commercial 3	19.54	19.93	18.45	18.15	9.36
Commercial 4	19.29	17.97	17.81	15.91	19.05
<i>Mean</i>	19.57	18.47	17.67	17.16	13.83
<i>s.e.</i>	0.517	0.456	0.417	0.404	
<i>Significance</i>	*	***	**	***	
	<i>Three-cut silage yield</i>				
Commercial 1	15.51	15.49	14.15	13.36	14.69
Commercial 2	15.82	15.24	14.13	13.50	15.75
Commercial 3	15.59	16.63	14.60	14.48	13.98
Commercial 4	15.33	14.69	13.93	12.29	21.59
<i>Mean</i>	15.56	15.51	14.20	13.41	16.50
<i>s.e.</i>	0.344	0.350	0.245	0.194	
<i>Significance</i>		***	*	***	

<sup>†</sup> Mixture names not provided due to confidentiality restriction.

<sup>‡</sup> Range in yield across years as a percentage of mean yield.

<sup>§</sup> Includes all growth to 30 April.

significant. When variation in 3-cut yield was regressed against the heading date range, however, the  $R^2$  value was 0.62. This indicated greater stability of silage yield as heading date range increased, as shown in Figure 2, after adjusting for overall year effects. For both total and 3-cut yields, around 68% of the variation could be associated with an increasing heading date range in the mixtures and the response showed less of a diminishing return as heading range increased.

A further aspect of mixture variability is summarised in Table 5 in which the differences, based on mean values over 4 years, between the mixture and its components for each of the seasonal growth periods were compared, under simulated grazing management. The swards from mixtures had a flatter seasonal profile than the components in monocultures as each mixture was always intermediate to the yields of its highest and lowest producing component variety in monoculture. There was

no evidence for synergistic effects between components in these mixtures, as mixture productivity was not raised above that of the seasonal yields of the best individual component.

The effects on sward composition of including an open- or dense-growing diploid in mixture with a tetraploid showed that by the second full growing season the tiller composition of both mixtures was relatively stable, with only small differences occurring between seasonal periods (Table 6). It was also clear that the tetraploid remained the dominant component and that the sowing density (2, 4 or 6 kg/ha) had little or no influence on sward composition after 2 full growing seasons. There was, however, a significant difference between the open and dense diploids as there was around 15% more of the open-growing diploid in the mixtures than the dense type.

The replacement sowing series, involving an erect-growing diploid with an open sward structure and a tetraploid, showed

**Table 5. Mean seasonal dry matter yield (t/ha) of mixtures, over 4 years, and differences from their components under simulated grazing management**

Mixture code <sup>†</sup>	Seasonal period			
	Spring (30 Apr) <sup>‡</sup>	Early summer (30 Jun)	Late summer (31 Aug)	Autumn (31 Oct)
Commercial 1				
Mean yield	3.00	5.91	3.99	1.76
Difference - minimum <sup>§</sup>	0.22	0.23	0.23	0.10
- maximum <sup>§</sup>	-0.27	-0.34	-0.25	-0.14
Commercial 2				
Mean yield	2.85	5.85	3.77	1.65
Difference - minimum	0.32	0.37	0.79	0.21
- maximum	-0.09	-0.34	-0.11	-0.04
Commercial 3				
Mean yield	3.10	5.70	3.91	1.70
Difference - minimum	0.19	0.01	0.13	0.06
- maximum	-0.08	-0.11	-0.14	-0.06
Commercial 4				
Mean yield	2.89	5.78	3.91	1.70
Difference - minimum	0.07	0.11	0.16	0.00
- maximum	-0.01	-0.12	-0.21	-0.04
<i>s.e.</i>	0.070	0.073	0.075	0.052
<i>Significance</i>	*	*	*	

<sup>†</sup> Mixture and variety names not provided due to confidentiality restriction.

<sup>‡</sup> Date of final cut in each seasonal period.

