CROPS AND SOILS RESEARCH PAPER Assessment of herbage yield and quality traits of perennial ryegrasses from a national variety evaluation scheme

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SUMMARY

Perennial ryegrass evaluation schemes categorize varieties into three maturity (early, intermediate and late) and two ploidy (diploid and tetraploid) groups, and compare the relative yield, persistence and nutritive quality of varieties within these groups. The present study compared these groups for herbage yield, dry matter (DM) concentration and, using near infrared reflectance spectroscopy (NIRS), four quality characteristics (in vitro content of digestible dry matter (CDDM), water-soluble carbohydrate (WSC) and crude protein (CP) concentrations, and buffering capacity). A total of 1208 plots were sown across 5 years in Irish Recommended List trials and then harvested 6-7 times in each of 2 harvest years. This also allowed an assessment of the effect of sward age. Maturity group had no significant effect on annual herbage yield, quality or DM concentration except for in vitro CDDM (P < 0.01) but differed significantly for in vitro CDDM (P < 0.01), WSC concentration (P < 0.01) and buffering capacity (P < 0.05) at the first silage harvest. Tetraploid swards had greater annual herbage yields (P < 0.001), in vitro CDDM values (P < 0.001) and WSC concentrations (P < 0.01), but lower CP and DM concentrations (P < 0.001) than diploids. Swards in their first full year produced an additional 5.17 t/ha DM (P < 0.001) and had a higher (P < 0.01) WSC concentration at the second silage harvest than in their second year, but did not differ significantly for in vitro CDDM and WSC, CP or DM concentrations. The present study showed that differences exist in yield, nutritive quality and ensilability indices between maturity and ploidy groups. These observations justify their assessment in variety comparative trials and facilitates particular groups being selected for individual farming systems to increase efficiency.

INTRODUCTION

Perennial ryegrass (*Lolium perenne* L.) is suited to many intensive ruminant production systems in temperate climates due to its high yield potential, persistency under grazing (Tallowin *et al.* 1995) and high digestibility (Wilkins 1997). These traits contribute to its potential to reduce feed costs (Finneran *et al.* 2011) and its majority representation in reseed mixtures (Rath & Peel 2005).

National perennial ryegrass variety evaluation schemes assess the relative agronomic performance of a large number of varieties, and only a select few that represent the most elite performing varieties are eventually recommended to farmers. Recommendations are primarily based on seasonal and annual herbage yield, persistence and, to a lesser extent, nutritive quality (Grogan & Gilliland 2011). Within the Irish Recommended List, perennial ryegrass varieties are categorized into three maturity groups based on mean heading date (DAFM 2012), and these groups differ in their suitability for meeting specific

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farming requirements (Frame 1991; Gilliland *et al.* 1995; Laidlaw 2004). Varieties are also categorized as either diploid or tetraploid and each of these types also has its own niche role in farming enterprises (Gilliland *et al.* 2002).

The agronomic quality of perennial ryegrass is difficult to define as swards may have several functions (e.g. grazing, conservation or both) and ultimately forage quality is expressed through animal productivity rather than simply indices measured in the laboratory (Casler 2001; Wilkins & Humphreys 2003; Humphreys 2005). These self-regenerating swards are harvested several times a year (Camlin 1997), and a loss in productivity or nutritive quality at one harvest may be compensated for at a later harvest (Gilliland 1997). Consequently, grass quality needs to be assessed throughout the growing season if a balanced overall judgement is to be made on a variety.

A major role of grass is to support animal production and its nutritive value is fundamental to fulfilling this requirement. *In vitro* content of digestible dry matter (CDDM) has been cited as the single best indicator of nutritional value (Casler 2001; Wilkins & Humphreys 2003), since it influences both grass intake and energy availability (Wilkins & Humphreys 2003). Thus, for example, Casler & Vogel (1999) reported an average 3·2% increase in live weight gain by beef cattle per 1% increase in grass digestibility.

Crude protein (CP) in grass is a major source of nitrogen for protein synthesis by microbes in the rumen. In grazed swards, perennial ryegrass CP concentration is relatively high among forages and is invariably above the threshold of 80 g CP/kg DM required for rumen function (Coleman & Moore 2003).

Water-soluble carbohydrate (WSC) concentration has attracted the attention of grass breeders and a sufficient increase in its concentration can increase intake and improve animal productivity (Humphreys 1989). Miller *et al.* (2001) reported an additional 2·7 kg daily milk yield when a perennial ryegrass variety with an additional 39 g WSC/kg DM rather than a control variety was supplied to late lactation heifers.

Conservation of ryegrass (e.g. as silage) provides the opportunity to fill seasonal gaps in feed availability (O'Kiely & Kaiser 2005). However, despite the widespread use of silage making on farms, insufficient attention is paid to the ensilability properties of grass varieties in national variety evaluation schemes (Conaghan *et al.* 2008*a*). Both WSC, the primary source of fermentable substrate during ensilage, and buffering capacity, which indicates the amount of fermentation acids and thus of fermentable substrate required, combine to provide such information (Buxton & O'Kiely 2003).

An understanding of the relationships between maturity and ploidy groupings for herbage yield and quality traits of perennial ryegrasses from a national variety evaluation scheme would facilitate the selection of varieties particularly suited to specific farm enterprise requirements.

Objectives

The primary objective of the present study was to assess the differences in four quality characteristics (*in vitro* CDDM, WSC, CP and buffering capacity) and herbage yield for maturity, ploidy and age of sward groups of perennial ryegrass varieties undergoing assessment in a national variety evaluation scheme.

MATERIALS AND METHODS

Field plots

Field trials were carried out at the Grass and Clover Variety Evaluation Centre at Backweston, Co. Kildare, Ireland ($53^{\circ}26'N$, $06^{\circ}30'W$, 50 m asl). The soil type is a clay loam with a pH of 7·2 and 63 g/kg organic matter (Grogan & Gilliland 2011). The trials were carried out for the Grass and Clover Recommended List Varieties for Ireland (DAFM 2012) under an established protocol (Grogan & Gilliland 2011).

Plot management

Perennial ryegrass varieties were arranged into three separate maturity groups (early, intermediate and late) based on mean date of ear emergence. Both intermediate and late groups were sown in separate trials each year while the early group was sown in alternate years. In late summer, plots $(7.0 \times 1.5 \text{ m})$ were sown as monocultures of perennial ryegrass varieties in the year prior to first harvest at a sowing rate of 31.0 and 41.0 kg/ha for diploids and tetraploids, respectively. Within each maturity group, plots were sown in a randomized complete block design with four replicate blocks. Inorganic nitrogen fertilizer was applied at an annual rate of 350 kg N/ha in all harvest years except for 2006, when 400 kg N/ha was applied. An initial 40 kg N/ha was applied in February and with 100–110, 90-110, 50-60, 35-45 and 25-35 kg N/ha applied after the first five harvests, sequentially.

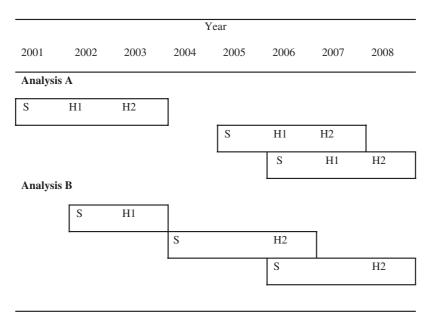


Fig. 1. Schematic outline of sowing and harvest year schedule used for analyses A and B. S, year of sowing; H1, year of first full season after sowing; H2, year of second full season after sowing.

