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## Persistency, yield, and silage quality of *Festulolium* cultivars over a consecutive five-year period under a mild Atlantic climate

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### Abstract

*Festulolium* are hybrids between species within the *Lolium* and *Festuca* genera. They are attractive candidates for hybridisation due to their complementary characteristics in the context of forage production and environmental challenges, with *Lolium* exhibiting greater digestibility and *Festuca* possessing superior resilience to abiotic stress. To establish the extent of the representation of these characteristics in different *Festulolium* hybrids, a five-year field experiment was undertaken in a mild Atlantic climate. Four alternative species combinations and six pure species (controls) were evaluated for dry matter yield and persistency from 2014 to 2018, and various nutritional parameters in the first silage cut were measured from 2015 to 2017. *Festulolium* hybrids derived from *L. multiflorum* were found to group with both *Lolium* and *Festuca* pure species for a mean ground score to evaluate persistency ( $P < 0.05$ ). Hybrid types of the *L. perenne* lineage were found to have ground score values more alike to their *Lolium* component and distinct from the *F. pratensis* pure species. In terms of dry matter yield, the majority of *L. multiflorum* hybrids observed formed a higher yielding group with the tetraploid *L. multiflorum* pure species and distinct from diploid *F. pratensis* pure species, whereas all other hybrid types and controls shared groups in common. For the nutrient content parameters including dry matter digestibility, water soluble sugars, and buffering capacity, *Lolium* pure species exhibited more favourable values, whereas hybrids were largely found to display intermediate to low values. *Festuca* pure species consistently exhibited poorer values with the exception of crude protein content where *F. pratensis* displayed the highest values and also the 2017 measurement of buffering capacity. In conclusion, the *Festulolium* hybrids shared characteristics with both parental types, but they often showed a greater likeness to the *Lolium* component.

Additional key words: *Festuca*, *Lolium*, ground cover, nutritional value.

### Introduction

Forages such as *Lolium* and *Festuca* represent a low cost source of feed in ruminant agriculture in temperate regions worldwide (Wilkins and Humphreys 2003). Areas in Europe, which traditionally support forages, are projected to experience increased instances and intensities of extreme weather such as drought and flooding due to climate change (Roudier *et al.* 2016). Forages with wide adaptability and also the productivity potential to support intensive grazing systems are required to meet these challenges (Lin 2011,

O'Donovan *et al.* 2011). *Festulolium* is natural and artificial interspecific hybrid derived from the genera *Festuca* and *Lolium*, and species from each of these genera exhibit such complementary characteristics in the context of forage production, qualifying them as suitable candidates for hybridisation (Thomas and Humphreys 1991). *Lolium* species are widespread in ruminant agriculture in temperate regions globally, with Italian ryegrass (*L. multiflorum*) and perennial ryegrass (*L. perenne*) cultivars being most widely sown (Wilkins and Humphreys 2003). Perennial ryegrass cultivars are considered high performance forage

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Abbreviations: BC - buffering capacity; CP - protein content; DMD - dry matter digestibility; DMY - dry matter yield; GC - ground cover score; Fa - *Festuca arundinacea*; Fg - *Festuca glaucescens*; Fp - *Festuca pratense*; Lm - *Lolium multiflorum*; Lp - *Lolium perenne*; PPI - pasture profit index; WSS - water soluble sugars.

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cultivars, withstanding extended periods of grazing and demonstrating favourable palatability and productivity in response to inputs. Italian ryegrass is a biennial forage crop with rapid establishment but comparatively lower persistency, suited for short-term swards. Fescues are not noted for their agronomic performance; they exhibit poorer palatability and lower digestibility relative to ryegrasses. Fescues, however, display great resistance to abiotic stresses, such as drought and flooding, attributed to well-developed root systems. Meadow fescue (*F. pratensis*) or tall fescue (*F. arundinacea*) most widely sown for forage, typically utilised species rich permanent pasture.

The level of integration of genomic material in hybrids is dependent on breeding strategy, through the generation of an amphiploid hybrid or by way of introgression. The former entails each parent donating at least one pair of chromosomes, though often resulting in *Lolium* chromatin dominating the proportion of genetic material (Kopecky *et al.* 2017). Introgression involves a backcrossing strategy with the ryegrass genome representing a greater proportion of the genomic material (Humphreys *et al.* 2003). Both methods allow for grasses with adequate yield and nutrition and increased hardiness and persistency providing ecosystem services (Macleod *et al.* 2013, Grogan *et al.* 2018). *Festulolium* has been shown to out yield perennial ryegrass on marginal land for biomass production, illustrating the potential of hybrids under suboptimal growing conditions (Meehan *et al.* 2017). Though the production model in Ireland is based on grazed grass, silage is a significant constituent in beef and dairy production because grass growth is seasonal and yield varies within the year. The role of silage is to “conserve” grass nutrients and meet demand in the production system during the winter period (Shalloo *et al.* 2004, Mayne and O’Kiely 2005, McEvoy *et al.* 2011). As it directly influences productivity, dry matter yield (DMY) has been the primary focus of breeding programmes in the improvement of perennial ryegrass cultivars, including those destined for silage production (McDonagh *et al.* 2016). Persistency refers to stability of yield and this relationship with production has led persistency to be identifiable as an economically important trait. It is assessed both for pasture profit index (PPI), the Irish index for economic value of cultivars and value cultivation and use, for cultivars to be published on recommended lists (Grogan and Gilliland 2011, O’Donovan *et al.* 2016). Persistency is widely quantified by ground score cover (GC), a 1 - 9 scale, for referral to recommended lists (Grogan and Gilliland 2011). Grass silage production involves mechanical cuts, with first and second cuts responsible for the greatest proportion of dry matter yield. Subsequent preservation under anaerobic conditions requires adequate amount water soluble sugars (WSS) to ensure production of lactic acid and fermentation through microbial activity, and a relatively low buffering capacity (BC; the acid required to lower the pH from 6 to 4) (Muck *et al.* 1988, Davies *et al.* 1998). Thus the nutrient profile of cultivars not only impacts the quality of animal nutrition but also the quality of silage production.

