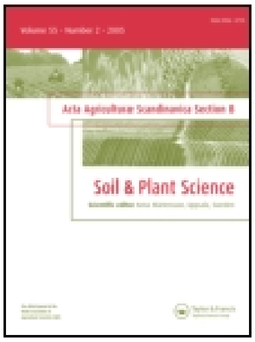

Acta Agriculturae Scandinavica, Section B - Soil & Plant Science

ISSN: 0906-4710 (Print) 1651-1913 (Online) Journal homepage: https://www.tandfonline.com/loi/sagb20

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To cite this article: Liv Østrem, Birger Volden & Arild Larsen (2013) Morphology, dry matter yield and phenological characters at different maturity stages of ×*Festulolium* compared with other grass species, Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 63:6, 531-542, DOI: 10.1080/09064710.2013.819440

To link to this article: https://doi.org/10.1080/09064710.2013.819440

Published online: 06 Aug 2013.

Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 2013
Vol. 63, No. 6, 531–542, http://dx.doi.org/10.1080/09064710.2013.819440

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ORIGINAL ARTICLE

Morphology, dry matter yield and phenological characters at different maturity stages of ×*Festulolium* compared with other grass species

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(*Received 13 August 2012; final version received 20 June 2013*)

The potential of ×*Festulolium* as a forage species for Nordic conditions was investigated by comparing Norwegian candivars (LpFp-T, LmFp-T, Lp^{Fp}-D-N, Lp^{Fp}-D-UK) differing in parental origin and ploidy level with commercial cultivars of ×*Festulolium* (Hykor, Felopa), the parental species perennial ryegrass (*Lolium perenne* L.) and meadow fescue (*Festuca pratensis* L.), and timothy (*Phleum pratense* L.). Plant development was observed as leaf:stem ratio (LSR) and standardised development stage, and dry matter yield (DMY) and digestibility were studied throughout two consecutive harvest seasons (2007 and 2008) as an effect of taking the first cut at four maturity stages (MSs), namely: (1) vegetative growth, (2) early heading, (3) heading and (4) anthesis, and then consecutive cuts throughout the season. Observations were made at two locations in Norway (61°N, 67°N). LSR and standardised development stage were affected by MSs and the loloid types of ×*Festulolium* (Felopa and the candivars) grouped between the parental species as to developmental pattern. The most extreme changes across MSs 1–4 in first cut were seen in the festucoid type Hykor (smallest) and in timothy (largest). Across MSs 2 and 3, for two years Hykor obtained higher DMY than the other entries, but overall digestible DMY was equal in Hykor and LpFp-T due to the better digestibility in LpFp-T. The lower DMY obtained in ×*Festulolium* Felopa, the diploid candivars and LmFp-T was mainly due to winter damages. Consistency was found between the locations for the entries investigated. For Nordic growing conditions, in which winter hardiness is required for commercial cultivars, the amphitetraploid breeding approach of perennial ryegrass and meadow fescue should be pursued. It is the parental origin of the candivar LpFp-T, which was comparable to Hykor, the best of the commercial ×*Festulolium* cultivars, and performed better than the cultivars of the parental species.

Keywords: breeding; digestibility; leaf:stem ratio; meadow fescue; mean stage by count; perennial ryegrass; timothy

Introduction

Grassland is of major importance for the agricultural industry in Europe, covering about 40% of total agricultural land in Europe and about 60% of that in Norway. Grass-based feeding systems dominate ruminant livestock farming in Norway, and feed energy yield per unit land area is higher from grasses than cereals in western and northern Norway (Ministry of Agriculture 2002).

Perennial ryegrass (*Lolium perenne* L.) is the dominant forage grass species in Europe due to its high regrowth capacity, rapid establishment, tolerance to frequent cutting and grazing, and high nutritive value for ruminant livestock (Wilkins & Humphreys 2003). Winter survival is still unreliable, but perennial ryegrass is being used increasingly in the Nordic region, and a future milder climate, as predicted for this area (Hanssen-Bauer et al. 2009), may increase the use of perennial ryegrass even more. Species within the genus *Festuca* have a higher level of general stress tolerance than perennial ryegrass. Since fescues and ryegrasses are closely related in evolutionary terms, they can be hybridised successfully. ×*Festulolium* hybrids have the potential of combining

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the superior forage quality of ryegrass species with the high persistency and stress tolerance of fescues in interspecific *Lolium* × *Festuca* hybrids (× *Festulolium*) (Humphreys et al. 2003; Thomas et al. 2003). × *Festulolium* includes all hybrids resulting from the crossing of a species of the genus *Festuca* with a species of the genus *Lolium* (Ghesquière et al. 2010).

Hybrids of × *Festulolium* have been developed and bred since the 1970s at least, but only recently have good cultivars entered the market in Europe (Ghesquière et al. 2010), i.e. cultivars of either the loloid type generated from crosses between ryegrass (*Lolium* spp.) and meadow fescue (*F. pratensis*) or of the festucoid type from crosses between Italian ryegrass (*L. multiflorum*) and tall fescue (*F. arundinacea*). So far, the most interesting cultivars for Nordic conditions are festucoid type × *Festulolium* cultivars due to their high yield ability and persistency (Østrem & Larsen 2008; Halling 2012). Similar results have been reported from the Baltic region (Gutmane & Adamovics 2008). Hybrids between perennial ryegrass and meadow fescue have a higher potential for winter survival and persistency at higher latitudes compared with hybrids between Italian ryegrass and meadow fescue. Therefore, development of hybrids between perennial ryegrass and meadow fescue is the main focus of the current × *Festulolium* breeding programme at Graminor Ltd. in Norway (Østrem et al. 2007). Future global climate change may lead to higher temperatures, a longer growing season and more water stress in many locations (Hanssen-Bauer et al. 2009), and this may affect the persistency of grasslands. The longer growing season during recent decades has led to a demand for leys that yield at least three cuts in most parts of Norway, and grass species with high tolerance to frequent cutting have a natural advantage in this regard.