The current study comprised 5 years of sowings (2001, 2002, 2004, 2005 and 2006) and each new set of plots was harvested in the two subsequent years, except for the sowings in 2002 and 2004 when only one subsequent harvest year was available for analysis (see Fig. 1). A total of 1208 plots were sown across the five sowing years. Each of these plots was harvested six or seven times throughout a growing season, in one or two harvest years resulting in a total of 11 580 plots harvested.

In each harvest year, plots underwent combined simulated grazing and conservation management. The first harvest was targeted for early April; however, if there were good growing conditions earlier in the season an additional harvest may have been taken prior to April. Each maturity group was harvested individually based on the productivity on plots, with a maximum gap of 1 week between early and late groups. The initial harvest(s) simulated spring grazing and the next two simulated conservation harvests taken at 7- and 6-week re-growth intervals, respectively. The final three harvests simulated grazing and were then taken after 4-, 5- and 6-week re-growth intervals. These were the targeted harvest intervals throughout the study; however, the targets were adjustable over the course of the study to reflect the changing weather and growing conditions. Plots were harvested to a stubble height of 50 mm above ground level using a Haldrup harvester (Haldrup, Logstor, Denmark) and herbage yield was estimated via an

on-board weighing scale. A sample of *c*. 300 g from each plot was oven dried at 80 °C for 16 h to determine dry-matter concentration. These were subsequently milled (Retch SM 100) through a 1 mm pore sieve prior to analysis of quality traits using near infrared reflectance spectroscopy (NIRS).

NIRS calibration

The NIRS calibrations were independently developed using 2076 samples that included a subset of the above perennial ryegrass samples as well as comparable Italian and hybrid ryegrass samples. These samples encompassed the range of climatic conditions, species, maturity, ploidy, genotype and age of sward occurring at this site. Absorbance (log 1/ reflectance) was measured from 400 to 2500 nm at 2 nm intervals using a NIRsystems 6500 or a standardized NIRsystems XDS (Foss UK Ltd, Warrington, UK).

Laboratory analysis

Each of the 2076 samples were analysed for four quality traits using reference methods. Buffering capacity was measured according to Playne & McDonald (1966), while CP (N×6·25) was determined using method 990-03 of the Association of Analytical Chemists (AOAC 1990) with a LECO FP-428 (Leco Corporation, St. Joseph, MI, USA). *In vitro* CDDM was assessed using the two-stage technique of Tilley & Terry (1963) modified by the final residue

	Analysis A	п	n^1	Analysis B	п	n^1
Maturity	Intermediate	80	3960	Early	18	408
,	Late	90	4452	Intermediate	85	1920
				Late	92	2076
Ploidy	Diploid	86	4252	Diploid	104	2340
,	Tetraploid	84	4160	Tetraploid	91	2064
Sowing year	2001	43	2064	2002	57	1368
07	2005	64	3072	2004	69	1380
	2006	63	3276	2006	69	1656

Table 1. Total number of perennial ryegrass variety units^{*} (n) and plot harvests (n^1) for various categories of variety for Analyses A and B

* A variety unit is defined as a unique variety within a sowing year (i.e. if a variety is sown in another year then it is a separate variety unit).

being isolated by filtration, and the automated anthrone method (Thomas 1977) was used to determine WSC concentration.

Chemometrics

Chemometric techniques were applied to the NIR spectra using WinISI version 4.00 (Infrasoft International, Port Matilda, PA, USA). Calibrations were developed across the wavelength range of 1108-2498 nm at 2 nm intervals. Permutations of several different chemometric techniques were applied to the spectra to form the calibration models. The spectra had two spectral pre-treatments, each with two options, applied prior to the application of regression techniques: (a) a scatter correction treatment of standard normal variate and de-trending (SNV-D; Barnes et al. 1989) was applied to spectra or no scatter correction treatment was applied, and (b) a curve smoothing of 1, 4, 4 or 2, 5, 10 derivations was carried out on the spectra, where the first value denotes the derivate, the second value denotes the gap over which the derivate is calculated and the third value denotes the number of data points. Three regression techniques (modified partial least squares, partial least squares and principal component regression) were then used to relate the reference data to the spectra. These combinations of spectral pre-treatments and regression techniques resulted in 12 permutations of calibration models per quality trait. Outliers were removed from each calibration model on the basis of chemical residual T > 2.5 or spectral global H > 10.0 using the WinISI software. A cross-validation was carried out in which 0.20 of the full calibration set was randomly removed as a validation set. The remaining samples were then used to form a new calibration model and this model

was applied to the validation set. This process was repeated five times and optimal calibration models were selected on the basis of high R^2 and low standard errors of cross-validation (Burns *et al.* 2011).

Statistical analysis

Since not all varieties were sown every year, the data were analysed using two separate analyses to optimize the use of information from the database. Analysis A comprised both the intermediate and late groups sown in 2001, 2005 and 2006 and harvested in each of the two subsequent years (Fig. 1). Analysis B comprised the early, intermediate and late groups sown in 2002, 2004 and 2006, and harvested in one of the two subsequent years (Fig. 1). This facilitated Analysis A assessing age of sward effects while having a larger sample size for assessing the effects of ploidy. Analysis B facilitated a comparison of the early group with the intermediate and late groups.

For Analysis A, 170 variety units (unique variety within a sowing year) had been sown (Table 1) and harvested in the two following years resulting in a total of 8412 plot harvests. Analysis B comprised 195 variety units (of which 63 were present in Analysis A) harvested in one of the 2 years following sowing resulting in 4404 plot harvests (of which 1512 were present in Analysis A). As different varieties were at different stages along their evaluation cycle during the present study not all varieties were present in each sowing. This resulted in 13 varieties that were common to the 3 years of sowings for Analysis A and 21 that were common to each of the sowings for Analysis B. The results from the harvests within a year were grouped as follows: Spring – harvests taken up to

17 April; Silage 1 – harvest taken on c. 29 May; Silage 2 – harvest taken on c. 10 July; Rest of year – combines the harvests taken on c. 7 August, 11 September and 23 October. Annual herbage yield was the cumulative total of the individual harvest values, while annual herbage quality trait values were based on the appropriate individual harvest values averaged using a weighting for individual harvests derived from their, respective, DM contributions to annual yield. Due to poor growing conditions, the first harvest in 2006 produced herbage yields below the reliable tolerance limits of the on-board weighing scales and less than was required for nutritive analysis so these samples were not included in yield or quality traits.

Analysis A comprised intermediate and late groups in an restricted maximum likelihood (REML) analysis with fixed effect terms for harvest year, maturity, ploidy and age of sward and random effect terms for trial, block within trial and variety within block within trial. A trial is defined as the maturity group within a sowing year (Fig. 1). Individual terms were added to the full fixed model and then maturity, ploidy, age of sward and the maturity × ploidy interaction were individually dropped from the full mixed model to assess the significance of each individual term. The random terms ensured that the effect of age of sward was tested against the error estimate at the age of sward within variety within block within trial level of the data. Analysis B included the early, intermediate and late groups with a REML analysis carried out, effectively on the trial-by-variety means, with fixed effect terms for harvest year, maturity and ploidy and random effect terms for trial and for variety within trial. Individual terms were added to the full fixed model and then maturity, ploidy and maturity × ploidy interaction were individually dropped from the full mixed model to assess the significance of each individual term.