This trial was one part of a multinational collaboration

coordinated by the Eucarpia Fodder Crops and Amenity Section *Festulolium* Working Group whereby the same cultivars were assessed across Europe in nine field trials and under contrasting growth conditions (Ghesquière *et al.* 2016). Partial results from this trial has been published previously, with data over a 3 year period (Grogan *et al.* 2018). The aim of this study was to further investigate the agronomic performance of *Festulolium* hybrids, in comparison to their *Lolium* and *Festuca* parental species, under conservation management over a five year period under an Atlantic climate. Specifically, persistency, dry matter yield, and nutrient value of cultivars of the hybrid types were observed and whether the merits of each species are represented in their hybrids was investigated.

## Materials and methods

**Experimental design:** This experiment was carried out in Athenry, Co. Galway, adjacent to the DAFM recommended list trial site (53°18’N, 8°45’W), on a peaty loam soil. Meteorological data (Fig. 1 Suppl.) were obtained from a weather station located on site.

Cultivars were donated, through *EUCARPIA*, from breeders across Europe, including six control species, representing *Lolium* and *Festuca* hybrid parental species, and 15 hybrids (Table 1 Suppl.). Trials were sown on 21<sup>st</sup> May, 2013 in field plots of 11.41 m<sup>2</sup> utilising a randomised complete block design with plots replicated in 3 blocks. Nitrogen (N) fertiliser (*High Sulphur CAN*, 27.5 % N + 7 % S) was applied 6 times for a total annual rate of 31 g(N) m<sup>-2</sup>. Based on annual soil analysis, no application of phosphorus was used, and potassium was applied twice a year to a total of 40 g(K) m<sup>-2</sup>. The trial was cut using a conservation/silage protocol, incorporating two silage cuts and three after-grass cuts per year. The first two cuts were taken when the trial had reached stem extension/heading growth stage, while the later harvests were cut to schedule at vegetative growth stages.

**Measurements:** Plots were harvested using a *Haldrup* plot harvester at cutting heights of *ca.* six cm. Total plot yield was recorded and a subsample (about 300 g) was oven-dried at 80 °C for 16 h to determine dry matter yield (DMY). Dried samples from the first silage cut in mid-May (in three years 2015, 2016 and 2017), were analysed utilising established wet chemistry methods at *Teagasc Grange*. A subsample was dried at 40 °C for 48 h, milled through a 1 mm screen and analysed for *in vitro* DM digestibility (DMD, Tilley and Terry 1963), crude protein content (CP, using the *LECO FP-628* nitrogen analyser), water WSS (using the anthrone method, Thomas 1977) and buffering capacity (BC, Playne and McDonald 1966). Ground cover scores on a 1 to 9 scale, where 1 represented bare ground and 9 a fully grass-covered plot, were recorded by visual assessment at the end of each harvest year (procedure of visual scoring described in Lynch *et al.* 2015).

**Statistical analysis:** Analysis was carried out in *R* (*R Core Team 2014, version 3.4.1; https://CRAN.R-project.org*)

package=emmeans). To investigate relationships between the measured parameters, type III analysis of variance (*ANOVA*) was implemented from the *car* package (Fox and Weisberg 2019).

To further investigate the response of DMY of cultivars over time, a generalised least squares model was carried out, where correlation between years was modelled using a repeated measures covariance structure in the *nlme* package (Pinheiro *et al.* 2020; R Core Team: *nlme*: Linear and Nonlinear Mixed Effects Models - R package version 3.1-148, <https://CRAN.R-project.org/package=nlme>). The response of total DMY was measured using the following models:

$$Y_{ijk} = \mu + T_i + R_j + C_k + (T_i \times C_k) + \epsilon_{ijk} \text{ (model 1)}$$

$$Y_{ijl} = \mu + T_i + R_j + H_l + (T_i \times H_l) + \epsilon_{ijl} \text{ (model 2)}$$

Where  $Y_{ijk}$  denotes the total DMY for the year (T) i (2013 to 2018), replicate (R) within a block j (1-3), cultivar (C) k (1-21) and  $\epsilon_{ijk}$  denotes the residual error. To investigate the response of DMY to hybrid type/pure species control the follow model was formulated where  $Y_{ijl}$  denotes the total DMY for the year i (2013 to 2018), replicate within a block j (1-3), hybrid type/pure species control 1 (1-10) and  $\epsilon_{ijl}$  denotes the residual error. A similar model was formulated to investigate the response of ground score to year:

$$P_{ijk} = \mu + T_i + R_j + C_k + (T_i \times C_k) + \epsilon_{ijk} \text{ (model 3)}$$

$$P_{ijl} = \mu + T_i + R_j + H_l + (T_i \times H_l) + \epsilon_{ijl} \text{ (model 4)}$$

Where  $P_{ijk}$  denotes the total ground score for the year (T) i (2013 to 2018), replicate (R) within a block j (1-3), cultivar (C) k (1-21) and  $\epsilon_{ijk}$  denotes the residual error. To investigate the response of DMY to hybrid type/pure species control (model 3). The follow model was formulated where  $Y_{ijl}$  denotes the total ground score for the year (T) i (2013 to 2018), replicate (R) within a block j (1-3), hybrid type/pure species control (H) denoted by 1 (1-10) and  $\epsilon_{ijl}$  denotes the residual error. A similar model to investigate the response of ground score to year (model 4). Post-hoc testing for significant differences between means was carried out using the *emmeans* package (Lenth 2020; <https://CRAN.R-project.org/package=emmeans>). Assumptions for analysis were checked and met.

## Results

Overall the trial had adequate ground cover (GC) for the first four years after field establishment, however, ground cover deteriorated in year 5 (Fig. 1). The mean GC for all years varied significantly from one another, besides the years 2015 and 2016, which were found not to be significantly different (Table 2 Suppl.). The lowest