To achieve high-quality herbage, primary growth has to be harvested at an early stage of maturity. Although plant maturity is the main factor affecting forage quality, the environment modifies the impact of plant maturity by altering growth rate, development rate, herbage yield and quality, senescence rate and amount of dead plant material. Thus, annual, seasonal and environmental variations related to geographical location alter forage quality, even when herbages are harvested at similar morphological stages (Buxton & Fales 1994). The decline in forage quality with age results primarily from a decrease in leaf:stem ratio (LSR) (Ugherughe 1986) and the decline in quality of the stem component from increased lignification (Nelson & Moser 1994). Immature grass stems are generally of high quality (Minson 1990), but stem quality decreases faster than that of leaves, especially as the plants approach maturity. Little is known about the variation in LSR in × *Festulolium* cultivars during the whole growing season. Since grass stems are more highly variable in forage quality than the leaf lamina, significant improvement of forage quality of grasses may be possible by selecting for stem quality (Illius & Gordon 1991), without compromising the seed yield potential. Plant breeding is an extremely cost-effective mechanism for increasing the nutritional value of forage crops. According to Casler and Jung (2006), relatively small increases in *in vitro* dry matter digestibility (IVDMD) can result in measurable improvements in animal performance and such increases in IVDMD may be obtained more easily than increases in forage yield (Casler 2000).

The aim of the present study was to examine the impact of MSs on phenological development, DM yield and digestibility of DM yield of × *Festulolium* candivars (promising breeding materials) differing in parental origin and ploidy levels in order to determine the potential for selection for Nordic growing conditions. The candivars were compared with commercial × *Festulolium* cultivars, the main commercial cultivars of the parental species perennial ryegrass and meadow fescue, and timothy (*Phleum pratense* L.), which is the main ley species in the region. Field experiments were performed at two locations with differing latitude and length of growing season during two ley years.

Materials and methods

Plant material

Candivars of × Festulolium

Four candivars from the plant breeding company Graminor Ltd. were included, all of which were exposed to two generations of seed propagation before being tested. They are denominated according to their parental origin; Lp = *L. perenne*, Lm = *L. multiflorum* and Fp = *F. pratensis*, with the abbreviation given as a superscript (e.g. $^{\mathrm{Fp}}$) when the species consists of segments within the *Lolium* genome. Ploidy level is denominated as either diploid (D) or tetraploid (T), and the geographical connection either Nordic (-N) or UK (-UK). The original candivar denominations from Graminor Ltd. are given in brackets.

LpFp-T (Candivar FuRs0463) was made from six plants which were selected after two winters in a nursery field at Bodø, northern Norway (67°17′N). The original synthetic population (FuRs9806), now listed as Fabel (Graminor Ltd., Norway), was established by intercrossing 64 surviving plants at Fureneset from 4 families (Ba-11356, Ba-11356-sel, Ba-11358 and Ba-11359), all originating from the Institute of Grassland and Environmental Research (IGER, now IBERS), Aberystwyth, UK.

Lp^{Fp}-D-N (Candivar FuRs0357) originates from a wide germplasm pool of several initial triploid hybrids prior to two backcrosses to diploid perennial ryegrass. The primary hybrids were made from either tetraploid ×*Festulolium* cv. Prior crossed with diploid perennial ryegrass cv. Riikka (Boreal Plant Breeding Ltd., Jokioinen, Finland), or crosses between the tetraploid perennial ryegrass population WIR40697 (obtained from the Vavilov Institute, St. Petersburg, Russia) or RAIGT5/Einar (Department of Genetics and Plant Breeding, Agricultural University of Norway, Ås, now the Norwegian University of Life Sciences) and meadow fescue cv. Fure, a Norwegian cultivar originating from Fure, Fjaler, western Norway (61°34′N). The initial hybrids were backcrossed twice onto diploid perennial ryegrass (cv. Norlea, the Canadian Government; cv. Gunne, Svalöf AB, Sweden; cv. Riikka; WIR35600 and WIR20258, the Vavilov Institute) to obtain progenies after the second backcross. The cultivar of ×*Festulolium* Prior (*L. perenne* ×*F. pratensis*) is an intergeneric amphiploid created in the early 1970s and is the product of crosses between colchicine-induced autotetraploids of the parental species perennial ryegrass and meadow fescue (Canter et al. 1999).

Lp^{Fp}-D-UK (Candivar FuRs0028) is a synthetic population based on backcrossed plants of initial hybrids of diploid perennial ryegrass and ×*Festulolium* cv. Prior, obtained from IGER in 1991, i.e. diploid ryegrass genome with small fescue segments after one generation of backcrossing. In spring 1995, following 3 winters in a nursery field at Fureneset, about 70 surviving plants were polycrossed and progenies from 3 of the original plants were backcrossed to diploid perennial ryegrass cv. Gunne. A polycross was performed with 35 plants with loloid-type panicles.

LmFp-T (Candivar FuRs0136) is a synthetic population made up of three plants from three crosses between tetraploid Italian ryegrass cv. Fabio (EuroGrass) and colchicine-induced tetraploid meadow fescue cvs. Fure and Salten, a synthetic population originating from Bodø, northern Norway. LmFp-T was completely dead in spring of the second ley year at the Bodø site (Østrem & Larsen 2010b) and was omitted from the total statistical analyses. Results for dry matter yield (DMY) and LSR from the Fureneset site are presented, however.