<sup>§</sup> Maximum and minimum refer to the differences between the mixture yield and the yield, in monoculture, of the highest and lowest yielding component.

realignment towards a more equal contribution of the two components after 2 years. When the tetraploids accounted for 10% and 30% at sowing the sward

composition moved to 32.2% and 56.8% tetraploid, respectively, after 2 years. When the mixture was sown with 70% and 90% tetraploid the sward was 66.0% and

**Table 6. Proportion<sup>†</sup> of tetraploid in mixtures involving an open-growing or a dense-growing diploid during second full growing season**

Seeding rate (kg/ha) of		Seasonal period <sup>‡</sup>				<i>Mean</i>
Diploid	Tetraploid	Spring	Early summer	Late summer	Autumn	
<i>Open growing diploid</i>						
2	33	0.75	0.77	0.62	0.66	0.70
4	31	0.68	0.59	0.57	0.66	0.62
6	29	0.66	0.79	0.74	0.55	0.68
<i>Mean</i>		0.70	0.72	0.64	0.62	0.67
<i>Dense growing diploid</i>						
2	33	0.75	0.96	0.74	0.79	0.81
4	31	0.83	0.77	0.89	0.77	0.81
6	29	0.78	0.91	0.76	0.74	0.80
<i>Mean</i>		0.79	0.88	0.80	0.77	0.81
<i>s.e.</i>						0.062
<i>Significance</i>						***

<sup>†</sup> Based on tiller counts.

<sup>‡</sup> See footnote, Table 5.

74.6% tetraploid, respectively, and when the components were sown in equal proportions the composition moved to a 65% tetraploid sward at the 2-year stage. These realignments between the components did not therefore result in similar composition for all mixtures as the effect of the different diploid to tetraploid proportions at sowing was still evident after 2 years.

### Discussion

It is a widely held belief in farming circles that mixtures express a synergism. This is not contrary to scientific principles, as it has long been accepted that complex ecosystems can be more efficient in capturing and utilizing available environmental resources than less complex ones (Harper 1967). More recently Kirwan *et al.* (2007) and Connolly *et al.* (2009) have reported increased biomass productivity with increased number of pasture species. The results from that work, which included the important temperate grasses and legumes in Europe, showed mixture yields in excess of that by any of the components grown in monoculture. Similar to Culleton *et al.* (1986), the current study did not reveal this degree of yield enhancement. The current study showed that it is incorrect to calculate an expected mixture yield as a weighted average based on the sowing proportions and the monoculture yields for the components. This was clearly due to the fact that the composition of swards based on ryegrass mixtures is not fixed at sowing. Clearly, composition changes must be accounted for when calculating expected yield, as performed using isozyme frequency changes in the current study. This conclusion is confirmed by results from several other studies (Gilliland 1995; Hazard and Ghesquière 1995; Quaite and Camlin 1986; Rhodes 1970), which showed similar composition changes and

yield responses. The use of isozyme frequencies to determine the actual composition of components in the sward brought the expected and actual yield back into close agreement. Moreover, the mixtures were not even higher yielding than the expected mean of the components in monoculture. It is possible that as the mixtures in the current study were combinations of varieties from the same species they may not involve the degree of complexity present in previous studies. It is notable that Nyfeler *et al.* (2009) observed that the degree of diversity needed to be sufficiently high before the performance of a mixture exceeded that of the best component when grown in monocultures. Furthermore, Rhodes (1970) found over-yielding to occur in ryegrass mixtures, but only under conservation management, not under simulated grazing, and in only 2 of 11 mixtures studied. Moreover, it did not occur in the more complex mixtures with 3 or 4 components, but only in binary mixtures. McBratney (1978) also found no synergistic responses in a range of perennial ryegrass mixtures with different combinations of maturity, compared to their monoculture yields. These studies would tend to indicate that increasing the number of ryegrass varieties in a mixture may not introduce the degree of complexity necessary to create the over-yielding recorded elsewhere.