Where a significant effect of maturity occurred in Analysis B, a student's *t*-test was applied to each paired combination of maturity groups to assess the significance between groups.

RESULTS

NIRS

The four calibration models that provided the most accurate and robust cross-validation statistics are displayed in Table 2 along with a summary of similar published calibration models. The most accurate calibration model was for predicting CP ($R^2 = 0.98$),

with WSC concentration and buffering capacity calibration models also being accurate ($R^2 = 0.96$, 0.95, respectively). The *in vitro* CDDM calibration model was least accurate ($R^2 = 0.86$). These were the four models used to predict *in vitro* CDDM, WSC, CP and buffering capacity of the perennial ryegrass samples from the plots.

Maturity

Within Analysis A, maturity had no significant effect on herbage yield (Table 3), *in vitro* CDDM (Table 4), WSC (Table 5), CP (Table 6) or DM (Table 8) at any individual seasonal period or for the annual value. For buffering capacity, the only effect was for 'Annual' where the intermediate group had lower (P < 0.05) values than the late group with the same relationship approaching significance (P = 0.053) at 'Silage 1'. However, with the inclusion of the early group (Analysis B), maturity had a significant effect on herbage yield in the 'Rest of Year' period (P < 0.05), with the early group having the lowest yield (Table 3).

In Analysis B, maturity also had an effect on *in vitro* CDDM in the 'Spring' (P < 0.01) period, with the late group being significantly greater than the early group, and in the 'Annual' (P < 0.01) period, where the late group was significantly higher than either the early or intermediate groups (Table 4).

A significant maturity × ploidy interaction occurred for *in vitro* CDDM in Analysis A in the 'Spring' (P<0·01), 'Silage 1' (P<0·01), 'Silage 2' (P<0·05) and 'Annual' (P<0·05) periods (Table 4), whereby intermediate diploids had lower values than intermediate tetraploids (783 v. 795 g/kg, respectively). The only other significant maturity × ploidy interactions for Analysis A were in 'Spring' for WSC (P<0·001) and CP (P<0·01) and 'Annual' for DM (P<0·05).

Ploidy

Tetraploid swards had greater herbage yields (P < 0.001), *in vitro* CDDM values (P < 0.001) and WSC concentrations (P < 0.01) than diploids in all seasons and annually. An exception to the above occurred for herbage yield in the 'Spring' period, where the difference was not significant (Table 3).

Diploid swards had a higher CP and DM concentration (P<0.001) than tetraploid swards over the growing season. This relationship occurred at all seasonal periods for DM concentration (Table 8) and in the 'Spring' (P<0.001), 'Silage 2' (P<0.05) and 'Rest

		Calibration					Validation			
Reference	Forage	N	R^2	Mean	S.E.	S.E.%	Ν	R^2	S.E.	s.e.%
In vitro CDDM (g/kg)										
Current study	Perennial, Italian or hybrid ryegrasses	1986	0.86	798	15.9	1.99	*	0.86	16.2	2.03
Parnell & White (1983)	Perennial ryegrass	49	0.96	_	12	-	33	0.96	23	_
Andueza <i>et al</i> . (2011)†	Perennial ryegrass	193	0.83	_	23	_	24	0.74	32	_
Park <i>et al</i> . (1998)	Fresh grass silage	90	0.90	686	19.9	2.90	46	0.85	23.3	3.40
Jafari <i>et al</i> . (2003)	Perennial ryegrass	62	0.96	783	13.2	1.69	82	0.92	16.1	2.06
WSC (g/kg DM)										
Current study	Perennial, Italian or hybrid ryegrasses	1941	0.96	182	10.4	5.71	*	0.98	10.8	5.93
Parnell & White (1983)	Perennial ryegrass	123	0.92	_	14	_	59	0.92	9.2	_
Park <i>et al</i> . (1998)	Fresh grass silage	136	0.91	2.93	0.37	12.63	*	0.81	0.56	19.11
Jafari <i>et al.</i> (2003)	Perennial ryegrass	56	0.98	226	8	3.53	90	0.96	11.9	5.26
Agnew <i>et al</i> . (2004)	Perennial ryegrass	116	1.00	194	3.9	2.04	*	0.99	5.1	2.62
CP (g/kg DM)										
Current study	Perennial, Italian or hybrid ryegrasses	1941	0.98	149	5.0	3.36	*	0.98	5.1	3.42
Parnell & White (1983)	Perennial ryegrass	108	0.98	_	7	_	33	0.92	8	_
Andueza <i>et al</i> . (2011)	Perennial ryegrass	195	0.98	_	5.4	_	24	0.95	9.4	_
Park <i>et al</i> . (1998)‡	Fresh grass silage	136	0.98	29	0.14	0.48	*	0.94	0.25	5.39
Jafari <i>et al</i> . (2003)	Perennial ryegrass	63	0.98	155	7.3	4.69	84	0.96	6.8	4.36
Agnew <i>et al</i> . (2004) ‡	Perennial ryegrass	108	0.97	172	6.1	3.45	*	0.97	6.7	3.77
Decruyenaere <i>et al.</i> (2009)	Perennial ryegrass or mixed forages	2765	0.98	148	8.6	5.82	*	-	8.6	5.82
Biewer et al. (2009)	Fresh perennial ryegrass and white clover	113	0.93	197	21	10.66	*	0.90	25.4	12.89
Buffering capacity (mEq/kg DM)										
Current study	Perennial, Italian or hybrid ryegrasses	1985	0.95	428	20.3	4.74	*	0.95	20.7	4.84
Park <i>et al.</i> (1998)	Fresh grass silage	136	0.81	222	14.2	6.40	*	0.68	18.6	8.37

Table 2. Comparison of NIRS calibration models and validations from the current study with values quoted in literature for quality traits of ryegrass

N: number of samples used in calibration/validation; R²: coefficient of determination; s.e.: standard error of prediction; s.e.%: standard error of prediction as a percentage of the mean.

* Cross-validation.

+ Organic matter.

+ Nitrogen concentration used in the literature, multiplied by 6.25 for ease of comparison. All samples dried and milled unless stated otherwise.

Table 3. REML analysis of seasonal and cumulative annual herbage yield (t/ha) for main effects of maturity,
ploidy, age of sward and maturity × ploidy interaction displaying effect means, s.E.D. and P-value for Analyses
A and B

	Spring	Silage 1	Silage 2	ROY	Annual
Analysis A					
Maturity					
Intermediate	1.41	5.81	3.25	4.65	14.65
Late	1.35	5.41	3.35	4.36	14.09
S.E.D.	0.046	0.269	0.279	0.535	0.232
Significance on 1, 2·1 D.F.	NS	NS	NS	NS	NS
Ploidy					
Diploid	1.38	5.48	3.20	4.40	14.03
Tetraploid	1.39	5.73	3.41	4.61	14.71
S.E.D.	0.019	0.045	0.035	0.025	0.071
Significance on 1, 661 D.F.	NS	P<0.001	P<0.001	P<0.001	P<0.001
Age of sward					
1	1.95	6.45	3.75	5.18	16.96
2	0.81	4.76	2.86	3.84	11.79
S.E.D.	0.057	0.325	0.336	0.638	0.286
F ^(Sig) on 1, 2.4 D.F.	P<0.001	P<0.05	NS	NS	P<0.001
Maturity×ploidy					
Significance on 1, 658 D.F.	P<0.01	NS	NS	NS	NS
Analysis B					
Maturity					
Early	1.31	6.47	3.13	3.59	14.08
Intermediate	1.11	6.29	3.72	3.84	14.58
Late	1.20	5.64	3.79	4.23	14.46
S.E.D.	0.098	0.290	0.335	0.157	0.210
Significance on 2, 3.0 D.F.	NS	NS	NS	P < 0.05	NS
Maturity×ploidy					
Significance on 2, 117 D.F.	NS	NS	NS	NS	NS

of Year' (P < 0.01) periods for CP concentration, while no significant effect was found in 'Silage 1' (Table 6).