Control species and cultivars

The candivars were compared against two commercial cultivars of ×*Festulolium*, two of meadow fescue (one at each site), one of perennial ryegrass and one of timothy. The two cultivars of ×*Festulolium*, cv. Felopa (*L. multiflorum* ×*F. pratensis*) and cv. Hykor (*L. multiflorum* ×*F. arundinacea*), are described by Zwierzykowski (2004). The two cultivars of meadow fescue are the main cultivars in the regions in which the two experimental fields were located: cv. Fure at the southern location and cv. Norild at the northern location. Norild is a synthetic population selected in Alta, Finnmark, northern Norway (69°55′N), from a local population collected in Harstad, Troms, northern Norway (68°47′N). The tetraploid perennial ryegrass cv. Napoleon (DLF-TRIFOLIUM, Denmark) was one of the main commercial cultivars in Norway when the field trials started. The timothy cv. Grindstad (origin Rakkestad, Østfold, Eastern Norway, 59°30′N), despite its southern Norwegian origins, is also well adapted to the northern location Bodø due to the breeding history of continual natural selection over decades. Timothy is the most important forage grass species in Scandinavia, being well adapted to Nordic farming conditions. It constitutes the main species in most seed mixtures for leys and is therefore decisive for time of harvest in many regions. However, timothy does not tolerate frequent cutting and its persistency is low under intensive management systems.

Field trial location, design, cuts and measurements

Field trials were established in 2006 at two coastal locations: Fureneset, Fjaler, western Norway (61°34′N, 5°21′E, 10 m.a.s.l.), and Vågønes, Bodø, northern Norway (67°17′N, 14°27′E, 35 m.a.s.l.). The experiment had a split-plot design, with MS on main plots and cultivars on sub-plots of 1.5 m × 4 m, all with three replicates. The experimental treatments were location, year, MS and entry. The sub-plots of entries were cut individually, i.e. on each entry separately, according to one of the four MSs: MS 1: continuous vegetative growth (approx. 15 cm); MS 2: early heading, i.e. when 1–2 cm of the inflorescence was visible above the leaf blade base; MS 3: heading, i.e. when 50% of the inflorescences had emerged; and MS 4: anthesis, i.e. peduncle node visible. In 2007, the entries were harvested at all four MSs, whereas in 2008 individual cuts were taken only at MS 2 and MS 3. Total amount of nitrogen (kg ha^{-1} year^{-1}) applied at Fureneset for the different MSs in 2007 and 2008 (in brackets) was MS 1: 230, MS 2: 280 (300), MS 3: 230 (240) and MS 4: 230. The corresponding nitrogen levels at Vågønes were MS 1: 230, MS 2: 240 (240), MS 3: 230 (230) and MS 4: 220. Each growth cycle was fertilised. Dates of growth start in 2007 and 2008, harvesting dates of three cuts, temperature sum (day degrees) and cumulative rainfall between growth start in spring and first cut and between succeeding cuts in

2007 (MS 1–4) and 2008 (MS 2–3) at each location are presented in Table 1. When each cultivar (separately assessed) reached the required MS, at least 45 tillers were cut at soil level from each of the subplots and for each individual cut standardised development stage (mean stage by count (MSC)) was assessed according to a phenological scale based on the number of tillers in the vegetative, elongative, regenerative or flowering stage (Moore et al. 1991). The collected tillers were divided into leaf and stem fractions to estimate the LSR. Plot harvesting was performed on the same day using a Haldrup forage plot harvester, and DMY was estimated. Herbage samples from the whole plot and leaf and stem fractions from all plots were dried at 60°C for 48 h. The dried samples were ground to pass a 1-mm stainless-steel mesh using a Cyclotec™ 1093 Sample Mill (Foss Tecator) and analysed by near infra-red spectroscopy for digestibility (Fystro & Lunnan 2006).

Statistical analysis

Data were analysed as a split-plot design with location, year, MS and entries as fixed factors using PROC GLM (SAS 2009). The model used was $y_{ijkl} = \mu + L_i$ (loc) + Y_j (year) + D_k (MS) + E_l (entries) + e_{ijkl}, where y_{ijkl} is the observation of LSR, MSC, DMY, digestible DM (DDM) and digestible DMY (DDMY), μ is the overall mean, L_i is the effect of location (i = 1–2), Y_j is the effect of year (j = 1–2), D_k is the effect of MS (k = 1, 2, 3, 4), E_l is the entry effect (l = 1–8) and e_{ijkl} = residual error. The number of entries relates to the two cvs. of meadow fescue being analysed as one entry when both locations were analysed and LmFp-T being omitted from the total analysis. Differences were considered significant at $P < 0.05$. The entry × MS interaction was tested against a pooled error including entry × MS and location × entry × MS, and similarly the entry × location against a pooled error including entry and entry × location. The year and year × location interaction effects were tested against the year × replication within location interaction, the location effect was tested against replication within location, the MS and MS × location effects were tested against the MS × replication within location effect and the year × location × MS interaction was tested against the year × MS × replication within location effect. Regression analysis was performed between DMY and DM digestibility using PROC REG (SAS 2009).

Meteorological conditions in the growing season

Air temperature (°C), cumulative precipitation (mm) and water deficit (mm) for Fureneset and

Table 1. Dates for growth start, dates for harvesting and sampling for analyses (HS), temperature sum (°Cd) and cumulative rainfall (mm) from growth start to first cut and between succeeding cuts at the field trial locations for four (in 2007) and two (in 2008) MSs.

Location		Fureneset						Vågønes					
Year, growth start*		2007 15 April			2008 16 April			2007 29 April			2008 3 May		
MS	Cut	HS**	°Cd	mm	HS**	°Cd	mm	HS**	°Cd	mm	HS**	°Cd	mm
1	1	24 May	318	215				31 May	232	79			
	2	13 June	282	26				19 June	204	31			
	3	4 July	297	11				7 July	299	6			
2	1	29 May	372	240	29 May	421	40	6 June	320	79	16 June	384	89
	2	20 June	318	1	30 June	414	188	6 July	396	37	23 July	462	54
	3	16 July	384	82	31 July	514	77	8 Aug	486	104	19 Aug	373	10
3	1	5 June	474	241	2 June	486	46	19 June	436	110	24 June	473	103
	2	29 June	341	7	4 July	424	183	23 July	526	90	23 July	373	40
	3	25 July	391	103	1 Aug	460	77	3 Sept	525	182	10 Sept	612	52
4	1	18 June	663	241				27 June	551	114			
	2	31 July	623	192				7 Aug	631	105			

Notes: *Growth start is estimated from soil temperature.
**Correspond to harvesting dates for ×*Festulolium* Hykor which was the first entry to be harvested in each cut.