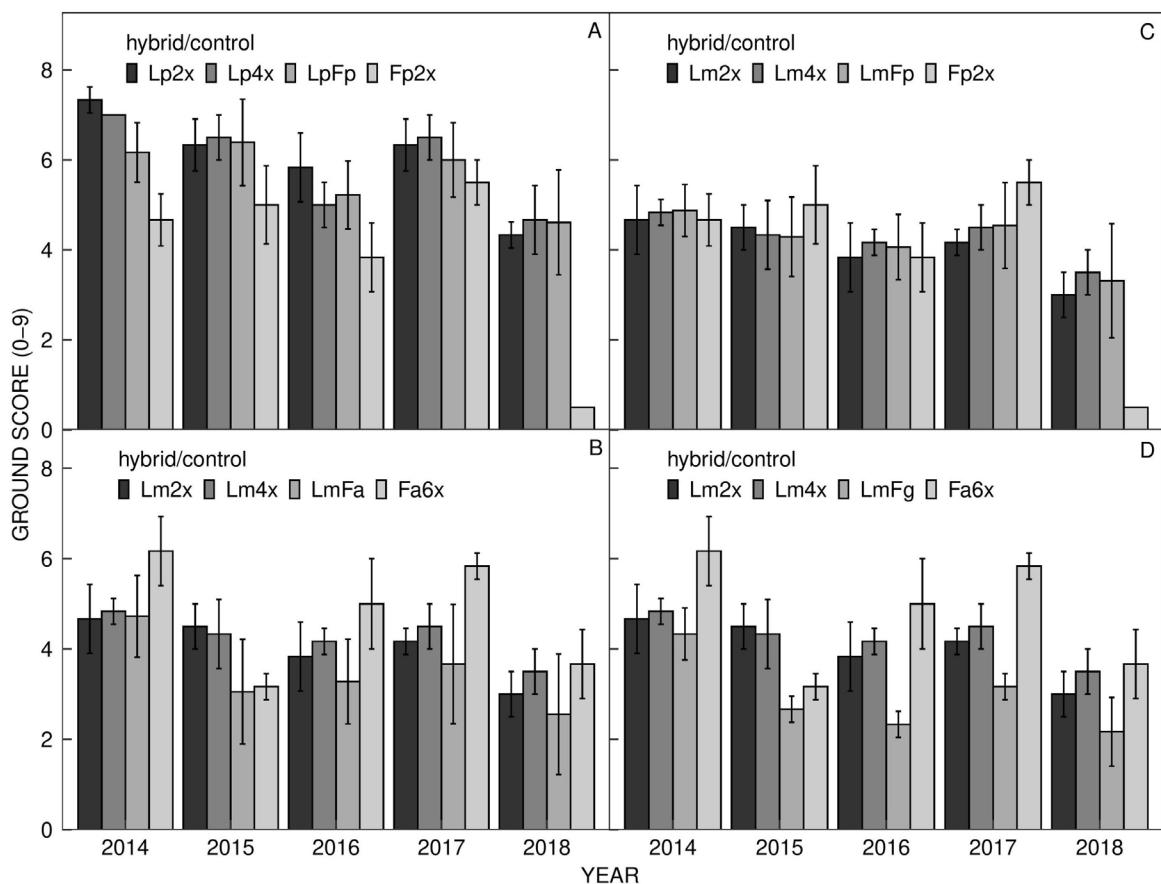


Fig. 1. Trends in the mean ground cover score for each *Festulolium* hybrid type: A - *Lolium perenne* × *Festuca pratensis*, B - *L. multiflorum* × *F. arundinacea*, C - *L. multiflorum* × *F. pratensis*, and D - *L. multiflorum* × *F. arundinacea* var. *glaucescens*, alongside their respective control species over a five-year period.

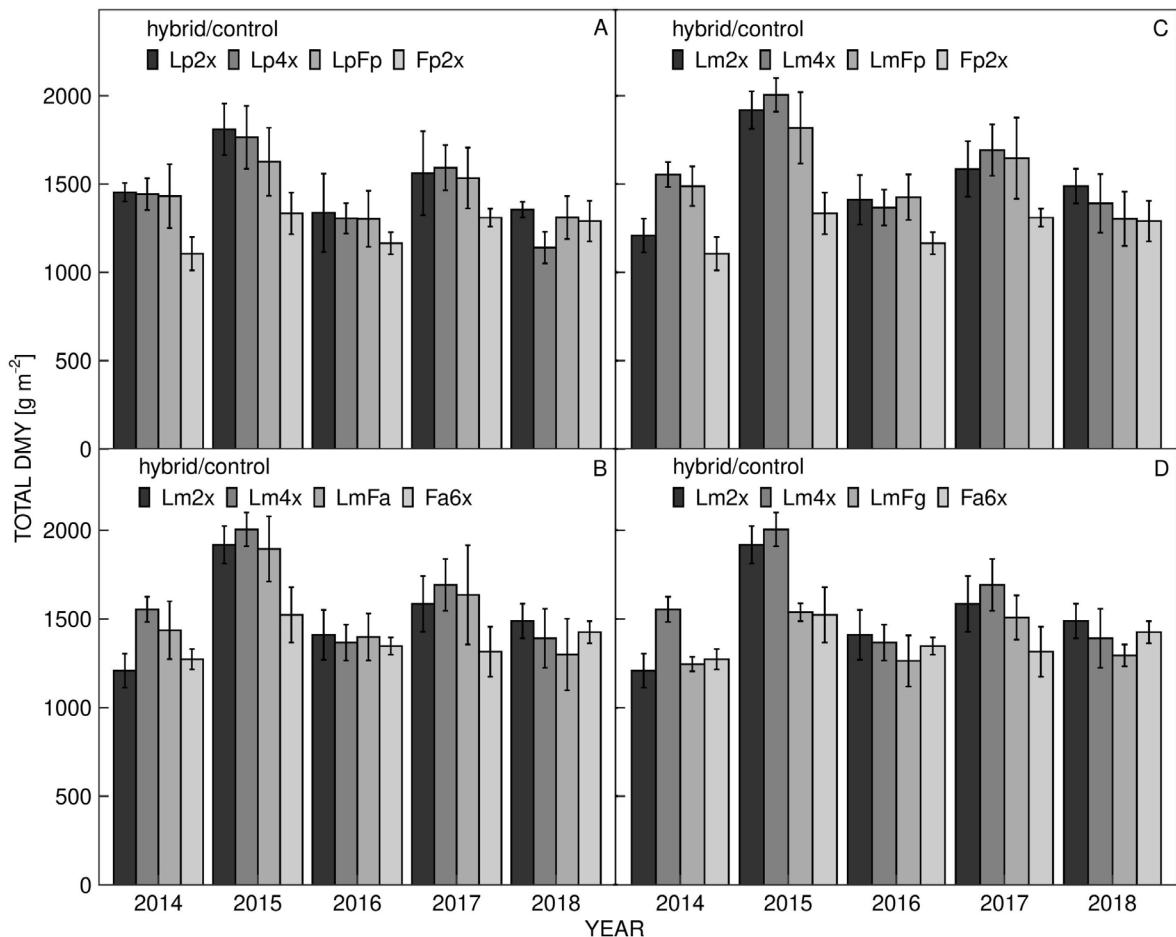


Fig. 2. Trends in the mean dry matter yield (DMY) for each Festulolium hybrid: A - *Lolium perenne* × *Festuca pratensis*, B - *L. multiflorum* × *F. arundinacea*, C - *L. multiflorum* × *F. pratensis*, and D - *L. multiflorum* × *F. arundinacea* var. *glaucescens* alongside with their respective control species over a five-year period.