Diploid swards had a significantly higher buffering capacity than tetraploids swards in 'Spring' (P < 0.01), but a lower value in the 'Silage 1' (P < 0.001), 'Silage 2' (P < 0.05) and 'Rest of Year' (P < 0.001) periods and in the 'Annual' (P < 0.05) period (Table 7).

Age of sward

In their first full season, swards had a higher herbage yield in 'Spring' (P < 0.001) and 'Silage 1' (P < 0.05) than in their second season. This resulted in a higher (P < 0.001) annual herbage yield in the first season after sowing (Table 3).

Age of sward had no effect (P > 0.05) on *in vitro* CDDM, WSC, CP or DM except for WSC at 'Silage 2' when 1-year-old swards had a higher (P < 0.01; Table 5) value. One-year-old swards had a higher

annual buffering capacity than 2-year-old swards (P < 0.05) reflecting higher (P < 0.05) values in the 'Spring' and 'Silage 2' seasonal periods, but with no significant difference in either the 'Silage 1' or 'Rest of year' periods (Table 7).

DISCUSSION

NIRS calibrations

As the current calibration set comprises samples from five harvest years and is based on 176 different ryegrass varieties, the model incorporates a wide range of growing conditions, plant genotypes and tissue development stages relevant to ryegrass variety evaluation at this one site.

The most accurate calibration models were for predicting CP: values in the literature have $R^2 = 0.93 - 0.99$; Table 2) and the current study compared

	Spring	Silage 1	Silage 2	ROY	Annual
Analysis A					
Maturity					
Intermediate	850	772	789	798	789
Late	844	791	788	799	796
S.E.D.	5.0	10.8	2.3	3.4	4.4
Significance on 1, 2.7 D.F.	NS	NS	NS	NS	NS
Ploidy					
Diploid	840	777	785	793	788
Tetraploid	854	787	792	804	798
S.E.D.	0.6	1.3	0.9	0.6	0.7
Significance on 1, 570 D.F.	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Age of sward					
1	854	778	791	802	795
2	839	786	787	795	791
S.E.D.	6.1	13.0	3.0	4.2	5.3
F ^(Sig) on 1, 2·8 D.F	NS	NS	NS	NS	NS
<i>Maturity</i> ×ploidy					
Significance on 1, 72 d.f.	P<0.001	P<0.01	P<0.05	NS	P<0.01
Analysis B					
Maturity					
Early	852	712	794	791	760
Intermediate	853	768	789	786	783
Late	846	802	783	796	797
S.E.D.	9.2	12.9	8.0	8.9	4.9
Significance on 2, 3.0 D.F.	NS	P<0.01	NS	NS	P<0.01
Maturity×ploidy					
Significance on 2, 116 D.F.	NS	NS	NS	NS	NS

Table 4. *REML* analysis of seasonal and weighted-average annual in vitro CDDM (g/ kg) for main effects of maturity, ploidy, age of sward and maturity \times ploidy interaction displaying effect means, s.E.D. P-value for analyses A and B

favourably with this range ($R^2 = 0.98$). The lower R^2 of *in vitro* CDDM in comparison with the other three quality traits may be related to the involvement of animal factors, thus incorporating additional variation (Coleman & Moore 2003). The *in vitro* CDDM calibration ($R^2 = 0.86$) was within the range of accuracy in other published work ($R^2 = 0.83-0.96$). The accuracy of these calibrations was satisfactory considering the larger calibration size (n = 1986; 90 outliers removed from full calibration set) compared to other studies (n = 49-193).

Maturity

Perennial ryegrass varieties are categorized into three maturity groups, although the mean heading dates of perennial ryegrass varieties form a continuum (Gilliland *et al.* 2002). This allows the harvest date of each group to be targeted to a physiological stage such as 0.5 ear emergence, to compare intrinsic varietal differences (Weddell et al. 1997). Aldrich & Dent (1963) have previously shown that there are differences in seasonal yield distribution across this continuum. The results of the present study confirm this, as there were only significant seasonal yield differences in the 'Silage 1' period. However, because the three maturity groups were cut at relatively similar times, it is noted that there is an underlying influence of differences in developmental stage at harvest on these comparisons. Even within a maturity group, such underlying effects cannot be entirely avoided unless each variety is harvested at the exact same developmental stage. The present results were, however, in contrast to the studies by Gilliland et al. (2002) and Laidlaw (2005), who found earlier groups to have a higher herbage yield in early April, corresponding to

	Spring	Silage 1	Silage 2	ROY	Annual
Analysis A					
Maturity					
Intermediate	242	172	157	181	178
Late	254	177	156	174	179
S.E.D.	23.6	5.8	2.9	14.7	6.8
Significance on $1, 2 \cdot 1$ D.F.	NS	NS	NS	NS	NS
Ploidy					
Diploid	236	168	153	172	172
Tetraploid	256	181	160	183	185
S.E.D.	1.3	1.5	1.9	1.3	1.1
Significance on 1, 604·2 D.F.	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Age of sward					
1	238	172	170	188	185
2	258	176	143	166	172
S.E.D.	28.2	7.2	4.0	17.6	8.2
Significance on 1, 2·9 D.F	NS	NS	P<0.01	NS	NS
Maturity × ploidy					
Significance on 1, 678 D.F.	P<0.001	NS	NS	NS	NS
Analysis B					
Maturity					
Early	213	166	181	161	175
Intermediate	212	200	180	161	187
Late	226	205	177	170	188
S.E.D.	18.8	4.5	10.3	11.2	6.3
Significance on 2, 3·0 d.f.	NS	P<0.01	NS	NS	NS
Maturity×ploidy					
Significance on 2, 116 D.F.	NS	NS	NS	NS	NS

Table 5. *REML* analysis of seasonal and weighted-average annual WSC (g/kg DM) for main effects of maturity, ploidy, age of sward and maturity × ploidy interaction displaying effect means, s.E.D. P-value for analyses A and B

the spring period of the present study; they also found that the late group had a higher herbage yield in the summer period coinciding with the timing of the silage cuts in the present study. Similarly, the lack of significant difference between the maturity groups for total annual herbage yield is in contrast to Gilliland *et al.* (2002), who found a significant difference in the annual herbage yield of the three maturity groups, and both Gowen *et al.* (2003) and O'Donovan & Delaby (2005), who found the late group to have a higher annual herbage yield than the intermediate group in grazed swards.

