Irish Journal of Agricultural and Food Research **50:** 23–30, 2011

Gains in dry matter yield and herbage quality from breeding perennial ryegrass

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During the last 100 years, in Western Europe and elsewhere, considerable effort has been devoted to improving perennial ryegrass (*Lolium perenne* L.) for agriculture. The first persistent cultivars to be widely used were more digestible than other common pasture species but were no higher yielding than the better wild populations of perennial ryegrass. Two main approaches (here called mainstream breeding and population improvement) have been used to further improve the species for the UK, but no recent experiments to assess progress have been published. In 2006, two plot trials were established at IBERS to compare the performance of some newer cultivars and candidate varieties with the first persistent cultivars to be widely used in the UK. One trial involved comparing 10 intermediate-heading (6 diploid and 4 tetraploid) cultivars and candidate varieties with the intermediate-heading cv. Talbot, and the other involved comparing 11 late-heading (4 diploid and 7 tetraploid) cultivars and candidate varieties with the late-heading cv. S23. During 2007 to 2009, one silage cut and 6 other cuts were harvested each year, annual dry matter (DM) yields were determined and DM samples analysed for *in vitro* DM digestibility (DMD), water soluble carbohydrate (WSC) and crude protein (CP) concentrations in the DM. Percentage ground covered by perennial ryegrass in November 2009 was estimated visually. Twenty of the 21 cultivars were significantly (12 to 38%) higher yielding, 15 were significantly (10 to 27 g/kg) higher in mean DMD, 15 were significantly (25 to 58 g/kg) higher in mean WSC and 7 (all diploids) were significantly higher in ground cover in autumn of the third harvest year than their respective control cultivars. There were no significant differences among the varieties in mean CP over all harvests. The newest intermediate-heading cultivar (the diploid Abermagic) produced 29% more DM, was 10 g/kg higher in DMD and 51 g/kg higher in WSC, and had significantly better ground cover at the end of the third harvest year than Talbot. The newest late-heading cultivar (the tetra-

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ploid Aberbite) produced 28% more DM than S23 and was 22 g/kg higher in DMD and 58 g/kg higher in WSC, although it was similar to S23 for ground cover at the end of the third harvest year. Both of these new varieties were developed entirely or partly by population improvement at IBERS over 26 years (1980 to 2005). These results suggest that the rates of gain in DM yield under nitrogen-limiting conditions and in herbage WSC concentration from perennial ryegrass breeding can be improved by utilizing new technologies and better breeding strategies.

Keywords: breeding; digestibility; DM yield; perennial ryegrass; population improvement

Introduction

The first major advance in the domestication of perennial ryegrass (*Lolium perenne* L.) was the introduction, in the 20th century, of leafy and persistent varieties bred from wild populations (ecotypes) that had adapted to intensive grazing (Beddows 1953). For the first time, it became profitable to reseed many permanent pastures with mixtures based on perennial ryegrass. Although similar in DM yield to some other common pasture grasses, these varieties were considerably more digestible. One such variety (cv. Perma) was between 45 and 144 g/kg higher in mean *in vitro* organic matter digestibility (OMD) over all harvests than the 10 other common pasture grass species and varieties evaluated by Frame (1991). Organic matter digestibility remains the best single predictor of ruminant animal production from high forage diets (Casler 2000), determining both the metabolisable energy concentration and voluntary intake of forage.

Effective regulation and certification of commercial seed was introduced in most Western European countries, both to encourage breeding by seed companies and to control the quality of seed purchased by farmers. This included Plant Breeders Rights (which involves testing candidate varieties and assessment for distinctiveness, uniformity and stability) that are awarded to give breeders protection against fraud and enable them to collect royalties from seed sales. The introduction of Value for Cultivation and Use (VCU) trials in member states of the European Union (EU) after 1972 (following directive 72/180/EEC) provided level playing fields for competition among breeders. Such trials, and any associated lists of recommended varieties, have become an integral part of the breeding process because, to a large extent, they determine breeding priorities. Thus, it is vital that such trials are regularly updated and kept relevant to the needs of the farming industry.