GC scores were observed in 2018, and the highest GC scores were found in 2014 (Table 3 Suppl.). Year, cultivar, hybrid type, and block were all found to have a significant effect on GC score, and there were significant interactions between year and cultivar, and between year and hybrid type (Tables 2 and 4 Suppl.).

No significant differences were observed within hybrid types for the first three years, however within the *L. multiflorum* × *F. pratensis* hybrid type, Sulino was found to vary significantly from AberNiche, Perseus, and Achilles for mean GC score in 2018 (Table 5 Suppl.). While within *L. multiflorum* × *F. arundinacea* hybrids, Lofa and FuRs0352 varied significantly in 2017 and 2018, and FuRs0352 and Bečva exhibited significant differences from one another in 2018 (Table 5 Suppl.). There were no significant differences observed between hybrids within *L. perenne* × *F. pratensis* for GC score within any experimental year (Table 5 Suppl.). Of the pure species, both tetraploid and diploid *L. perenne* cultivars exhibited the highest GC over time, followed by pure *F. pratensis* (Table 6 Suppl.).

From the *Festulolium* hybrids the best GCs were obtained from *L. perenne* × *F. pratensis* hybrids, with the cv. Fabel exhibiting the highest GC in comparison

to all other cultivars (Table 6 Suppl.). The poorest GC from the beginning to the end of the trial was obtained by the *Festulolium* hybrid Lueur of the *L. multiflorum* × *F. arundinacea* var. *glaucescens* type, while FuRs0352, a *L. multiflorum* × *F. arundinacea* hybrid, was the cultivar with the lowest over all mean GC (Table 6 Suppl.). Three statistically significant groups are present from mean GC across 5 years, with tetraploid *L. perenne* and hybrid *L. perenne* × *F. pratensis* belonging to the highest performing group, while the remaining hybrids and *F. pratensis* allocated in the group with the lowest GC. The remaining pure species overlap with these groups and share a group among themselves (Table 6 Suppl.).

The first cut of silage accounted for, on average, 46.6 % of total DMY, while the second cut accounted for an average of 26.2 % of total DMY. The remaining percentage of total DMY was comprised of the subsequent three cuts. Similar trends in total DMY production were observed across hybrid types, with the establishment year exhibiting lower yields, increasing in 2015, declining in 2016, recovering in 2017, and then finally declining in 2018 (Fig. 2). This was reflected when comparing the mean yield for each year, the mean yields for 2014 and 2016 did not differ significantly, likewise, the mean yield

Table 1. The mean total dry matter yield (DMY, g m<sup>-2</sup>) per cultivar for each year, and the overall mean for a five-year period, including a mean and a standard deviation (SD) for yield per hybrid type for each year and a five-year mean. Fa - *Festuca arundinacea*, Fg - *Festuca glaucescens*, Fp - *Festuca pratense*, Lm - *Lolium multiflorum*, Lp - *Lolium perenne*. Means within a column with different letters in superscript differ significantly ( $P < 0.05$ ).

Cultivars	2014	2015	2016	2017	2018	Mean	SD
Pure species							
AberBite (Lp4x)	1443	1764	1306	1592	1140	1449 <sup>a,b</sup>	246
AberMagic (Lp2x)	1453	1810	1337	1561	1355	1503 <sup>a,b</sup>	226
Caballo (Lm4x)	1554	1004	1367	1692	1391	1607 <sup>b</sup>	262
Podium (Lm2x)	1209	1918	1411	1585	1488	1522 <sup>a,b</sup>	263
Fure (Fp2x)	1106	1334	1165	1310	1290	1241 <sup>a</sup>	121
Kora (Fa6x)	1273	1523	1348	1315	1425	1377 <sup>a,b</sup>	127
LpFp							
Fabel	1504	1650	1282	1574	1307	1464	221
FuRs0142	1457	1611	1377	1484	1320	1450	220
Prior	1332	1612	1250	1543	1305	1410	180
Mean	1431	1626	1303	1534	1311	1441 <sup>a,b</sup>	-
SD	181	193	158	172	121	-	204
LmFa							
FuRs0352	1267	1698	1250	1543	1304	1396	240
Bečva	1547	1949	1486	1744	1237	1593	380
Lofa	1496	2036	1436	1823	1261	1610	299
Mean	1437	1894	1398	1636	1300	1533 <sup>b</sup>	-
SD	163	183	132	132	202	-	285
LmFp							
AberNiche	1383	1864	1402	1499	1105	1451	279
Agula	1455	1704	1285	1453	1251	1430	243
Felopa	1479	1775	1398	1582	1224	1492	178
Sulino	1426	1704	1362	1480	1332	1461	252
Hostyn	1534	1925	1577	1869	1396	1660	252
Perseus	1619	1936	1515	1872	1475	1683	217
Perun	1483	1753	1438	1770	1368	1544	246
Achilles	1522	1882	1426	1644	1368	1568	234
Mean	1488	1818	1425	1646	1303	1536 <sup>b</sup>	-
SD	112	201	129	230	154	-	246
LmFg							
Lueur	1245	1538	1263	1508	1294	1370 <sup>a,b</sup>	153

for 2018 and 2016 did not exhibit significant differences (Table 7 Suppl.). Year and cultivar had a significant effect on DMY, with the interaction among the cultivars was not found to be significant. Statistically significant differences in yield were also observed amongst hybrid types with a significant interaction with year. *L. multiflorum* × *F. pratensis* hybrids Hostyn and Perseus outperformed other cultivars of the same hybrid type in yield, though there was no statistical significance between the cultivars for each year. Similarly, the *L. multiflorum* × *F. arundinacea* hybrid Bečva performed well across years in comparison to *Lolium* pure species, however mean DMY for the cultivar declined significantly from 2015 to 2016, with 2018 yields also found to be significantly different from 2015. Lower

yielding cultivars and pure species exhibited no such significant decrease in yield. Overall, cultivars, which exhibited lower yield in earlier years remained consistent, while higher yielding cultivars declined in yield in 2018.