The present study imposed a different management regime to any of these previous studies as it did not involve animal grazing and tested a much larger and different set of varieties (43–69 varieties annually compared to 9–12 in the previous studies). As Gilliland & Mann (2000) have shown, the management regime imposed on the sward can significantly affect

productivity patterns and strong genotype× environment interactions are known to occur for productivity (Conaghan *et al.* 2008*b*) and quality traits, particularly WSC concentration (Evans *et al.* 2011). This highlights the challenges encountered when evaluating varieties for agronomic use and the limitations in extrapolating results from variety trials onto farm level, as reported by Conaghan *et al.* (2008*b*).

When the nutritive value parameters were compared between maturity groups, a number of significant differences were found. In 'Silage 1', intermediate and late groups had significantly higher *in vitro* CDDM than the early group. This is an important observation, as first-cut silage contributes the most conserved forage of any harvest in the growing season in Ireland (Connolly *et al.* 2003). This suggests it may be possible to grow varieties from the late maturing group to a higher yield than earlier maturing types,

Spring	Silage 1	Silage 2	ROY	Annual
164	116	149	148	134
153	119	143	155	135
19.2	6.1	4.9	10.0	2.3
NS	NS	NS	NS	NS
161	118	147	153	136
156	117	144	151	133
0.6	0.7	0.9	0.5	0.5
P<0.001	NS	P<0.001	P<0.001	P<0.001
157	110	144	146	131
160	125	147	157	138
22.9	7.3	5.9	11.9	2.9
NS	NS	NS	NS	NS
<i>P</i> <0.01	NS	NS	NS	NS
177	97	147	162	128
183	102	136	163	129
182	117	133	155	135
7.1	5.8	6.4	8.3	3.1
NS	NS	NS	NS	NS
<i>P</i> <0.001	NS	NS	NS	NS
	164 153 19·2 NS 161 156 0·6 $P < 0.001$ 157 160 22·9 NS $P < 0.01$ 177 183 182 7·1 NS	164 116 153 119 19·2 6·1 NS NS 161 118 156 117 0·6 0·7 $P < 0.001$ NS 157 110 160 125 22·9 7·3 NS NS $P < 0.01$ NS 177 97 183 102 182 117 7·1 5·8 NS NS	164 116 149 153 119 143 19·2 6·1 4·9 NS NS NS 161 118 147 156 117 144 0·6 0·7 0·9 $P < 0.001$ NS $P < 0.001$ 157 110 144 160 125 147 22·9 7·3 5·9 NS NS NS $P < 0.01$ NS NS 177 97 147 183 102 136 182 117 133 7·1 5·8 6·4 NS NS NS	164 116 149 148 153 119 143 155 19·2 6·1 4·9 10·0 NS NS NS NS 161 118 147 153 156 117 144 151 0·6 0·7 0·9 0·5 P<0·001

Table 6. *REML* analysis of seasonal and weighted-average annual CP (g/kg DM) for main effects of maturity, ploidy, age of sward and maturity × ploidy interaction displaying effect means, s.E.D. and P-value for analyses A and B

while still maintaining an adequate digestibility, and this is in accord with Humphreys & O'Kiely (2006).

A significant increase in WSC concentration was also recorded in 'Silage 1' and this probably contributed to the differences in in vitro CDDM at this harvest. There are, however, other important implications of increased WSC concentration. When WSC concentration is expressed on an aqueous phase basis and considered together with buffering capacity (DM basis) they provide a good index of ensilability (O'Kiely & Muck 1998). The overall mean WSC and buffering capacity values among all 'Silage 1' and 'Silage 2' herbages (49 g WSC/l and 367 mEq/kg DM) indicated that crops had a considerable surplus of fermentable substrate and should have been quite straightforward to preserve properly as silage. Herbage maturity group had no overall impact on ensilability in the 'Silage 2' harvest, with the lower

WSC concentration among the early group (45.7 g/l) than among the intermediate (55.4 g/l) or the late (59.2 g/l) groups being offset by the correspondingly lower buffering capacity values associated with the early group (i.e. 322 v. 349 and 408 mEq/kg DM, respectively). Thus, maturity categorization had no impact on ensilability. Across all three maturity groups, the ensilability indices of herbages from 'Silage 1' indicated they would be easier to preserve than 'Silage 2' herbages.

Notably, there were no significant differences in CP concentrations between maturity groups. Binnie *et al.* (2001) have shown that high application rates of inorganic N fertilizer can raise CP concentration, with the relatively high rates in the present study (350–400 kg N/ha per annum) possibly masking any differences that might exist between maturity groups. In addition, as perennial ryegrass is known for its high

	Spring	Silage 1	Silage 2	ROY	Annual
Analysis A					
Maturity					
Intermediate	413	423	378	420	412
Late	425	452	374	430	424
S.E.D.	17.5	7.7	4.0	11.5	3.0
Significance on $1, 2.2$ D.F.	NS	P = 0.053	NS	NS	P < 0.05
Ploidy					
Diploid	426	434	374	421	415
Tetraploid	412	442	378	429	420
S.E.D.	1.5	2.1	1.9	1.6	1.4
Significance on 1, 625·4 D.F.	P<0.001	P<0.001	P<0.05	P<0.001	P<0.001
Age of sward					
1	467	442	385	426	427
2	370	433	367	424	408
S.E.D.	21.0	9.5	5.3	13.8	3.8
Significance on 1, 2.8 D.F.	P < 0.05	N.S.	P < 0.05	N.S.	P < 0.05
Maturity×ploidy					
Significance on 1, 626 D.F.	NS	NS	NS	NS	NS
Analysis B					
Maturity					
Early	466	322	389	420	372
Intermediate	453	349	366	431	384
Late	461	408	377	424	409
S.E.D.	7.9	22.7	9.6	4.0	12.6
Significance on 2, 2.8 D.F.	NS	P<0.05	NS	NS	NS
Maturity×ploidy					
Significance on 2, 116 D.F.	NS	NS	NS	NS	NS

Table 7. *REML* analysis of seasonal and weighted-average annual buffering capacity (*mEq/kg DM*) for main effects of maturity, ploidy, age of sward and maturity × ploidy interaction displaying effect means, s.E.D. and P-value for analyses A and B

CP concentration, with swards normally exceeding the 80 g CP/kg DM concentration thought to be limiting to rumen function (Coleman & Moore 2003), the lack of differences may also reflect the low priority of CP as a trait for ryegrass breeders (O'Donovan *et al.* 2011).

Ploidy

Gilliland *et al.* (2007) reported an increase in the usage of tetraploid cultivars of perennial ryegrass over the past 25 years in Northern Ireland, and these now account for almost 0.30 of all perennial ryegrass seed sold. This increased usage was attributed to the perceived increased yield potential and nutritive value of tetraploid varieties. This advantage was repeated in the present study, as tetraploids conferred a small but statistically significant DM yield advantage in all seasonal periods except 'Spring', which gave a total yield increase of 0.66 t/ha per annum. This was similar to the findings of Gilliland et al. (2002), who found no seasonal differences between ploidies but an additional 1 t DM/ha per annum from tetraploids. Under animal grazing conditions, however, Gowen et al. (2003) and Balocchi & Lopez (2009) found diploids swards to have a higher annual herbage yield than tetraploids. Although additional environmental and management factors are likely to affect the yield potential of diploid and tetraploid swards, these inconsistencies raise concern regarding how representative simulated-grazing is of actual grazing. This is clearly not a simple issue, as Wims et al. (2010) found no significant re-ranking of varieties when comparing animal grazed and simulated grazed plots.