Subsequent breeding was facilitated by the introduction of new technologies during the second half of the 20th century: efficient plot harvesters to measure DM yield, inexpensive computers to record and rapidly analyse data, infrared reflectance spectroscopy to predict herbage quality traits, and affordable flow cytometers to determine ploidy level. Doubling the chromosome number from diploid to tetraploid, by the use of colchicine, widened the range of perennial ryegrass types available. However, none of the current commercial varieties are transgenic and, as far as we know, none have been produced by using marker-assisted selection.

Breeding strategies

Perennial ryegrass breeding is complicated by a wide range of variation in ear emergence (heading) date (varying from early April to mid June in the UK) and the existence of two ploidy levels, diploid and tetraploid. In the UK there are significant markets for four categories of cultivar: intermediate-heading diploids, late-heading diploids, intermediate-heading tetraploids and late-heading tetraploids. Each category must be bred separately. Two different approaches have been used to raise the agronomic performance and herbage quality of perennial ryegrass beyond that of the best ecotypes.

The first, which we will call 'mainstream breeding', is designed to make as much gain as possible from a single breeding cycle. A typical cycle begins with up to 1000 pair crosses among plants selected mainly from the breeder's newest varieties and from competitor's varieties. Seed of the resultant families is multiplied outdoors in small plots separated by rye to reduce pollen flow between families. A large number (often exceeding 100000) of individual spaced plants reared from seed of these families is evaluated visually over two or three harvest years. Selected plants are clonally replicated. Subsequently they are evaluated as clonal rows or the clones are allowed to intercross and the progeny families are evaluated as plots. Four or more of best clones with similar heading date are later inter-crossed to produce the first generation seed of each new candidate variety. Several candidate varieties are produced and evaluated as small plots to identify the best for commercial development and for use as parents in further breeding. Depending on the exact procedure, each cycle takes between 9 and 15 years to complete. The process can be started again the following year, although this could involve plants in a different heading date category, or with a different ploidy.

The second approach, 'population improvement', is to form populations based on 8 to 20 carefully selected diploid parents and to progressively increase the frequency of desirable alleles within each population by recurrent selection of individuals and their progeny families. Each generation of selection takes at least 4 years. One or more candidate varieties can be produced following each selection cycle. Recurrent selection within restricted breeding populations has proved to be highly effective in accumulating desirable alleles across the genome that work well together. It has been used both to achieve very high levels of expression of single traits (e.g., the oil and protein concentrations of maize grain; Dudley and Lambert 1992) and to combine several different traits (e.g., resistances to late blight and white potato cyst nematode, good fry colour and high dry matter yield in potato; Bradshaw, Dale and Mackay 2009). But this is not easy to apply to perennial ryegrass. Pollination must be controlled strictly, selection must be sustained continuously over successive generations, and all important traits (including plot persistency) must be evaluated during each cycle of selection to avoid progressive deterioration in the population mean of any of them. As far as we know, the perennial ryegrass breeding programme at Aberystwyth (formerly the Welsh Plant Breeding Station and now incorporated in IBERS at Aberystwyth University) is the only one to sustain population improvement for 30 years (starting in 1980).

With both mainstream and population improvement procedures that involve progeny testing, it is possible to test the families in more than one way to ensure adequate seed yield, or at more than one site to ensure adequate tolerance or resistance to the range of environmental stresses, diseases and pests. Some tetraploid varieties (including Aberbite and the tetraploid candidate variety Ba13798) have been produced by a combination of recurrent selection and mainstream breeding (Wilkins and Lovatt 2006).

Progress from breeding

The best way to assess gains from breeding is to compare directly the performance of the newest perennial ryegrass varieties with old cultivars or with a sample of indigenous wild populations. This was done in Belgium, where the old cultivar Vigor (a late-heading cultivar, formerly called Melle Pasture) has been maintained and included as a control in VCU trials over a 40-year period (from 1963 to 2007; Chaves *et al*. 2009). The results were similar among varieties within the intermediate-heading and late-heading groups and within diploids and tetraploids. Relative to Vigor, total annual DM yield of candidate varieties, over 2 to 3 harvest years under infrequent cutting, increased by 3.1% per decade, ground cover in autumn increased by 21%, and resistance to crown rust increased by 44%. Herbage quality traits were not measured. In France, 21 late-heading cultivars were compared with 7 natural populations under simulated grazing at 4 sites (Sampoux *et al.* 2011). Annual dry matter yield was increased by 3.2% per decade and there were also significant improvements in persistency and resistance to crown rust.