Two distinct groups were observed when the mean yield of hybrid types were compared, *L. multiflorum* tetraploid control was allocated to the highest yielding group, along with the *Festulolium* hybrids *L. multiflorum* × *F. pratensis* and *L. multiflorum* × *Festuca* (Table 1). *Festuca pratensis* diploid plant was categorised into the second, lower yielding group, with the remaining hybrids/pure species overlapping with either group.

Year, cultivar, and hybrid type all had a highly significant effect ( $P < 0.001$ ) on all recorded nutritional

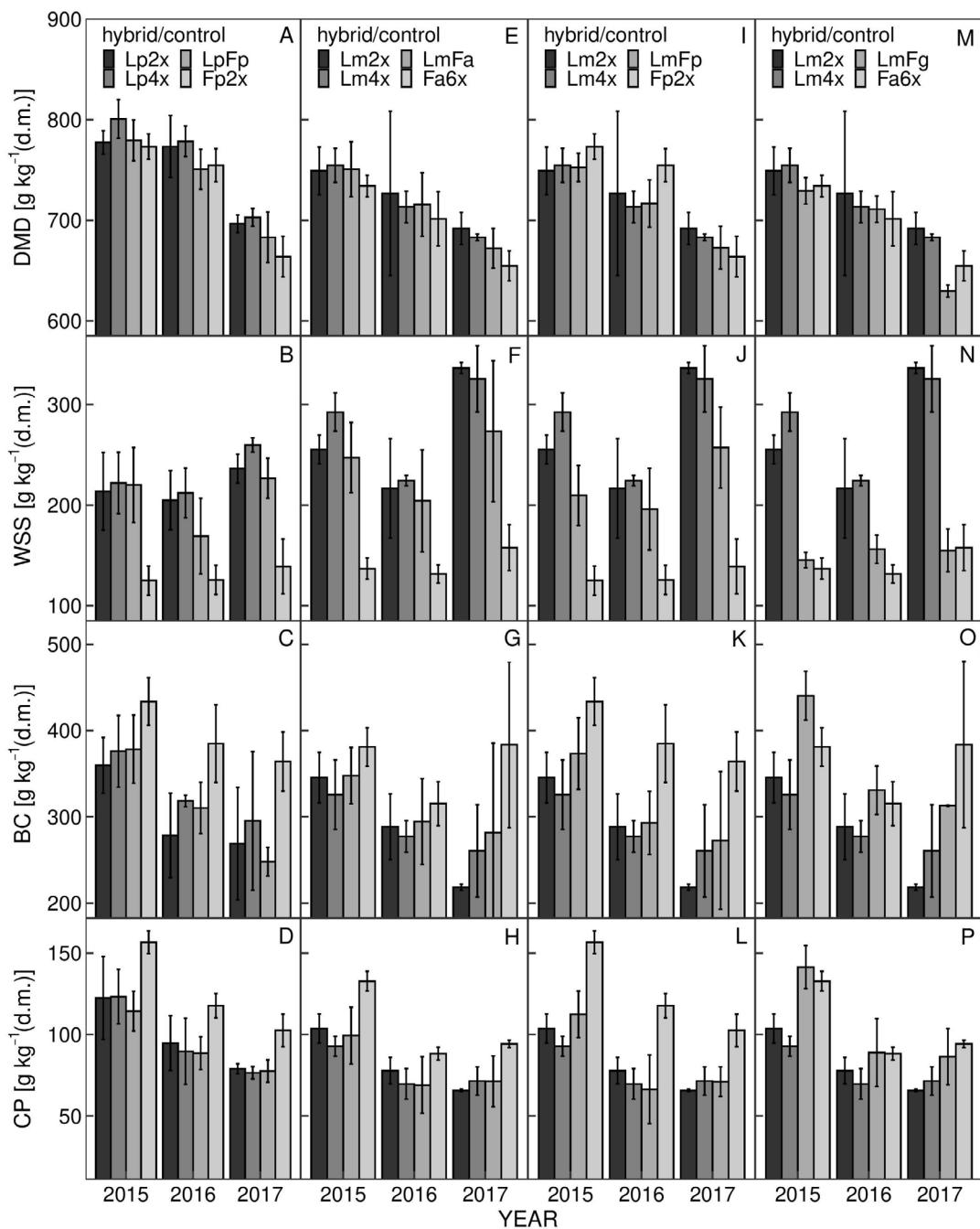


Fig. 3. Quality parameters over three years, dry matter digestibility (DMD), water soluble sugars (WSS) buffering capacity (BC), and crude protein (CP) for each *Festulolium* hybrid: A - D - *Lolium perenne* × *Festuca pratensis*, E - H - *L. multiflorum* × *F. arundinacea*, I - L - *L. multiflorum* × *F. pratensis*, M - P - *L. multiflorum* × *F. arundinacea* var. *glaucescens* alongside with their respective control species over a three year period.

parameters of the first silage cuts over a three year period (2015 to 2017) (Tables 2 and 4 Suppl.). There were no significant interactions between year and cultivar or year and hybrid type for any for the parameter, with the exception of a significant interaction observed in WSS between year and cultivar.

Dry matter digestibility steadily declined over the three year period, across all hybrid types and pure species (Fig. 3). The hybrids exhibiting the highest mean DMDs in 2015,

2016, 2017 were observed to be *L. perenne* × *F. pratensis* [mean = 689.96 g kg⁻¹(d.m.), standard deviation = 46.90] and the pure species; and highest mean over all was the tetraploid *L. perenne* [mean = 760.67 g kg⁻¹(d.m.), SD = 46.24]. *L. multiflorum* × *F. arundinacea* var. *glaucescens* was the hybrid type which exhibited the lowest mean DMD [mean = 689.96 g kg⁻¹(d.m.), SD = 46.90], and *F. arundinacea* was the control with lowest DMD over all three years [mean = 696.70 g kg⁻¹(d.m.)],

SD = 38.23].