The higher nutritive value of tetraploid varieties is well established (Wilkins 1991; Gilliland *et al.* 2002;

	Spring	Silage 1	Silage 2	ROY	Annual
Analysis A					
Maturity					
Intermediate	250	198	186	210	202
Late	263	190	180	205	197
S.E.D.	24.1	8.7	6.1	6.6	5.1
Significance on 1, 2.6 D.F.	NS	NS	NS	NS	NS
Ploidy					
Diploid	264	200	190	217	207
Tetraploid	250	188	176	199	192
S.E.D.	0.9	1.0	1.2	0.7	0.7
Significance on 1, 625·4 D.F.	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Age of sward					
1	248	189	176	196	193
2	266	199	191	220	206
S.E.D.	28.7	10.5	7.4	7.9	6.1
Significance on 1, 3.0 D.F	NS	NS	NS	NS	NS
Maturity×ploidy					
Significance on 1, 615 D.F.	N.S.	N.S.	N.S.	N.S.	<i>P</i> <0·05
Analysis B					
Maturity					
Early	228	216	193	192	202
Intermediate	242	217	199	212	211
Late	273	224	204	208	216
S.E.D.	3.42	10.22	12.62	8.46	6·18
Significance on 2, 3.0 D.F.	P<0.001	NS	NS	NS	NS
Maturity×ploidy					
Significance on 2, 117 D.F.	<i>P</i> <0.01	NS	NS	NS	NS

Table 8. *REML* analysis of seasonal and weighted-average DM (g/kg) for main effects of maturity, ploidy, age of sward and maturity × ploidy interaction displaying effect means, s.E.D. and P-value for analyses A and B

Balocchi & Lopez 2009), although it is unclear as to the mechanism(s) that induce higher nutritive value. A proposed explanation is that the increased ratio of cell contents to cell wall in tetraploids allows for increased intra-cellular components without a proportional increase in potentially lower nutritive value cell wall material (Wilkins & Sabanci 1990). However, this possible explanation cannot be the single causal factor as the CP concentration of tetraploids was found to be lower.

The increased *in vitro* CDDM of tetraploids as shown throughout the growing season in the current study may be partially attributed to the increased WSC concentration (Humphreys 1989). However, it could equally have been due to a decrease in fibrous material (O'Donovan *et al.* 2011) or a change in fibre digestibility.

The increased WSC concentration of tetraploids may have resulted in the decreased CP concentration.

Smith *et al.* (2001) reported altered carbon partitioning between intracellular components in grass varieties bred for high WSC concentration.

Tetraploid varieties are often suggested as being optimal for ensilage, reflecting the higher yield potential under mechanical harvested systems and the increased WSC concentration. In 'Silage 1', the higher DM and lower WSC (g/kg DM) concentrations associated with diploid compared to tetraploid varieties meant that when expressed on an aqueous phase basis the WSC concentrations were numerically quite similar (42.0 and 41.9 g/l for diploids and tetraploids, respectively). Although buffering capacity was lower for the diploid varieties, the magnitude of this effect was very small (434 v. 441 mEq/kg DM). In 'Silage 2', the higher DM and lower WSC (g/kg DM) concentrations associated with diploid compared to tetraploid varieties meant that when expressed on an aqueous phase basis the WSC concentrations were 35.9 and 34.2 g/l, respectively. There was no effect of ploidy on buffering capacity. In terms of ensilability indices the increased WSC concentration of tetraploids is effectively counteracted by the decreased DM concentration when WSC concentration is reported on an aqueous phase basis. Although 'Silage 1' diploids had a marginally lower buffering capacity the effect of ploidy on silage fermentation is likely to be minimal.

Age of sward

As a sward ages, its yield and quality has been shown to decrease due to the invasion of secondary grasses and weeds (Wilkins 1997). The present study had a decreased herbage yield of 31% between the first and second year swards; however, it was not clear whether this was a result of weather conditions in the year of harvest, age of plant material or the residual effects of soil fertility and structure following sowing (Parsons et al. 2011). There was, notably, no decline in digestibility and little deterioration in other nutritional quality traits between 1- and 2-year-old swards, with the only difference being WSC concentration in 'Silage 2'. Here again it is possible that the high application rates of inorganic nitrogen fertilizer masked any deterioration in CP concentration, as Keady et al. (2000) have shown that increasing the application rate of inorganic nitrogen fertilizer increases the CP concentration in the sward.

Age of sward did not affect ensilability in 'Silage 1', while in 'Silage 2' the slightly higher WSC values in 1-year-old swards ($36 \cdot 3 \ v. \ 33 \cdot 8 \ g/l$) was counterbalanced by a correspondingly higher buffering capacity ($385 \ v. \ 367 \ mEq/kg \ DM$). Thus, there was little indication of any effect of age of sward on grass ensilability.

It is noted, however, that the present study only examined swards up to 2 years after sowing and the longer-term effects of sward aging may not be reliably predicted from the current dataset.

Implications

Although differences have been reported on a statistically significant basis, any changes in nutritive quality need to be extrapolated to the animal interface to determine their true value to ruminant enterprises. Small increases in digestibility can be valuable as they can have a beneficial effect on grass intake and energy availability from the sward (Casler 2001; Coleman & Moore 2003; Wilkins & Humphreys 2003), with a

3.2% increase in beef production being reported per 1% increase in digestibility (Casler & Vogel 1999). The required scale of increase in WSC concentration is less clear as Miller et al. (2001) found that 40 g WSC/kg DM provided increased milk production and quality, while Taweel et al. (2005) found no benefit in milk production or quality when heifers were fed a perennial ryegrass cultivar with increased WSC concentration (24-31 g WSC /kg DM greater than control). A complicating factor as reported by Parsons et al. (2011) is that increases in nitrogen use efficiency (NUE) are dependent on the ratio of energy (in the form of WSC) to nitrogen (including CP sources). Although this is a continuum response in which any increase in WSC will lead to an increase in NUE, detection in field trials requires a substantial increase in WSC. In the present study, the largest difference between ploidy groups was reported in the 'Spring' period when tetraploids had 22 g/kg DM higher WSC than diploids. Nonetheless, there was a maximum range of 90 g/kg DM in annual WSC concentrations between individual ryegrass varieties (data not presented) representing the difference between the worst and elite performing varieties. This scale of differences would be expected to confer significant animal production benefits.

The main conclusions from the present work were that although significant differences exist between ploidy and maturity for herbage yield, nutritive quality traits and ensilability indices, there is justification for the use of these groups in evaluation trials as they permit like-for-like comparisons between varieties. It also shows that despite breeders' achievements in creating considerable differences between individual varieties, as evident in recommended list publications, ploidy and maturity group characteristics remain an influence on performance. As these differences between groups, and particularly between ploidies, were sufficiently large to affect animal productivity and efficiency, end-users are also justified in selecting varieties from within a specific group to get the performance profile that suits their individual farming enterprise. The seasonal differences between maturity groups at the first silage cut suggests that timing this harvest differently for the three groups in the variety trials might better reflect best management practice. The detectable sward age effects between the first and second harvest years could be the start of a progressive decline in performance of swards following sowing or equally evidence that the first year after reseeding does not reflect the longer-term potential of perennial ryegrass swards. A longer-term study of sward age should easily resolve which of these two possibilities is the causal factor of this observation.