The present report concerns the results of two trials (one of intermediate-heading and the other of late-heading cultivars and candidate varieties) that were sown at IBERS in 2006. In each trial, an old control cultivar of perennial ryegrass was included. These were the first persistent cultivars to be widely used in the UK, the intermediate-heading cv. Talbot and the late-heading cv. S23. Except for cv. Lasso, which was dropped from the list after the plots were sown, the other named cultivars are currently recommended for use in England and Wales (www.herbagevarietiesguide.co.uk). Recent research (reviewed by Edwards *et al.* 2007) shows that increasing the ratio of water-soluble carbohydrate (WSC) to crude protein (CP) in perennial ryegrass herbage reduces the output of N in the urine of grazing animals, which is likely to lead to lower losses of nitrous oxide to the atmosphere and of nitrate N (NO_3-N) to ground water. As well as DM yield and *in vitro* dry matter digestibility (DMD), the WSC and CP concentrations of the herbage were determined at every harvest over the first 3 harvest years.

Materials and Methods

The original samples of the old cultivars Talbot and S23 were early generation seed supplied by the breeders (Van De Have and the Welsh Plant Breeding Station) and stored at –12 to –16 °C at IBERS. During 2003 to 2005, fresh seed of the old cultivars was generated by isolating approximately 100 randomly-selected plants of each in separate glasshouse compartments ventilated with pollen-free air. The seed was stored at 3 °C and 30 to 40% relative humidity. In August 2006, 12 intermediate-heading perennial ryegrass varieties were sown in Trial 1 (10 cultivars, 1 candidate variety and Talbot) and 13 late-heading varieties were sown in Trial 2 (8 cultivars, 4 candidate varieties and S23). In both trials seed was drilled into a fine seedbed (August 2006) in randomized block (4 replicates) plot trails, randomized block plot trials, with guard plots at both ends of each block, and the plots were rolled. In October, the plots were mowed and marked with herbicide, each plot measuring 3 m × 1.25 m. During each of the following 3 years (2007 to 2009) the plots were harvested, at 5 cm above ground level using a Haldrup plot harvester, on 7 occasions: once in April, once shortly after ear emergence (the silage cut) and on

5 subsequent occasions at approximately 4-week intervals. All plots in the same trial were harvested on the same day. The fresh herbage from each plot was weighed, a sample (300 to 400 g) was oven-dried (80 °C) and DM yield was determined. Plots were fertilized with a compound fertilizer (23:4:13:7; $N:P_2O_5:K_2O:SO_3$) in late February and after each harvest, except the last, at rates equivalent to 57, 86, 57, 57, 57, 35 and 35 kg/ha N. *In vitro* DMD, WSC and CP concentrations of milled samples of oven-dried herbage from every harvest were determined by Near Infrared Reflectance Spectroscopy (NIRS). Dry matter digestibility was determined in the first harvest year only, while WSC and CP were determined in all three harvest years. Ten days after the final harvest in November 2009, the percentage of ground covered by perennial ryegrass in each plot was estimated visually by the same observer on a 1 to 9 scale (1=10% cover, 9=90% cover). Variance analysis of the data was carried out using GENSTAT (Genstat 9 or 10; VSN International Ltd., Hemel Hempstead, UK).

Results

Except for Lasso, which now has been removed from the list of recommended varieties for England and Wales, the cultivars and candidate varieties were significantly higher in mean total annual DM yield over the first three harvest years than their respective control variety. In the intermediate-heading group (Table 1; Trial 1) varieties yielded 12 to 29% more than the old cv. Talbot, and in the late-heading group (Table 2; Trial 2) they yielded 15 to 38% more than the old cv. S23. However, the results indicate that the relative contribution of the first silage cut and the other (leafy) cuts to the total DM yield varied considerably among some of the varieties.