The one characteristic of the ryegrasses that was associated with differential mixture performance was the heading date range in the mixture. It was interesting that mixture composition changes were converse under the two different managements. This was likely to have been driven by the key role of the first 2 silage cuts in the conservation management system. These harvests are highly influenced by the interaction between heading date and timing of cut, whereby the earlier heading

component is most aggressive at the first cut and weakest at the second cut, with a decline in overall aggressivity at the third cut (Gilliland 1995). This study showed that the greater the range in heading date the greater the competitive interactions between the mixture components. Competitive hierarchies that increase with greater heading date range could also explain the tendency for mixture yields to fall below the predicted yield when the range in heading date of the conservation-managed mixture was greater than around 7 days. The relationship between simulated grazing yield differences and heading date range showed a somewhat different tendency by remaining or rising slightly until the range in heading date was around 15 days. This different response pattern can be interpreted as a consequence of the management imposed. The simulated grazing management does not involve the accumulation of large yields in the May-June period, when reproductive growth is predominant, in contrast with the conservation management system. Therefore, the more frequent defoliation interrupts competitive interactions and hence the overall level of competitive pressure is reduced.

The heading date range among the components was also found to be positively associated with yield stability across years, and the mixtures were also less reactive to growing conditions at the different seasonal growth periods. Greater annual stability would be advantageous for grassland management and may also indicate a wider climatic tolerance, which supports the popular belief that ryegrass mixtures have a wider geographical range for peak performance than monocultures (Ingram 1997). The lower response of mixtures to seasonal growing conditions compared to the best monoculture component is clearly advantageous. Dillon *et al.* (2002) have

demonstrated clear advantages from a higher proportion of grazed grass in the diet of cows in spring, making increased productivity in spring potentially the most valuable of the year. The results from the current study indicate that any perennial ryegrass mixture will have a diluting effect, failing to achieve the biological potential of the component with the highest productivity in spring.

The competitive interactions in the two diploid/tetraploid sowing rate studies were generally in good agreement. In both studies, the tetraploid component was the more aggressive, both when plants were sampled on the basis of tiller presence and on canopy composition. As Charles (1961) showed that competition between grass plants can result in as little as 20% seedling survival 2 months after sowing and as few as 10% after 12 months, it is likely that competition for space was entirely completed after 2 years. So these mostly tetraploid-dominant mixture compositions should be stable at the end of the second full season, at least in terms of plant-to-plant competition. The competitive advantage for the tetraploid was not maturity influenced as the components all had the same heading date, but could be related to canopy structure. For example, tetraploid perennial ryegrasses have longer leaves and it is notable that Hazzard and Ghesquière (1995) found that this gave a competitive advantage in ryegrass mixtures cut at a frequency similar to that used in the current study. This would also explain the competitive advantage of the more open-growing diploid compared to the dense growing one, because the open-growing diploid was an erect growing type with longer leaves.

The overall conclusion from these mixture studies is that while component interactions can be complex and not easily observed, the use of isozyme frequencies to determine the actual composition at the time of examination is a valuable tool which

assists in exposing the influence of specific parameters on the competitive interactions. As the current study only involved a small number of mixtures, it is possible that the results may express some degree of specificity to these compilations, and this should not be ignored. Nonetheless, the productivity responses and hierarchies identified are likely to operate in other ryegrass mixtures, albeit at different magnitudes, and so this study provides a basis for further and more detailed experimentation.