Values for WSS declined in 2016 and recovered in 2017, and similar to the trends in DMD, trends were largely conserved across all hybrid types and pure species. Higher WSS was observed in *L. multiflorum* pure species, *L. perenne* and hybrids exhibiting intermediate to lower WSS, and *Festuca* pure species the lowest (Fig. 3). The hybrid type which had the highest observed mean WSS was *L. multiflorum*  $\times$  *F. arundinacea* [mean = 241.67 g kg<sup>-1</sup>(d.m.), SD = 59.36], with tetraploid *L. multiflorum* the pure species control with the highest mean WSS [mean = 280.82 g kg<sup>-1</sup>(d.m.), SD = 45.59]. The hybrid *L. multiflorum*  $\times$  *F. arundinacea* var. *glaucescens* exhibited the lowest WSS in 2015, 2016, 2017 [mean = 152.22 g kg<sup>-1</sup>(d.m.), SD = 14.25] and the pure species *F. pratensis* [mean = 129.92 g kg<sup>-1</sup>(d.m.), standard deviation = 18.38].

Higher BC was observed in *Festuca* pure species and *Festulolium* hybrids than in *Lolium* pure species and *L. multiflorum*  $\times$  *F. arundinacea* var. *glaucescens* [mean = 361.44 g kg<sup>-1</sup>(d.m.), SD = 62.99] and pure species control *F. arundinacea* the control *F. pratensis* [mean = 394.28 g kg<sup>-1</sup>(d.m.), standard deviation = 44.13]. A decrease in BC was observed over time (Fig. 3), with some exceptions *F. arundinacea*, gaining BC from 2016 to 2017 [means = 315.32 g kg<sup>-1</sup>(d.m.) and 383.64 g kg<sup>-1</sup>(d.m.), SDs = 25.52 and 96.33]. Lowest BC was observed in the *L. multiflorum* pure species and intermediate BC in *L. multiflorum* and *L. perenne* hybrids (Fig. 3).

Trends in CP were similar to that of BC; there was a steep decline between 2015 and 2016 with a more gradual between 2016 and 2017, and though there was generally consistency in this trend among pure species and hybrid types there were some exceptions. In the pure species *F. arundinacea* increased CP between 2016 to 2017 was observed and [mean = 88.28 and 94.26 g kg<sup>-1</sup>(d.m.), SDs = 3.91 and 2.2, respectively] and slightly increased in *L. multiflorum*  $\times$  *F. arundinacea* [means = 68.99 and 71.20 g kg<sup>-1</sup>(d.m.), SDs = 17.37 and 96.33, respectively] and in *L. perenne*  $\times$  *F. pratensis* [means = 66.36 and 71.06 g kg<sup>-1</sup>(d.m.), SDs = 21.08 and 0.79, respectively] (Fig. 3). Consistently across all years, *F. pratensis* [mean = 125.63 g kg<sup>-1</sup>(d.m.), SD = 25.23] exhibited the highest CP. The lowest mean CP across three years was observed in the tetraploid *L. multiflorum* [mean = 77.92 g kg<sup>-1</sup>(d.m.), SD = 13.17], followed by the hybrid *L. multiflorum*  $\times$  *F. arundinacea* [mean = 79.85 g kg<sup>-1</sup>(d.m.), SD = 21.43].

## Discussion

*Festulolium* hybrids are of interest for forage production due to their complementary characteristics, with *Lolium* exhibiting greater quality attributes and *Festuca* possessing superior resilience to abiotic stress. We carried out a five year field experiment to evaluate dry matter yield, ground cover and persistency, and nutritional parameters of *Festulolium* hybrids under a conservation management in a mild Atlantic climate.

Persistency is a complex trait controlled by many interacting genetic and environmental factors, and it has

been identified as a trait for selection in perennial ryegrass breeding programmes due its economic importance associated with sward stability and renewal (Conaghan and Casler 2011). The trends in persistency were conserved among hybrid types, and they most aligned more closely to the *Lolium* pure species controls. Values for ground score, however, demonstrated comparability between *L. multiflorum* pure species, hybrid types, and *Festuca* pure species when five year means were grouped. *L. perenne* pure species exhibited greater persistency, this was expected (Wilkins and Humphreys 2003), while the hybrid *L. perenne*  $\times$  *F. pratensis* also displayed ground score and similar to that of its *Lolium* component. *L. multiflorum* pure species were expected to be the least persistent, however, they were found to have intermediate persistency (Wilkins and Humphreys 2003). There was some deviation within hybrid types, with some cultivars deviating significantly from hybrid groups in later years of the trial. The *Festulolium* hybrid cv. Spring Green demonstrated superior ground cover in colder climate in a multisite study in the USA, while performing poorer in warmer regions in comparison to the *Festulolium* hybrids from which it was derived (Casler *et al.* 2002) This indicates the variation in adaptability of *Festulolium* in terms of persistency. *Festuca* pure species exhibited poorer persistency based on ground score. Due to the resilience to abiotic stress observed in *Festuca*, it was expected for these cultivars to exhibit enhanced persistency. However, ground score cover as a measure of abundance and does not necessarily reflect productivity as demonstrated by the *F. pratensis* cv. Fure. Although ground score decreases, DMY increases after an extended period, perhaps due to the broad leaf morphology of *Fescue* species in comparison to *Lolium* species (Gymer and Whittington 1973). Despite the presence of open ground indicated by the low GC score, the larger leaf area and subsequent larger volume of biomass may account for the disparity between GC score as a measure of persistency and the observed yield. Though GC score is the widely accepted measurement of persistency for national list in Ireland, it is not without limitations. Ground score measures the abundance of a species within a sward rather than the biomass of the surviving plants, failing to account for yield stability. Methods have been proposed such as reporting the relationship between short term and medium term yields to describe persistency (Dodd *et al.* 2018).

In addition to ground cover scores, the change in dry matter yield over time is also an important component of persistency. The results from this trial for DMY demonstrate that *Festulolium* hybrids perform well in terms of productivity and in some cases out yield *Lolium* pure species. Similarly, Meehan *et al.* (2017) found that *Festulolium* hybrids along with *F. arundinacea* were capable of producing greater DMY than *L. perenne* under suboptimal conditions, over a two year period for biomass production. Studied years tended to exhibit poorer yields in grasses as well as sharp declines in DMY in 2016 and 2018. Though DMY during these years declined, higher yielding hybrid types still exhibited higher DMY values than more stable yielding cultivars. Yield stability is more