Table 1. Mean values (over 3 years) for dry matter yield, *in vitro* dry matter digestibility (DMD) and concentrations of water soluble carbohydrate (WSC) and crude protein (CP) in dry matter, and the mean percentage of ground covered at the end of the third year for 11 intermediate-heading perennial ryegrass cultivars and candidate varieties and the control variety Talbot

Variety§	Dry matter yield‡ (t/ha)			DMD (g/kg)	WSC (g/kg)	CP (g/kg)	Ground cover (%)
	Silage cut	Other cuts	Total annual				
Talbot	5.57 (100)†	7.44 (100)	13.01 (100)	763	199	157	49
Premium	6.67 (120)	7.86 (106)	14.53 (112)	756	195	152	54
Aberdart	6.02 (108)	9.27 (125)	15.30 (118)	766	226	147	76
Aberstar	6.32 (113)	9.70 (130)	16.02 (123)	768	229	147	67
Abermagic	6.69 (120)	10.14 (136)	16.83 (129)	773	250	142	61
Abersweet	6.33 (114)	9.31 (125)	15.64 (120)	775	250	142	57
Abergreen	5.83 (105)	10.33 (139)	16.16 (124)	769	243	139	70
Aubisque (T)	6.63 (119)	8.28 (111)	14.91 (115)	777	217	156	55
Magician (T)	6.21 (111)	8.62 (116)	14.83 (114)	777	213	144	47
Aberglyn (T)	6.57 (118)	8.50 (114)	15.07 (116)	767	201	159	49
Ba13743 (T)	6.44 (116)	8.51 (114)	14.95 (115)	771	203	158	49
$LSD_{0.05}$	0.631	0.851	1.242	8.3	24.9	–	6.9
Significance	**	***	***	***	***		***

§ T denotes tetraploid.
‡ 7 cuts per annum.
† Relative (%) to cv. Talbot.

The intermediate-heading cv. Abergreen was similar in DM yield to cv. Talbot at the first silage cut but was 39% higher at the other cuts and the late-heading variety Aberchoice was similar to cv. S23 at the first silage cut but 36% higher yielding at the other cuts. This indicates that the percentage gains in DM yield over the respective control varieties and the exact ranking of some of these varieties in total annual DM yield would have been different if a management (such as simulated grazing throughout) that altered the contribution of the first silage cut to total annual yield had been applied. But other varieties (Abermagic, Aberglyn, Abercraigs and Ba13798) showed a similar proportional yield improvement over their respective control varieties at both the first silage cut and at the other cuts.

Differences among varieties in mean *in vitro* DMD over all harvests in 2007 were highly statistically significant in both trials (Tables 1 and 2). In the Trial 1, only 4 (the diploids AberMagic and Abersweet, and the tetraploids Aubisque and Magician) were significantly (10 to 14 g/kg) higher in DMD than cv. Talbot. All the varieties in the late-heading group were significantly (16 to 27 g/kg) higher in mean DMD than cv. S23. Differences in mean WSC concentration over all harvests were also highly statistically significant and were greater than for DMD. Five diploid varieties (Aberdart, Aberstar, Abermagic, Abersweet and Abergreen) were significantly higher (27 to 51 g/kg) in mean WSC than cv. Talbot, and all the late-heading varieties, except Lasso, were significantly higher (35 to 58 g/kg) in mean WSC than cv. S23. In both trials, differences among varieties in mean CP concentration over all harvests were small and not statistically significant.

Table 2. Mean values (over 3 years) for dry matter yield, *in vitro* dry matter digestibility (DMD) and concentrations of water soluble carbohydrate (WSC) and crude protein (CP) in dry matter, and mean percentage of ground covered by ryegrass at the end of the third year for 11 late-heading perennial ryegrass cultivars and candidate varieties and the control variety S23