Vågønes during the main growing period April–August in 2007 and 2008 are shown in Table 2. Water deficit was calculated monthly as rainfall - ETo (mm day^{-1}) (Riley & Berentsen 2009), and the service was provided by Bioforsk (www.bioforsk.no). During the period 1961–1990 at Vågønes, mean annual precipitation was 1020 mm, the number of growing day degrees (above 5°C) was 170, and in total there were 880 day degrees. The corresponding values for Fureneset were 2010 mm, 208 days and 1129 day degrees (Olsen 1993; Norwegian Meteorological Institute 2013).

Results

Mean stage by count

Plant development in the four cuts in 2007, assessed as MSC, was affected only by MS (all $P < 0.0004$) and entry differences (all $P < 0.0001$). In the first cut there was an even increase in MSC from MS 1 to MS 4 averaged across entries (1.58, 1.93, 2.11, 2.51). Averaged across all MS in the first cut, the ranking of entries from highest to lowest was cv. Grindstad (2.47) > Lp^{Fp}-D-UK, cv. Napoleon, cv. Felopa, Lp^{Fp}-D-N > LpFp-T, cv. Fure/Norild, cv. Hykor (Figure 1). In the second cut, MSC declined evenly from MS 1 to MS 4 (1.93, 1.85, 1.77, 1.71), and similarly in the third cut from MS 1 to MS 3 (1.77, 1.68, 1.58). The MSC values averaged over MS 2 and MS 3 for two years and two locations are shown in Table 3 for the entries investigated. In most cases entries would be harvested at a similar MSC. Due to the number of species involved in our study and the expected variation within the entries, pre-determined phenological stages (MS 1–4) were used for determining harvest time and MSC became a post-harvest assessment reflecting the amount of generative as opposed to vegetative tillers. Entries of ×*Festulolium* had a low amount of generative tillers, especially in the first cut. All entries of ×*Festulolium* except cv. Hykor and perennial ryegrass cv. Napoleon demonstrated a uniform decrease in MSC from the first to the fourth cut, whereas in ×*Festulolium* cv. Hykor, meadow fescue cvs. Fure/Norild and timothy cv. Grindstad, only small changes in MSC were observed for the last three cuts.

Leaf:stem ratio

The LSR in the first three cuts of 2007 was affected by MS (all $P < 0.0002$) and entry differences (all $P < 0.0001$). An even decrease in LSR from MS 1 to MS 4 (1.71, 0.99, 0.68, 0.41) was observed in the first cut. ×*Festulolium* cv. Hykor demonstrated the highest LSR value (1.44) averaged over MS, which was significantly different only from timothy cv. Grindstad (0.63) (Figure 1). The LSR values in the second cut averaged over all entries were 1.21, 1.16, 1.56, 1.78 for MS 1, 2, 3 and 4, respectively, and the corresponding values for the third cut were 1.28, 2.08, 2.03 (no MS 4 observation). The LSR averaged over two years and two locations (MS 2 and MS 3) (Table 4) revealed growth differences in the entries investigated, especially in the second cut, in which ×*Festulolium* Lp^{Fp}-D-N, Lp^{Fp}-D-UK and cv. Felopa as well as perennial ryegrass cv. Napoleon all displayed low LSR values due to a high degree of heading in the regrowth. As Figure 1 demonstrates, there were similarities in MSC and LSR pattern between entries, e.g. ×*Festulolium* cv. Hykor and meadow fescue cvs. Fure/Norild displayed small changes for both MSC and LSR across MS, in contrast to the group consisting of ×*Festulolium* cv. Felopa and the candivars.

Dry matter yield

Total DMY in the first-year ley depended on MS ($P < 0.0001$) and entry differences ($P < 0.0001$),

Table 2. Weather conditions at the locations Fureneset (Fu) and Vågønes (Vå) for the field trial period April–August in 2007 and 2008.

Attribute	Year	Location	April	May	June	July	August
Mean air temperature (°C)	2007	Fu	6.8	8.8	14.4	14.7	13.3
		Vå	3.4	7.2	11.8	16.0	12.4
	2008	Fu	6.6	10.7	12.8	16.6	14.8
		Vå	3.7	7.3	11.3	14.2	12.4
Cumulative rainfall (mm)	2007	Fu	158	158	5	185	257
		Vå	148	76	36	86	166
	2008	Fu	74	15	185	76	86
		Vå	30	77	36	32	29
Water deficit (mm)	2007	Fu	106	76	−113	113	210
		Vå	104	−16	−92	−42	111
	2008	Fu	28	−82	97	−31	23
		Vå	−15	−1	−76	−84	−44