### References

- Anon. 2009. Forage legumes in temperate pasture-based systems. *Irish Journal of Agricultural and Food Research* **48**: 115–281.
- Barnes, R.F., Nelson, C.J., Moore, K.J. and Collins, M. 2007. “Forages: The Science of Grassland Agriculture”, 6th Edition, Volume II, Wiley-Blackwell, 791 pages.
- Camlin, M.S. 1981. Competitive ability of cultivars of perennial ryegrass (*Lolium perenne* L.). In: “Plant Physiology and Herbage Production” (ed. C.E. Wright). *Occasional Symposium No. 13, British Grassland Society*, pages. 137–142.
- Charles, A.H. 1961. Differential survival of cultivars of *Lolium*, *Dactylis* and *Phleum*. *Journal of the British Grassland Society* **16**: 69–75.
- Connolly, J., Finn, J.A., Black, A.D., Kirwan, L., Brophy, C. and Lüscher, A. 2009. Effects of multi-species swards on dry matter production and the incidence of unsown species at three Irish sites. *Irish Journal of Agricultural and Food Research* **48**: 243–260.
- Culleton, N, Murphy, W.E. and O’Keeffe, W.F. 1986. The role of mixtures and seeding rates in ryegrass productivity. *Irish Journal of Agricultural Research* **25**: 229–306.
- Culleton, N. and Cullen, T. 1992. Trends in herbage seed use in Ireland. *Plant Varieties and Seeds* **5**: 63–69.
- Dillon, P., Crosse, S., O’Brien, B. and Mayes, R.W. 2002. The effect of forage type and level of concentrate supplementation on the performance of spring-calving dairy cows in early lactation. *Grass and Forage Science* **57**: 212–223.
- Donald, C.M. 1963. Competition among crop and pasture plants. *Advances in Agronomy* **15**: 1–18.
- Elliot, R.H. 1943. “The Clifton Park System of Farming and Laying Down Land to Grass”. 5th Edition, Faber and Faber Limited, London, 261 pages.
- England, F. 1968. Competition in mixtures of herbage grasses. *Journal of Applied Ecology* **5**: 227–242.
- FERA (The Food and Environment Research Agency). 2011. The Food and Environment Research Agency web site – VCU Procedures for Grasses (Perennial, Italian and Hybrid Ryegrass, Timothy and Festulolium) Available from: [www.fera.defra.gov.uk/plants/plantVarieties/national-Listing/documents/protocolGrasses.pdf](http://www.fera.defra.gov.uk/plants/plantVarieties/national-Listing/documents/protocolGrasses.pdf) [Accessed 19 November 2011].
- Gilliland, T.J. 1983. Identification of ryegrass cultivars by classification of PGI allozymes/genotypes. *Proceedings of an International Seed Testing Association Symposium on biochemical tests for Cultivar Identification*, National Institute of Agricultural Botany, Cambridge, United Kingdom, pages 85–95.
- Gilliland, T.J. and Watson, S. 1987. Study of complex *Lolium* cultivar seed mixtures by maximum likelihood analysis of isozyme frequencies. *Proceedings of the 2nd International Seed Testing Association Symposium on Biochemical Identification of Varieties, Leningrad, Russia*, pages 1–8.
- Gilliland, T.J., Johnston, J. and Connolly, C.P. 2007. A review of forage grass and clover seed use in Northern Ireland. *Grass and Forage Science* **62**: 1–8.
- Gilliland, T.J. 1995. Effect of harvest date and cultivar maturity on the composition of perennial ryegrass (*Lolium perenne* L.) mixtures. *Irish Journal of Agricultural and Food Research* **34**: 143–150.
- Gilliland, T.J. 2009. “Recommended Grass and Clover Varieties for Northern Ireland 2009/10”, Department of Agriculture and Rural Development, Northern Ireland, ISBN 978–1–84807–120–9, pages 33.
- Gilliland, T.J. 2010. Control of cultivar release and distribution. In: “Handbook of Plant Breeding”, Volume **5**: Fodder Crops and Amenity Grasses. (eds. B. Boller, U.K. Posselt and F. Veronesi). Springer Science & Business Media B.V., XI, ISBN: 978–1–4419–0759–2, pages 523.
- Green, J.O., Corral, A.J. and Terry, R.A. 1971. Grass species and varieties: relationships between the stage of growth, yield and forage quality. The Grassland Research Institute Technical Report, No. 8. Hurley, United Kingdom.
- Greenaway, T.E. and Budden, M. 1958. A discussion on the influence of relative moisture availability on the swards resulting from standard grass seeds mixtures, I. Mixture of the cockle park type. *Grass and Forage Science* **13**: 222–228.
- Greenaway, T.E. and Budden, M. 1959. A discussion on the influence of relative moisture availability on the swards resulting from standard grass seeds