prevalent in lower yielding hybrids and similar results were found in two Swedish regions where *Festulolium* cultivars which yielded higher declined more quickly, while *Festulolium* cultivars with lower yields remained stable (Halling 2012). In a Lithuanian climate *Festulolium* exhibited superior yields to both *Festuca* and *Lolium* with *L. perenne* displaying much lower yields (Lemežienė 2004). The decline in DMY in 2016 and 2018 observed in this study was likely due to low rainfall during the late spring and early summer months (April 2016 = 49.2 mm, May 2016 = 56.7 mm, May 2018 = 62.5 mm, June 2018 = 25.2 mm). Based on historical data, low summer rainfall is generally not a limiting factor in Irish climatic conditions, except when values for total monthly rainfall were between 20 - 60 mm during the late spring to summer period, as observed in both 2016 and 2018 (Hurtado-Uria *et al.* 2014). The climatic influence causing variability among hybrids over a single year was observed in a multisite study including that described here (Ghesquière *et al.* 2016). In terms of yield in this study, the hybrids yields appear to more closely follow their *Lolium* component than their *Festuca* component. This was also observed when foliar characteristic and DMY were observed in *Festulolium* hybrids and compared to that of *Lolium* pure species (Humphreys *et al.* 2014, Muhandiram *et al.* 2020) This may be due to breeding strategy, genome instability after successive generations, or disparity between integration of source genomes, such as amphiploid or introgressed hybrids. We did observe some variability among hybrid cultivars, where some cultivars exhibited comparable yields to *Festuca* while others out yield *Lolium*, though these cases were not found to be significant.

In general, *Lolium* has been observed to possess higher digestibility, therefore, in this study we expected that *Lolium* pure species would exhibit relatively higher quality, followed by *Festulolium* hybrids and finally, *Festuca* pure species with the lowest quality (Wilkins and Humphreys 2003, Banzhaf and Boberfeld 2005). We observed that all pure species cultivars and hybrids were affected equally by different experimental years which points towards a good silage quality under marginal conditions. *Festulolium* cultivars exhibited intermediate levels for both WSS and BC parameters, indicating they would be suitable for silage production. Similarly, no significant differences between *Festulolium* hybrids *Lolium perenne* pure species were observed in a previous study when digestibility of hybrids was investigated (Kamau *et al.* 2020) The *F. arundinacea* cultivar Kora had the highest levels of CP across all years. This was replicated in a Danish study where cv. Kora was found to have higher CP content in the first cut in comparison to *Festulolium* hybrid (Solati *et al.* 2018) with CP declining similarly in both cultivars in the autumn. This is likely due to maturity as the range of heading dates will affect the move from vegetative to reproductive phase.

Overall, *Festulolium* hybrids performed at a very similar level as *Lolium* species in terms of persistency, yield, and ensilability in this five year study. This makes *Festulolium* hybrids very suitable to be grown as a silage crop under a mild Atlantic climate, even under marginal conditions.

## References

Banzhaf, K., Boberfeld, W.O.V.: Ensilability and silage quality of different *Festulolium* hybrids in comparison to *Festuca arundinacea*. - In: Park, R.S., Stronge, M.D.: XX International Grassland Conference: Offered paper 472, Wageningen Academic Publishers, Wageningen 2005.

Casler, M.D., Peterson, P.R., Hoffman, L.D., Ehlke, N.J., Brummer, E.C., Hansen, J.L., Mlynarek, M.J., Sulc, M.R., Henning, J.C., Undersander, D.J., Pitts, P.G.: Natural selection for survival improves freezing tolerance, forage yield, and persistence of *Festulolium*. - *Crop Sci.* **42**: 1421-1426, 2002.

Conaghan, P., Casler, M.D.: A theoretical and practical analysis of the optimum breeding system for perennial ryegrass. - *Irish J. Agr. Food Res.* **50**: 47-63, 2011.

Davies, D.R., Merry, R.J., Williams, A.P., Bakewell, E.L., Leemans, D.K., Tweed, J.K.S.: Proteolysis during ensilage of forages varying in soluble sugar content. - *J. Dairy Sci.* **81**: 444-453, 1998.

Dodd, M.B., Chapman, D.F., Ludemann, C.I., Griffiths, W., Tozer, K.N., Donnelly, L.: The measurement of perennial ryegrass persistence. - *J. New Zeal. Grassland* **80**: 161-168, 2018.

Fojtik, A.: Methods of grass improvement used at the Plant Breeding Station Hladke Zivotice. - *Genet. Pol.* **35**: 25-31, 1994.

Fox, J., Weisberg, S.: A Companion to Applied Regression. Third Edition. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/Sage> 2019.

Ghesquière, M., Emile, J.C., Jadas Hécart, J., Mousset, C., Trainau, R., Poisson, C.: First *in vivo* assessment of feeding value of *Festulolium* hybrids derived from *Festuca arundinacea* var. *glaucescens* and selection for palatability. - *Plant Breed.* **115**: 238-244, 1996.

Ghesquière, M., Baert, J., Barth, S., Černoch, V., Grogan, D., Humphreys, M.W., Murray, P., Østrem, L., Sokolović, D., Paszkowski, E., Zwierzykowski, Z.: Enhancing the productivity in forage grasses on the European scale using interspecific hybridization. - In: Roldán-Ruiz, I., Baert, J., Reheul, D. (ed.): Breeding in a World of Scarcity. Pp. 199-204. Springer, Cham 2016.

Grogan, D., Gilliland, T.J.: A review of perennial ryegrass variety evaluation in Ireland. - *Irish J. Agr. Food Res.* **50**: 65-81, 2011.

Grogan, D., Barth, S., Grant, J., O'Riordan, E., Hanley, M.: *Festulolium* in Ireland – seasonal yield and quality assessment. - In: Horan, B., Hennessy, D., O'Donovan, M., Kennedy, E., McCarthy, B., Finn, J.A., O'Brien, B. (ed.): Sustainable Meat and Milk Production from Grasslands. Pp. 206-208. Wageningen Academic Publishers, Wageningen 2018.

Gymer, P.T., Whittington, W.J.: Hybrids between *Lolium perenne* and *Festuca pratensis* II. Comparative morphology. - *New Phytol.* **72**: 801-865, 1973.

Halling, M.A.: Yield stability of *Festulolium* and perennial ryegrass in southern and central Sweden. - *Grassland Sci. Eur.* **17**: 118-120, 2012.

Houdek, I.: *Festulolium 'Perseus'*. - *Czech J. Genet. Plant Breed.* **41**: 35-36, 2005.

Humphreys, M.W., Canter, P.J., Thomas, H.M.: Advances in introgression technologies for precision breeding within the *Lolium* *Festuca* complex. - *Ann. appl. Biol.* **143**: 1-10, 2003.