Variety[§]	Dry matter yield[‡] (t/ha)			DMD (g/kg)	WSC (g/kg)	CP (g/kg)	Ground cover (%)
	Silage cut	Other cuts	Total annual				
S23	5.57 (100)[†]	7.86 (100)	13.43 (100)	765	182	163	46
Lasso	6.08 (109)	8.39 (107)	14.47 (108)	767	189	158	51
Aberavon	6.68 (120)	10.08 (128)	16.76 (125)	781	217	154	62
Aberchoice	5.54 (99)	10.69 (136)	16.23 (121)	784	239	150	52
Ba13942	5.46 (98)	10.36 (132)	15.82 (118)	781	237	148	58
Condesa (T)	5.50 (99)	9.91 (126)	15.42 (115)	788	223	154	48
Twymax (T)	6.72 (121)	9.70 (123)	16.42 (122)	787	218	153	48
Abercraigs (T)	7.05 (127)	9.64 (123)	16.69 (124)	792	218	155	46
Ba13744 (T)	5.91 (106)	9.53 (121)	15.44 (115)	784	211	154	44
Ba13798 (T)	7.64 (137)	10.94 (139)	18.58 (138)	790	240	146	46
Aberbite (T)	6.70 (120)	10.49 (133)	17.18 (128)	787	236	150	45
Ba13851 (T)	6.27 (113)	10.39 (132)	16.56 (123)	787	224	152	40
$LSD_{0.05}$	0.362	1.143	1.275	6.2	17.8	–	5.4
Significance	***	***	***	***	***		***

[§] T denotes tetraploid.
[‡] 7 cuts per annum.
[†] Relative (%) to cv. S23.

Some diploid varieties, but none of the tetraploid varieties, were significantly higher for ground cover at the end of the third harvest year than their respective diploid control cultivar. This is a good indicator of relative persistency. In the intermediate-heading group AberDart, Aberstar, Abermagic, Abersweet and Abergreen were 8 to 27 percentage points higher in ground cover than cv. Talbot, and in the late-heading group Aberavon, Aberchoice and Ba13942 were 6 to 16 percentage points higher than cv. S23. Only one variety (the late-heading tetraploid Ba13851) was significantly lower for ground cover than its respective control variety (S23).

Discussion

These results come from only one site and one particular management regime. Thus, the gains in annual DM yield of the currently recommended cultivars over the old cultivars Talbot and S23 should be interpreted with caution. Nevertheless, they do indicate a substantial improvement in yield from breeding. In earlier trials, Talbot was similar in dry matter yield to locally-adapted Belgian ecotypes (Limbourg and Leconte 1997), and thus these results give an indication of the increase in yield that could be expected following the reseeding of an old ryegrass-dominated pasture with the newest cultivars available. Except for Premium and Lasso, the diploid varieties had a higher ground cover than cv. Talbot or cv. S23 at the end of the third harvest year. Ranking in ground cover scores have agreed well with ranking in shoot density in previous experiments (for example Wilkins, Lovatt and Hayes 2007). Thus the diploids can be expected to persist better and thus to have a greater advantage over the old varieties in dry matter in subsequent harvest years. However, none of the tetraploid varieties were significantly higher in ground cover than their respective control cultivar.

Some intermedate-heading varieties (including the high yielding and persistent Abermagic) and most of the late-heading varieties showed modest improvement in mean DMD over all harvests in 2007 compared with their respective control cultivar. This can be attributed primarily to higher WSC concentration, WSC being completely digested. Such differences, combined with improvements in dry matter yield, can have substantial effects on animal production. Charolais cross steers grazing cv. Aberdart (mean WSC 155 to 231 g/kg) gained between 18% and 35% more live weight than those grazing cv. Fennema (mean WSC 133 to 205 g/kg) (Marley *et al*. 2005). Recent research results on sheep (http://randd.defra.gov.uk/Document.aspx?Document=AC0209_10114_FRP.pdf) showed that using cultivars with high WSC reduced methane emissions per unit of live weight gain by approximately 20%.