- mixtures, 2. Swards produced from a cocksfoot/timothy/meadow-fescue mixture. *Grass and Forage Science* **14**: 117–123.
- Harper, J.L. 1967. A Darwinian approach to plant ecology. *Journal of Ecology* **55**: 247–250.
- Hazard, L. and Ghesquière, M. 1995. Evidence from the use of isozyme markers of competition in swards between short-leaved and long-leaved perennial ryegrass. *Grass and Forage Science* **50**: 241–248.
- Humphreys, J. and O’Kiely, P. 2006. Amount and quality of grass harvested for first-cut silage for differing spring-grazing frequencies of two mixtures of perennial ryegrass cultivars with contrasting heading date. *Grass and Forage Science* **61**: 77–88.
- Humphreys, J. and O’Kiely, P. 2007. Effects of two mixtures of perennial ryegrass cultivars with contrasting heading dates, and differing in spring-grazing frequency and silage harvest date, on characteristics of silage from first-cut swards. *Grass and Forage Science* **62**: 389–404.
- Ingram, J. 1997. Some theoretical aspects of mixtures. In: “Seeds of Progress” (ed. J.R. Weddell), British Grassland Society Occasional Symposium No. 31, 1997, ISBN 0905944496, p: 176–188.
- Kennedy, S.J., Gardiner, S.J., Gilliland, T.J. and Camlin, M.S. 1985. The use of electrophoretic techniques to distinguish perennial ryegrass cultivars when sown in mixtures. *Journal of Agricultural Science* **104**: 1–9.
- Kirwan, L., Lüscher, A., Sebastia, M.T., Finn, J.A., Collins, R.P., Porqueddu, C., Helgadottir, A., Baadshaug, O.H., Brophy, C., Coran, C., Dalmannsdottir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, Golinski, B.E., Grieu, P., Gustavsson, A.M., Hoglind, M., Huguenin-Elie, O., Iliadis, C., Jorgensen, M., Kadziulienė, Z., Karyotis, T., Lunnan, T., Malengier, M., Maltoni, S., Meyer, V., Nyfeler, D., Nykanen-Kurki, P., Parente, J., Smit, H.J., Thumm, U. and Connolly, J. 2007. Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology* **95**: 530–539.
- Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Connolly, J. and Lüscher, A. 2009. Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology* **46**: 683–691.
- McBratney, J.M. 1978. Production of perennial ryegrass cultivars of different maturity types when grown as pure swards and in certain combinations. *Grass and Forage Science* **33**: 283–288.
- Pearson, C.J. and Ison, R.L. 1997. *Agronomy of Grassland Systems*. 2nd Edition, Cambridge University Press, Cambridge, United Kingdom, ISBN 1–57808–005–3, 222 pages.
- Quaite, E. and Camlin, M.S. 1986. The use of electrophoretically labelled populations for intra-specific competition studies in perennial ryegrass (*Lolium perenne* L.) swards. *Journal of Agricultural Science* **106**: 99–106.
- Rhodes, I. 1970. The production of contrasting genotypes of perennial ryegrass (*Lolium perenne* L.) in monocultures and mixed cultures of varying complexity. *Journal of the British Grassland Society* **25**: 285–288.
- Simmonds, N.W. 1962. Variability in crop plants, its use and conservation. *Biological Review of the Cambridge Philosophical Society* **37**: 442–465.
- Steiner, M.A., Kruse, M. and Leist, N. 2008. ISTA method validation 2007: a historical retrospect. *Seed Testing International* **136**: 30–33.
- Trenbath, B.R. 1974. Biomass productivity of mixtures. *Advances in Agronomy* **26**: 177–210.
- Weddell, J.R., Gilliland, T.J. and McVittie, J. 1997. Evaluation procedures: Past, present and future. In: “Seeds of Progress” (ed. Weddell, J.R.), British Grassland Society Occasional Symposium No. 31, ISBN 0905944496, pages 202–225.
- Woodford, E.K. 1966. The need for a fresh approach to the place and purpose of ley. *Journal of the British Grassland Society* **21**: 109–115.