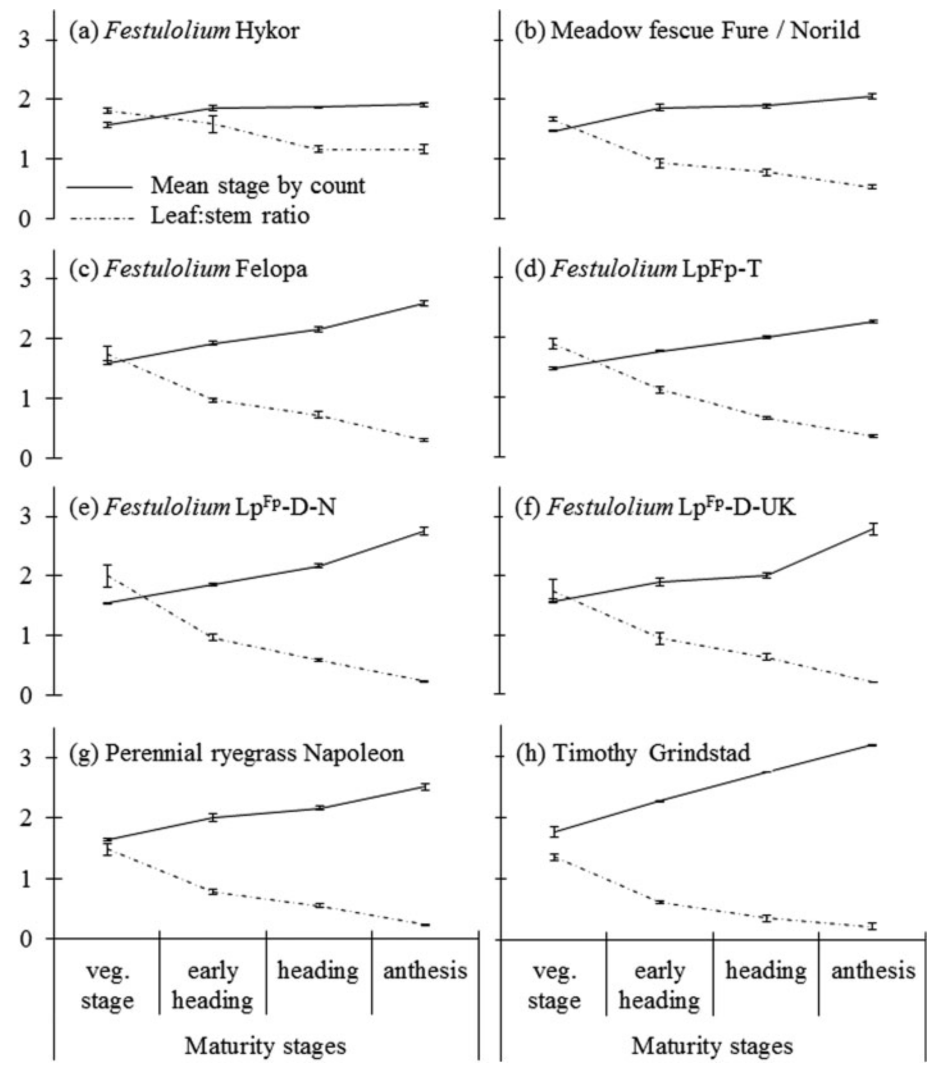

Figure 1. The effect of MS of entries of ×*Festulolium*, meadow fescue, perennial ryegrass and timothy on a standardised developmental stage expressed as mean stage by count (MSC) (solid line) and leaf:stem ratio (LSR) (dotted line) in cut 1 in first-year ley (2007) averaged over two locations.
Note: Vertical bars are standard errors of mean. N = 6.

Table 3. Standardised developmental stage in entries of ×*Festulolium*, perennial ryegrass, meadow fescue and timothy expressed as mean stage by count (MSC) in cuts 1–4 averaged over two maturity stages (2 and 3), two locations (Vågønes and Fureneset) and two ley-years (2007 and 2008).

		MSC							
Species	Entry	Cut 1		Cut 2		Cut 3		Cut 4	
×*Festulolium*	Hykor	2.05	d	1.45	e	1.55	c	1.37	e
×*Festulolium*	Felopa	2.38	b	2.07	a	1.87	a	1.51	bc
×*Festulolium*	LpFp-T	2.16	cd	1.76	cd	1.56	c	1.40	de
×*Festulolium*	Lp^{Fp}-D-N	2.33	bc	1.80	cd	1.58	c	1.44	d
×*Festulolium*	Lp^{Fp}-D-UK	2.46	b	1.95	b	1.69	b	1.46	cd
Per. ryegrass	Napoleon	2.49	b	1.87	bc	1.71	b	1.52	b
Meadow fescue	Fure/Norild	2.29	bc	1.44	e	1.45	d	1.40	de
Timothy	Grindstad	2.77	a	1.71	d	1.61	bc	1.61	a

Notes: N = 24. Different letters within columns indicate significant differences between entries ($P \leq 0.05$).

Table 4. Leaf:stem ratio (LSR) in entries of ×*Festulolium*, perennial ryegrass, meadow fescue and timothy as mean values in four cuts averaged across two maturity stages (MS 2 and 3), two locations (Vågønes and Fureneset) and two ley-years (2007 and 2008).

Species	Entry	LSR Cut 1		Cut 2		Cut 3		Cut 4	
×*Festulolium*	Hykor	1.13	a	2.99	a	3.64	a	3.33	a
×*Festulolium*	Felopa	0.67	bc	0.87	d	1.22	d	2.48	bc
×*Festulolium*	LpFp-T	0.78	b	1.50	c	2.09	c	2.43	bcd
×*Festulolium*	Lp^{Fp}-D-N	0.62	bc	1.09	d	1.73	c	2.37	cd
×*Festulolium*	Lp^{Fp}-D-UK	0.55	cd	0.75	d	1.14	d	2.08	de
Perennial ryegrass	Napoleon	0.52	cd	0.99	d	1.33	d	2.12	cde
Meadow fescue	Fure/Norild	0.64	bc	2.58	b	2.47	b	2.77	b
Timothy	Grindstad	0.42	d	1.74	c	1.77	c	1.93	e
×*Festulolium*	LmFp-T*	1.02		0.59		0.52		1.27	

Notes: N = 24. Different letters within columns indicate significant differences between entries ($P \leq 0.05$).
* ×*Festulolium* LmFp-T at Fureneset only (N = 12), not included in the statistical analyses.

Table 5. Dry matter yield (DMY) (t ha^{-1}) at four plant maturity stages (MS 1–4) in entries of ×*Festulolium*, perennial ryegrass, meadow fescue and timothy averaged over two locations (Vågønes and Fureneset) in first-year ley (2007).