The two experiments included six diploid cultivars that were produced by 'population improvement' but only two that were produced by 'mainstream breeding' (Premium and Lasso). Newer diploid varieties, produced by 'mainstream breeding', with higher DM yield have become available recently (www.herbagevarieties-guide.co.uk). Thus the current results do not provide a firm basis for comparing progress from the two breeding methods. They do, however, provide a measure of progress during 26 years of population improvement at IBERS (1980 to 2005). The newest intermediate-heading cultivar (the diploid Abermagic) produced 29% more DM, had 10 g/kg higher DMD and 51 g/kg higher WSC, and had significantly higher ground cover at the end of the third harvest year than cv. Talbot. The newest late-heading cultivar (the tetraploid Aberbite) produced 28% more dry matter than cv. S23 had 22 g/kg higher DMD and 58 g/kg higher WSC, although it was similar to cv. S23 for ground cover. These gains in annual DM yield of approximately 9% per decade compare well with the gains of approximately 3% per decade from breeding perennial ryegrass over the previous 40 years (Chaves *et al.* 2009; Sampoux *et al*. 2011). Furthermore, they were accompanied by significant improvement in herbage quality.

Acknowledgements

This work was funded jointly by the UK Department for Environment, Food and Rural Affairs (Defra) and Germinal Holdings Ltd.

References

Beddows, A.R. 1953. The ryegrasses in British agriculture; a survey. Welsh Plant Breeding Station Bulletin Series H, No. 17, Aberystwyth, Wales.

Bradshaw, J.E., Dale, M.F.B. and Mackay G.R. 2009. Improving the yield, processing quality and disease and pest resistance of potatoes by genotypic recurrent selection. *Euphytica* **170:** 215–227.

Casler, M.D. 2000. Breeding forage crops for increased nutritional value. *Advances in Agronomy* **71:** 51–107.

Chaves, B., De Vliegher, A., Van Waes, L., Carlier, L. and Marynissen, B. 2009. Change in agronomic performance of *Lolium perenne* and *Lolium multiflorum* varieties in the past 40 years based on data from Belgium VCU trials. *Plant Breeding* **128:** 680–690.

Dudley, J.W. and Lambert, R.J. 1992. Ninety generations of selection for oil and protein content in maize. *Maydica* **37:** 81–87.

Edwards, G.R., Parsons, A.J., Rasmussen, S. and Bryant, R.H. 2007. High sugar ryegrasses for livestock systems in New Zealand. *Proceedings of the New Zealand Grassland Association* **69:** 161–171.

Frame, J. 1991. Herbage production and quality of a range of secondary grass species at five rates of fertilizer nitrogen application. *Grass and Forage Science* **46:** 139–151.

Limbourg, P. and Leconte, P.H. 1997. Compared productivity of local ecotypes and selected cultivars of perennial ryegrass (*Lolium perenne* L.) in high Belgium. Proceedings of the 18th International Grassland Congress, Canada, **1:** 15–16.

Marley, C.L., Davies, D.A., Vale, J.E., Evans, J.G., Scollan, N.D., Moorby, J.M., Macrae, J.C. and Theodorou, M.K. 2005. Effects of upland pastures sown with two contrasting *Lolium perenne* varieties on the performance of beef steers when compared to steers grazing permanent pastures. *Proceedings of the British Society of Animal Science Annual Conference, York*, p. 194.

Sampoux, J.P., Bandouin, P., Bayle, B., Béguier, V., Bourdon, P., Chosson, J.-F., Deneufbourg, F., Galbrun, C., Ghesquière, M., Noël, D., Pietraszek, W., Tharel, B. and Viguié, A. 2011. Breeding perennial ryegrass for forage usage: an experimental assessment of trait changes in diploid perennial ryegrass (*Lolium perenne* L.) cultivars released in the last four decades. *Field Crop Research* **123:** 117–129.

Wilkins, P.W. and Lovatt, A.J. 2006. Chromosome doubling as a means of exploiting heterosis in perennial ryegrass. In "Breeding and Seed Production for Conventional and Organic Agriculture". *Proceedings of the 26th EUCARPIA Fodder Crops and Amenity Grasses Section and the 16th Medicago spp. Group Joint Meeting, Perugia, Italy,* pages 52–55.

Wilkins, P.W., Lovatt, J.A. and Hayes, R.C. 2007. Effect of a stay green locus on the dry matter yield, nitrogen yield and shoot density of perennial ryegrass. *Grass and Forage Science* **62:** 453–458.