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REFERENCES

- AGNEW, R. E., PARK, R. S., MAYNE, C. S. & LAIDLAW, A. S. (2004). Potential of near infrared spectroscopy to predict the voluntary intake of grazed grass. *Animal Feed Science and Technology* **115**, 169–178.
- ALDRICH, D. T. A. & DENT, J. W. (1963). The interrelationships between heading date, yield, chemical composition and digestibility in varieties of perennial ryegrass, timothy, cocksfoot and meadow fescue. *Journal of the Institute* of Agricultural Botany **9**, 261–281.
- ANDUEZA, D., PICARD, F., JESTIN, M., ANDRIEU, J. & BAUMONT, R. (2011). NIRS prediction of the feed value of temperate forages: efficacy of four calibration strategies. *Animal* 5, 1002–1013.
- AOAC (1990). *Official Methods and Analysis*. First Supplement (1990) to the 15th edition, Method 990-03. Arlington, VA: AOAC.
- BALOCCHI, O. A. & LOPEZ, I. F. (2009). Herbage production, nutritive value and grazing preference of diploid and tetraploid perennial ryegrass cultivars (*Lolium perenne* L.). *Chilean Journal of Agricultural Research* **69**, 331–339.
- BARNES, R. J., DHANOA, M. S. & LISTER, S. J. (1989). Standard normal variate transformation and de-trending of nearinfrared diffuse reflectance spectra. *Applied Spectroscopy* 43, 772–777.
- BIEWER, S., FRICKE, T. & WACHENDORF, M. (2009). Development of canopy reflectance models to predict forage quality of legume–grass mixtures. *Crop Science* **49**, 1917–1926.
- BINNIE, R. C., MAYNE, C. S. & LAIDLAW, A. S. (2001). The effects of rate and timing of application of fertilizer nitrogen in late summer on herbage mass and chemical composition of perennial ryegrass swards over the winter period in Northern Ireland. *Grass and Forage Science* **56**, 46–56.
- BURNS, G. A., GILLILAND, T. J., GROGAN, D. & O'KIELY, P. (2011). Using chemometrics to develop near infrared spectroscopy (NIRS) calibrations to predict ryegrass quality. In *Agricultural Research Forum 2011*, p. 135. Tullamore, Ireland: Agricultural Research Forum.
- BUXTON, D. R. & O'KIELY, P. (2003). Preharvest plant factors affecting ensiling. In *Silage Science and Technology*. *Agronomy Monograph No. 42* (Eds D. R. Buxton, R. E. Muck & J. H. Harrison), pp. 199–250. Madison, WI, USA: ASA, CSSA & SSSA.

- CAMLIN, M. S. (1997). Plant breeding achievement and prospects; grasses. In *Proceedings of the British Grassland Symposium No. 31: Seeds of Progress* (Ed. J. R. Weddell), pp. 2–14. Nottingham, UK: British Grassland Society.
- CASLER, M. D. (2001). Breeding forage crops for increased nutritional value. *Advances in Agronomy* **71**, 51–107.
- CASLER, M. D. & VOGEL, K. P. (1999). Accomplishments and impact from breeding for increased forage nutritional value. *Crop Science* **39**, 12–20.
- COLEMAN, S. W. & MOORE, J. E. (2003). Feed quality and animal performance. *Field Crops Research* **84**, 17–29.
- CONAGHAN, P., CASLER, M. D., MCGILLOWAY, D. A., O'KIELY, P. & DOWLEY, L. J. (2008b). Genotype×environment interactions for herbage yield of perennial ryegrass sward plots in Ireland. *Grass and Forage Science* **63**, 107–120.
- CONAGHAN, P., O'KIELY, P., HOWARD, H., O'MARA, F. P. & HALLING, M. A. (2008a). Evaluation of *Lolium perenne* L. cv. AberDart and AberDove for silage production. *Irish Journal of Agricultural and Food Research* **47**, 119–134.
- CONNOLLY, L., KINSELLA, A. & QUINLAN, G. (2003). Teagasc National Farm Survey 2002. Carlow, Ireland: Teagasc. Available online at: http://www.teagasc.ie/publications/ 2002/farmsurvey2002.asp (verified 16 Feb 2012).
- DECRUYENAERE, V., LECOMTE, P., DEMARQUILLY, C., AUFRERE, J., DARDENNE, P., STILMANT, D. & BULDGEN, A. (2009). Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. *Animal Feed Science and Technology* **148**, 138–156.
- Department Of Agriculture, Food And The Marine (DAFM) (2012). Grass and Clover: Recommended List Varieties for Ireland 2011. Celbridge, Co. Kildare, Ireland: DAFM. Available online at: http://www.agriculture.gov.ie/ media/migration/farmingsectors/crops/seedcertification/ cropvarietyevaluationcvepublications/2012/GrassClover RecommendedList2012.pdf (verified 17 Feb 2012).
- EVANS, J. G., FRASER, M. D., OWEN, I. & DAVIES, D. A. (2011). An evaluation of two perennial ryegrass cultivars (AberDart and Fennema) for sheep production in the uplands. *Journal* of Agricultural Science, Cambridge **149**, 235–248.
- FINNERAN, E., CROSSON, P., O'KIELY, P., SHALLOO, L., FORRISTAL, D. & WALLACE, M. (2011). Stochastic simulation of the cost of home-produced feeds for ruminant livestock systems. *Journal of Agricultural Science, Cambridge* **150**, 123–139.
- FRAME, J. (1991). Herbage production and quality of a range of secondary grass species at 5 rates of fertilizer nitrogen application. *Grass and Forage Science* **46**, 139–151.
- GILLILAND, T. J. (1997). Changes induced by defoliation in the yield and digestibility of leaves and stems of perennial ryegrass (*Lolium perenne* L.) during reproductive development. *European Journal of Agronomy* **6**, 257–264.
- GILLIAND, T. J., BARRETT, P. D., MANN, R. L., AGNEW, R. E. & FEARON, A. M. (2002). Canopy morphology and nutritional quality traits as potential grazing value indicators for Lolium perenne varieties. *Journal of Agricultural Science, Cambridge* **139**, 257–273.
- GILLILAND, T. J., CAMLIN, M. S. & JOHNSTON, J. (1995). Effect of harvest date and cultivar maturity on perennial ryegrass

(Lolium perenne L.) yield and digestibility. *Irish Journal of Agricultural and Food Research* **34**, 133–142.