Species	Entry	DMY (t ha^{-1}) MS 1*		MS 2*		MS 3*		MS 4*	
×*Festulolium*	Hykor	8.0	a	9.7	ab	9.7	ab	9.9	a
×*Festulolium*	Felopa	6.6	bc	8.1	cd	8.1	bc	7.9	c
×*Festulolium*	LpFp-T	7.5	ab	10.0	a	10.7	a	9.6	ab
×*Festulolium*	Lp^{Fp}-D-N	6.7	bc	9.2	abc	9.4	ab	9.5	abc
×*Festulolium*	Lp^{Fp}-D-UK	5.6	c	7.8	d	7.5	c	8.5	abc
Perennial ryegrass	Napoleon	7.3	ab	8.5	bcd	9.7	ab	8.8	abc
Meadow fescue	Fure/Norild	7.2	ab	8.7	abcd	9.5	ab	8.1	bc
Timothy	Grindstad	6.6	bc	9.0	abcd	9.7	ab	9.6	ab
×*Festulolium*	LmFp-T**	4.4		6.8		4.1		6.1	

Notes: N = 6. Different letters within columns indicate significant differences between entries ($P \leq 0.05$).
*No. of cuts within each maturity stage (MS 1: 5 cuts, MS 2: 4 cuts, MS 3: 4 cuts, MS 4: 2 cuts).
** ×*Festulolium* LmFp-T at Fureneset only (N = 3), not included in the statistical analyses.

Table 6. Dry matter yield (DMY) (t ha^{-1}) in four cuts in entries of ×*Festulolium*, perennial ryegrass, meadow fescue and timothy averaged across two maturity stages (2 and 3), two locations (Vågønes and Fureneset) and two years (2007 and 2008).

Species	Entry	DMY (t ha^{-1}) Cut 1		Cut 2		Cut 3		Cut 4	
×*Festulolium*	Hykor	4.4	ab	2.5	a	2.7	a	1.9	b
×*Festulolium*	Felopa	2.6	c	2.2	bc	2.3	b	1.8	bc
×*Festulolium*	LpFp-T	4.4	ab	2.3	abc	2.3	b	2.2	a
×*Festulolium*	Lp^{Fp}-D-N	4.0	ab	2.3	bc	2.2	b	1.9	b
×*Festulolium*	Lp^{Fp}-D-UK	2.9	c	1.8	d	2.2	b	1.7	cd
Perennial ryegrass	Napoleon	3.7	b	2.4	ab	2.3	b	1.9	b
Meadow fescue	Fure/Norild	4.1	ab	2.1	c	2.2	c	1.6	d
Timothy	Grindstad	4.6	a	1.7	d	1.7	c	1.8	bc
×*Festulolium*	LmFp-T*	0.7		2.0		2.0		1.8	

Notes: N = 24 (18 in cut 4). Different letters within columns indicate significant differences between entries ($P \leq 0.05$).
* ×*Festulolium* LmFp-T at Fureneset only (N = 12), not included in the statistical analyses.

and DMY at MS 3 was significantly higher than at MS 2 and MS 1, and also higher than at MS 4, although not significantly (Table 5). Ranking of entries for DMY (four MS, four cuts) from highest to lowest was as follows: LpFp-T, cv. Hykor > cv. Fure/Norild, cv. Napoleon, cv. Grindstad, Lp^{Fp}-D-N,

Table 7. Digestible dry matter (DDM) ($g\ kg^{-1}$ DM) in cut 1 in entries of ×*Festulolium* (*FL*), perennial ryegrass (PRG), meadow fescue (MF) and timothy (Tim) at four maturity stages (MS 1–4).

Species	Entry	DDM ($g\ kg^{-1}$ DM)								Days 1–4	Red day^{-1}
		MS 1		MS 2		MS 3		MS 4			
FL	Hykor	807	d	784	c	785	c	735	a	25	2.88
FL	Felopa	879	ab	855	a	830	a	724	ab	28	5.54
FL	LpFp-T	889	a	863	a	818	ab	723	ab	32	5.19
FL	Lp^{Fp}-D-N	868	ab	852	a	816	ab	692	c	32	5.50
FL	Lp^{Fp}-D-UK	845	bc	840	a	822	a	690	c	28	5.54
PRG	Napoleon	853	bc	845	a	810	ab	697	bc	31	5.03
MF	Fure/Norild	857	abc	816	b	795	bc	713	abc	25	5.76
Tim	Grindstad	826	cd	768	c	716	d	649	d	31	5.03

Notes: No. of days between harvesting at MS 1 and MS 4 (days 1–4), and daily reduction of DDM during this period (Red day^{-1}). Mean for two locations (Fureneset, Vågønes) in first-year ley (2007). N = 6. Different letters within columns indicate significant differences between entries ($P \leq 0.05$).

cv. Felopa, >Lp^{Fp}-D-UK. A significant location × entry interaction ($P < 0.0002$) due to differential ranking of the entries at the two locations was mainly caused by timothy cv. Grindstad, which was within the top three at Fureneset mainly because winter damage in spring 2007 favoured timothy. At Vågønes, where the winter survival was generally good, timothy cv. Grindstad demonstrated significantly lower DMY than the remaining entries. A significant MS × entry interaction ($P < 0.0096$) was due to differential ranking of the entries in MS 1–4, mainly due to timothy cv. Grindstad and meadow fescue cv. Fure/Norild, which respectively raised and lowered their rankings from MS 1 to MS 4. Averaged over both years and sites, DMY for four cuts across MS 2 and MS 3 was affected by year ($P = 0.0062$) and entry ($P < 0.0001$) (Table 6). Total DMY in 2008 (10.2 t ha^{-1}) was significantly higher than in 2007