Humphreys, M.W., O'Donovan, S.A., Farrell, M.S., Gay, A.P., Kingston Smith, A.H.: The potential of novel *Festulolium* (2n= 4x= 28) hybrids as productive, nutrient use efficient fodder for ruminants. - *Food Energy Security* **3**: 98-110, 2014.

Hurtado-Uria, C., Hennessy, D., Shalloo, L., O'Connor, D., Delaby, L.: Relationships between meteorological data

and grass growth over time in the south of Ireland. - *Irish Geography* **46**: 175-201, 2013.

Kamau, S., Belanche, A., Davies, T., Rees Stevens, P., Humphreys, M., Kingston-Smith, A.H.: A route to decreasing N pollution from livestock: Use of *Festulolium* hybrids improves efficiency of N flows in rumen simulation fermenters. - *Food Energy* **9**: e209, 2020.

Kopecký, D., Šimoníková, D., Ghesquière, M., Doležel, J.: Stability of genome composition and recombination between homoeologous chromosomes in *Festulolium* (*Festuca* × *Lolium*) cultivars. - *Cytogenet. Genome Res.* **151**: 106-114, 2017.

Lemežienė, N., Kanapeckas, J., Tarakanovas, P., Nekrošas, S.: Analysis of dry matter yield structure of forage grasses. - *Plant Soil Environ.* **50**: 277-282, 2004.

Lewis, E.J., Tyler, B.F., Chorlton, K.H.: Development of *Lolium*-*Festuca* hybrids. - *Annu. Rep. Welsh Plant Breed. Stat.* for **1972**: 34-37, 1973.

Lin, B.B.: Resilience in agriculture through crop diversification: adaptive management for environmental change. - *BioScience* **61**: 183-193, 2011.

Lynch, T.M.H., Barth, S., Dix, P.J., Grogan, D., Grant, J., Grant, O.: Ground cover assessment of perennial ryegrass using digital imaging. - *Agron. J.* **107**: 2347-2352, 2015.

Macleod, C., Humphreys, M., Whalley, W.R., Turner, L., Binley, A., Watts, C.W., Skøt, L., Joynes, A., Hawkins, S., King, I.P., O'Donovan, S., Haygarth, P.M.: A novel grass hybrid to reduce flood generation in temperate regions. - *Sci. Rep.* **3**: 1-7, 2013.

Mayne, C.S., O'Kiely, P.: An overview of silage production and utilisation in Ireland (1950–2005). - In: Park, R.S., Stronge, M.D. (ed.): *Proceedings of the XIV international silage conference*. Pp. 19-34. Wageningen Academic Publishers, Wageningen 2005.

McDonagh, J., O'Donovan, M., McEvoy, M., Gilliland, T.J.: Genetic gain in perennial ryegrass (*Lolium perenne*) varieties 1973 to 2013. - *Euphytica* **212**: 187-199, 2016.

McEvoy, M., O'Donovan, M., Shalloo, L.: Development and application of an economic ranking index for perennial ryegrass cultivars. - *J. Dairy Sci.* **94**: 1627-1639, 2011.

Meehan, P., Burke, B., Doyle, D., Barth, S., Finn, J.: Exploring the potential of grass feedstock from marginal land in Ireland: does marginal mean lower yield? - *Biomass Bioenerg.* **107**: 361-369, 2017.

Muck, R.E.: Factors influencing silage quality and their implications for management. - *J. Dairy Sci.* **71**: 2992-3002, 1988.

Muhandiram, N.P., Humphreys, M.W., Fychan, R., Davies, J.W., Sanderson, R., Marley, C.L.: Do agricultural grasses bred for improved root systems provide resilience to machinery derived soil compaction?. - *Food Energy Security* **9**: 227, 2020.

O'Donovan, M., Lewis, E., O'Kiely, P.: Requirements of future grass-based ruminant production systems in Ireland. - *Irish J. Agr. Food Res.* **50**: 1-21, 2011.

O'Donovan, M., McHugh, N., McEvoy, M., Grogan, D., Shalloo, L.: Combining seasonal yield, silage dry matter yield, quality and persistency in an economic index to assist perennial ryegrass variety selection. - *J. agr. Sci.* **155**: 556-568, 2016.

Østrem, L., Volden, B., Larsen, A.: Morphology, dry matter yield and phenological characters at different maturity stages of × *Festulolium* compared with other grass species. - *Acta agr. Scand. B Soil Plant Sci.* **63**: 531-542, 2013.

Playne, M.J., McDonald, P.: The buffering constituents of herbage and of silage. - *J. Sci. Food Agr.* **17**: 264-268, 1966.

Roudier, P., Andersson, J.C., Donnelly, C., Feyen, L., Greuell, W., Ludwig, F.: Projections of future floods and hydrological droughts in Europe under a + 2°C global warming. - *Climatol. Change* **135**: 341-355, 2016.

Shalloo, L., Dillon, P., O'Loughlin, J., Rath, M., Wallace, M.: Comparison of a pasture based system of milk production on a high rainfall, heavy clay soil with that on a lower rainfall, free draining soil. - *Grass Forage Sci.* **59**: 157-168, 2004.

Solati, Z., Manevski, K., Jørgensen, U., Labouriau, R., Shahbazi, S., Lærke, P.E.: Crude protein yield and theoretical extractable true protein of potential biorefinery feedstocks. - *Ind. Crops Prod.* **115**: 214-226, 2018.

Thomas, H., Humphreys, M.O.: Progress and potential of interspecific hybrids of *Lolium* and *Festuca*. - *J. agr. Sci.* **117**: 1-8, 1991.

Thomas, T.A.: An automated procedure for the determination of soluble carbohydrates in herbage. - *J. Food Agr.* **28**: 639-642, 1977.

Tilley, J.M.A., Terry, R.A.: A two stage technique for the *in vitro* digestion of forage crops. - *Grass Forage Sci.* **18**: 104-111, 1963.

Wilkins, P.W., Humphreys, M.O.: Progress in breeding perennial forage grasses for temperate agriculture. - *J. agr. Sci.* **140**: 129-150, 2003.

Zwierzykowski, Z., Tayyar, R., Brunell, M., Lukaszewski, A.J.: Genome recombination in intergeneric hybrids between tetraploid *Festuca pratensis* and *Lolium multiflorum*. - *J. Hered.* **89**: 324-328, 1998.