- GILLILAND, T. J., JOHNSTON, J. & CONNELLY, C. (2007). A review of forage grass and clover seed use in Northern Ireland, UK between 1980 and 2004. *Grass and Forage Science* **62**, 239–254.
- GILLILAND, T. J. & MANN, R. L. (2000). Effect of sward cutting management on the relative performance of perennial ryegrass varieties. *Journal of Agricultural Science, Cambridge* **135**, 113–122.
- GOWEN, N., O'DONOVAN, M., CASEY, I., RATH, M., DELABY, L. & STAKELUM, G. (2003). The effect of grass cultivars differing in heading date and ploidy on the performance and dry matter intake of spring calving dairy cows at pasture. *Animal Research* **52**, 321–336.
- GROGAN, D. & GILLILAND, T. J. (2011). A review of perennial ryegrass variety evaluation in Ireland. *Irish Journal of Agricultural and Food Research* **50**, 65–81.
- HUMPHREYS, J. & O'KIELY, P. (2006). Amount and quality of grass harvested for first-cut silage for differing springgrazing frequencies of two mixtures of perennial ryegrass cultivars with contrasting heading date. *Grass and Forage Science* **61**, 77–88.
- HUMPHREYS, M. O. (1989). Water-soluble carbohydrates in perennial ryegrass breeding 0.3. Relationships with herbage production, digestibility and crude proteincontent. *Grass and Forage Science* **44**, 423–429.
- HUMPHREYS, M. O. (2005). Genetic improvement of forage crops past, present and future. *Journal of Agricultural Science, Cambridge* **143**, 441–448.
- JAFARI, A., CONNOLLY, V., FROLICH, A. & WALSH, E. J. (2003). A note on estimation of quality parameters in perennial ryegrass by near infrared reflectance spectroscopy. *Irish Journal of Agricultural and Food Research* **42**, 293–299.
- KEADY, T. W. J., MAYNE, C. S. & FITZPATRICK, D. A. (2000). Prediction of silage feeding value from the analysis of the herbage at ensiling and effects of nitrogen fertilizer, date of harvest and additive treatment on grass silage composition. *Journal of Agricultural Science, Cambridge* 134, 353–368.
- LAIDLAW, A. S. (2004). Effect of heading date of perennial ryegrass cultivars on tillering and tiller development in spring and summer. *Grass and Forage Science* **59**, 240–249.
- LAIDLAW, A. S. (2005). The relationship between tiller appearance in spring and contribution to dry-matter yield in perennial ryegrass (*Lolium perenne* L.) cultivars differing in heading date. *Grass and Forage Science* **60**, 200–209.
- MILLER, L. A., MOORBY, J. M., DAVIES, D. R., HUMPHREYS, M. O., SCOLLAN, N. D., MACRAE, J. C. & THEODOROU, M. K. (2001). Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. *Grass and Forage Science* 56, 383–394.
- O'KIELY, P. & KAISER, A. G. (2005). Overcoming seasonality of production; opportunities offered by forage conservation technologies. In *Grassland: A Global Resource* (Ed. D. A. McGilloway), pp. 123–136. Wageningen, The Netherlands: Wageningen Academic Publishers.

- O'KIELY, P. & MUCK, R. E. (1998). Grass silage. In *Grass for Dairy Cows* (Eds J. H. & D. J. R. Cherney), pp. 223–251. Wallingford, UK: CABI Publications.
- O'DONOVAN, M. & DELABY, L. (2005). A comparison of perennial ryegrass cultivars differing in heading date and grass ploidy with spring calving dairy cows grazed at two different stocking rates. *Animal Research* **54**, 337–350.
- O'DONOVAN, M., LEWIS, E. & O'KIELY, P. (2011). Requirements of future grass-based ruminant production systems in Ireland. *Irish Journal of Agricultural and Food Research* **50**, 1–21.
- PARK, R. S., AGNEW, R. E., GORDON, F. J. & STEEN, R. W. J. (1998). The use of near infrared reflectance spectroscopy (NIRS) on undried samples of grass silage to predict chemical composition and digestibility parameters. *Animal Feed Science and Technology* **72**, 155–167.
- PARNELL, A. & WHITE, J. (1983). The use of near infrared reflectance analysis in predicting the digestibility and the protein and water soluble carbohydrate contents of perennial ryegrass. *Journal of the National Institute of Agricultural Botany* **16**, 221–227.
- PARSONS, A. J., EDWARDS, G. R., NEWTON, P. C. D., CHAPMAN, D. F., CARADUS, J. R., RASMUSSEN, S. & ROWARTH, J. S. (2011). Past lessons and future prospects: plant breeding for yield and persistence in cooltemperature pastures. *Grass and Forage Science* 66, 153–172.
- PLAYNE, M. J. & McDONALD, P. (1966). The buffering constituents of herbage and of silage. *Journal of the Science of Food and Agriculture* **17**, 264–268.
- RATH, M. & PEEL, S. (2005). Grassland in Ireland and the UK. In *Grassland: A Global Resource* (Ed. D. A. McGilloway), pp. 13–27. Wageningen, The Netherlands: Wageningen Academic Publishers.
- SMITH, K. F., SIMPSON, R. J., CULVENOR, R. A., HUMPHREYS, M. O., PRUD'HOMME, M. P. & ORAM, R. N. (2001). The effects of ploidy and a phenotype conferring a high water-soluble carbohydrate concentration on carbohydrate accumulation, nutritive value and morphology of perennial ryegrass (Lolium perenne L.). Journal of Agricultural Science, Cambridge 136, 65–74.
- TALLOWIN, J. R. B., BROOKMAN, S. K. E. & SANTOS, G. L. (1995). Leaf growth and utilization in four grass species under steady state continuous grazing. *Journal of Agricultural Science, Cambridge* **124**, 403–417.
- TAWEEL, H. Z., TAS, B. M., SMIT, H. J., ELGERSMA, A., DIJKSTRA, J. & TAMMINGA, S. (2005). Effects of feeding perennial ryegrass with an elevated concentration of water-soluble carbohydrates on intake, rumen function and performance of dairy cows. *Animal Feed Science and Technology* **121**, 243–256.
- THOMAS, T.A. (1977). An automated procedure for the determination of soluble carbohydrates in herbage. *Journal of the Science of Food and Agriculture* **28**, 639–642.
- TILLEY, J. M. A. & TERRY, R. A. (1963). A two-stage technique for the *in vitro* digestion of forage crops. *Grass and Forage Science* **18**, 104–111.
- WEDDEL, J. R., GILLILAND, T. J. & MCVITTIE, J. (1997). Evaluation procedures; past present and future. In *Proceedings of the*

British Grassland Symposium No. 31: Seeds of Progress (Ed. J. R. Weddell), pp. 202–225. Nottingham, UK: British Grassland Society.

- WILKINS, P.W. (1991). Breeding perennial ryegrass for agriculture. *Euphytica* **52**, 201–214.
- WILKINS, P.W. (1997). Useful variation in *in vitro* digestibility within perennial ryegrass. *Euphytica* **93**, 249–255.
- WILKINS, P.W. & HUMPHREYS, M.O. (2003). Progress in breeding perennial forage grasses for temperate agriculture. *Journal of Agricultural Science, Cambridge* **140**, 129–150.
- WILKINS, P. W. & SABANCI, C. O. (1990). Genetic variation in leaf epidermal cell size and shape in *Lolium perenne*. *Euphytica* **47**, 233–239.
- WIMS, C., MCEVOY, M., BOLAND, T. & O'DONOVAN, M. (2010).
 A comparison between cut and intensively grazed swards on dry matter yield of perennial ryegrass. In *Grassland in a Changing World: Proceedings of the 23rd General Meeting of the European Grassland Federation* (Eds H. Schnyder, J. Isselstein, F. Taube, K. Auerswald, J. Schellberg, M. Wachendorf, A. Herrmann, M. Gierus, N. Wrage & A. Hopkins), pp. 578–580. Kiel, Germany: European Grassland Federation.