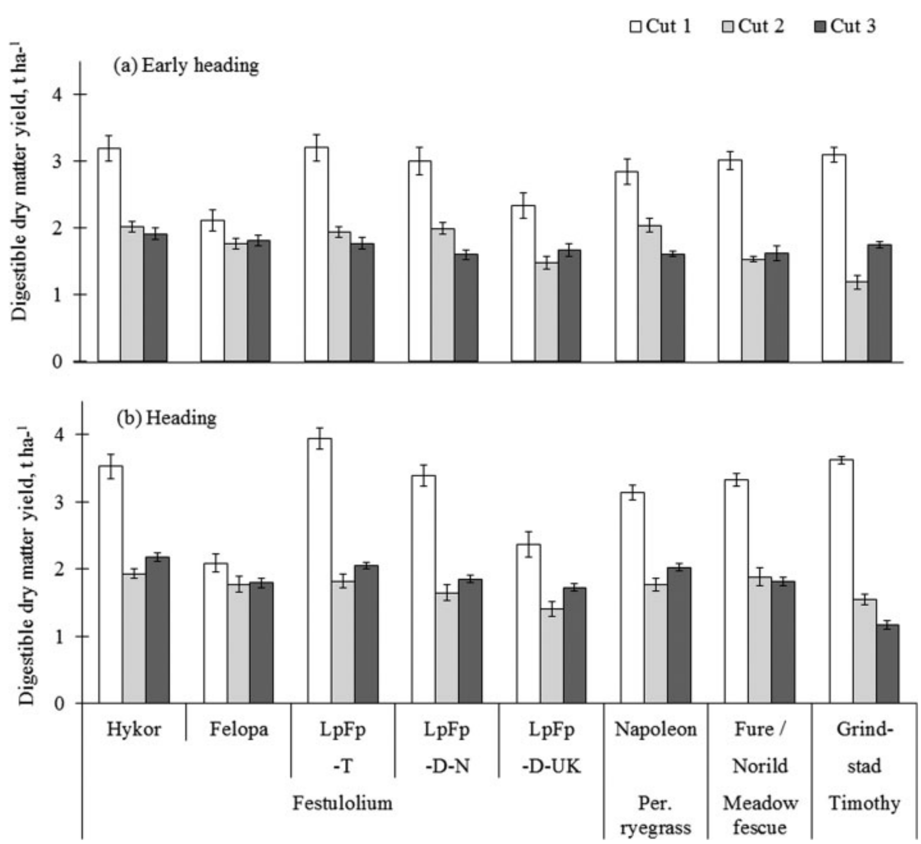

Figure 2. The effect of MS of entries of ×*Festulolium*, meadow fescue, perennial ryegrass and timothy on digestible dry matter yield (DDMY) (t ha^{-1}) at two locations during two successive years.
Note: N = 12.

(9.0 t ha^{-1}) for the four cuts combined. For three cuts, DMY was higher at Vågønes (8.3 t ha^{-1}) than at Fureneset (8.1 t ha^{-1}), but the difference was not statistically significant.

Digestible dry matter

In the first-year ley, DDM was affected by MS ($P < 0.0001$ for first and second cut, $P = 0.04$ for third cut), with a considerable decrease from MS 1 to MS 4 for all cuts. Averaged over all entries, DDM of the first cut decreased from 852 g kg^{-1} DM at MS 1 to 703 g kg^{-1} DM at MS 4. The relative ranking of entries for DDM in the first cut (highest to lowest, averaged over all MS) was as follows: cv. Felopa, LpFp-T > Lp^{Fp}-D-N, cv. Napoleon, Lp^{Fp}-D-UK, cv. Fure/Norild > cv. Hykor > cv. Grindstad. A location × entry interaction in the first cut was due to minor differences in ranking of the entries at the two locations. In the second cut, meadow fescue cvs. Fure/Norild and ×*Festulolium* LpFp-T presented the highest digestibility. Analysis of the rate of decrease in DDM from MS 1 to MS 4 in the first cut revealed that the DDM reduction was considerably lower in ×*Festulolium* cv. Hykor than in the remaining entries (Table 7).

Digestible dry matter yield

Total DDMY for three cuts averaged over two years and two locations for MS 2 and MS 3 depended on year ($P = 0.0048$) and entry differences ($P < 0.0001$). The ranking of entries for DDMY in mean of treatments was as follows (highest to lowest): cv. Hykor, LpFp-T > Lp^{Fp}-D-N, cv. Napoleon, cv. Fure/Norild, cv. Grindstad > cv. Felopa, Lp^{Fp}-D-UK (Figure 2). From MS 2 to MS 3, DDMY increased by 10% and 6% for the first and third cut, respectively, whereas for the second cut only minor changes were observed (−1%). The increase in DDMY between MS 2 and MS 3 in the first cut was especially evident for ×*Festulolium* LpFp-T and timothy cv. Grindstad, whereas ×*Festulolium* cv. Felopa and Lp^{Fp}-D-UK showed almost no increase. The relationship between DMY and DDM was significant for all cuts ($P < 0.0001$) and proved negative in the first (−2.10), second (−1.13) and third (−0.56) cuts when simple regression analysis was performed across MS 2 and MS 3 in 2007 and 2008.

Discussion

When plant breeding activities on *Lolium* × *Festuca* hybrids for Nordic conditions were started, the main aim was to transfer stress tolerance from fescues into the ryegrass genome with higher forage nutritive quality. The main breeding strategy used to date has been introgression breeding between species, where primary hybrids between tetraploid perennial ryegrass and diploid meadow fescue are backcrossed to diploid perennial ryegrass. After repeated backcrossing, diploid hybrid derivatives with small fescue segments in a ryegrass background are obtained, as generally described by Humphreys (1989). However, the introgression breeding method has not proven very successful, because it appears as though too many of the chromosomal regions or genes from fescue determining winter survival and disease resistance are lost or inactivated during the backcrossing generations or in the following generations of seed propagation (Østrem et al. 2007). Investigations of introgressed material using *in situ* hybridisation have identified very small fescue segments in the genomes of the hybrid derivatives (Lideykytė et al. 2006). Bartoš et al. (2011) showed that ×*Festulolium* Lp^{Fp}-D-N (FuRs0357) contains genotypes with a high frost tolerance and were able to map and associate DArT markers to quantitative trait loci (QTLs) for frost tolerance. However, few fescue chromosomal segments were present in the frost tolerant genotypes and both diploid and tetraploid plants were found. Therefore, in order to succeed in a ×*Festulolium* breeding strategy, we need to understand how the transfer of stress tolerance genes can be better controlled in an introgression breeding programme, and to ensure that resistance genes from fescues are fully expressed in a ryegrass genomic background.

Another way to combine the traits of *Lolium* and *Festuca* is amphiploidy whereby whole genomes of parental species are maintained in the hybrids (Humphreys et al. 2003), and ×*Festulolium* cultivar development so far has largely been through the amphiploidy approach (Ghesquière et al. 2010). This approach has also been used in the Norwegian plant breeding programme for transferring stress tolerance from the fescues into ryegrass when combining perennial ryegrass with meadow fescue. To a lesser degree, Italian ryegrass has been combined with meadow fescue to take advantage of valuable traits such as high regrowth and forage quality from the ryegrass in the amphiploid hybrid in addition to the fescue stress tolerance. Experiences show that the amphiploid approach is better for Nordic growing conditions than the introgression method, mainly due to better winter survival, and for persistency reasons perennial ryegrass should be used as the ryegrass parent to generate hybrids (Østrem & Larsen 2010a). The results obtained for ×*Festulolium* LpFp-T and LmFp-T in the present study support this. They demonstrate the stress tolerance present in the fescues, but also the importance of the adaptational level of winter survival in perennial ryegrass as compared with Italian ryegrass. However, with the amphiploid approach a genetic drift is clear and investigations have revealed significant and progressive loss of fescue chromosomes throughout generations (Zwierzykowski et al. 2006).

The inherent potential for adaptation is always important in plant breeding material. The ×*Festulolium* candivars Lp^{Fp}-D-UK and Lp^{Fp}-D-N have been generated by similar introgression methodology, the main difference being in the origin of the parental material. It originates from material adapted to either UK or Nordic conditions, which might be the main reason behind the better performance of Lp^{Fp}-D-N. A few generations of natural selection in Norway of the UK-material have not been sufficient to match the Nordic material as regards adaptation level, but over a longer time span such southern adapted material will be valuable in future breeding programmes. Of the parental species tested here, meadow fescue had overall high persistence, but the adaptation differences, and thus stress tolerance, were highly variable within perennial ryegrass in contrast to Italian ryegrass and should be emphasised. In official Norwegian variety testing, the amphiploid entries have demonstrated less winter damage, and thus better DMY, than the diploid hybrid derivatives (Nesheim & Langerud 2010; Nesheim & Langerud 2011). In our study the lower DMY in ×*Festulolium* cv. Felopa and Lp^{Fp}-D-UK was mainly caused by low winter survival (Østrem & Larsen 2010b). High DMY is usually obtained at the southern location (Fureneset) and the occurrence of winter damage after the first winter is in most years minimal at this location. However, perennial ryegrass and ×*Festulolium* are quite vulnerable to low-temperature fungal diseases. In spring 2007 (first-year ley), some attack by snow mould (*Microdoccium nivale*) was observed and spring growth was delayed at Fureneset. This was followed by a wet, cold May and a warm and extremely dry June. At the northern location (Vågønes), the two experimental years were fairly similar and, in the absence of any severe fungal attack, high DMY was obtained.

Natural selection for field survival of ×*Festulolium* strains compared with unselected tetraploid parental populations has proven successful in obtaining higher adaptation to forage production environments in harsh USA climates (Casler et al. 2002). For amphiploid ×*Festulolium*, natural selection should be pursued in the breeding programme, as was done to generate LpFp-T. High genetic variation for frost resistance in amphiploid ×*Festulolium* populations has been experienced and the mother population of LpFp-T (cv. Fabel) has thus been established at five locations in Norway from 59°N to 69°N for selection of plants surviving in different climate conditions for future ×*Festulolium* breeding purposes.

The ×*Festulolium* cv. Hykor was included in the present study due to its market position within the Nordic countries, to which it was introduced initially as a tall fescue cultivar. The initial hybrid of Italian ryegrass × tall fescue was re-crossed with selected tall fescue varieties (http://www.pbhz.cz/michal/variety/english/fl/hykoren.pdf) and should be considered more as an introgressive form than a true ryegrass × fescue amphiploid (Ghesquière et al. 2010). In contrast, ×*Festulolium* cv. Felopa as a loloid type is comparable with the candivars investigated. Timothy was included since due to its persistency, it will continue to be an important forage grass species in ley mixtures in the Nordic countries in the foreseeable future. Timothy is an important species in mixtures and the agricultural community is likely to demand late-heading cultivars of other species as companion grasses for timothy for which ×*Festulolium* cvs. are valuable options.

The DDMY data in this study provide an overall picture of the production potential of the entries investigated, with only minor changes in the DMY ranking of the entries for the two most relevant MSs, MS 2 and MS 3. When harvesting at these MSs the DDM is generally at a satisfactory level and the general yield ability is of overall importance. Due to high DMY in all harvests, ×*Festulolium* cv. Hykor kept its top position when DMY was adjusted for DDM, although the higher DDM values in ×*Festulolium* LpFp-T evened out the DMY differences between these two entries. This demonstrates the importance of forage quality in forage grass breeding although this trait is not yet included in the national variety testing in Norway.

Fertility and seed productivity are crucial aspects in grass breeding and lower seed set is reported for amphiploid ×*Festulolium* compared with their parental species (Ghesquière et al. 2010; Kosmala et al. 2010). Seed production has not yet been evaluated in the entries investigated here, but since the amount of stems is one of the several characters determining seed productivity, the LSR may indicate the potential in the ×*Festulolium* candivars. When averaged over MS 3 and MS 4 in the first cut in 2007, LSR was fairly equal in ×*Festulolium* cv. Felopa and LpFp-T (above 0.5), and above 0.4 in the two diploid candivars, and 0.39 in perennial ryegrass cv. Napoleon, which indicates lower seed productivity of the ×*Festulolium* candivars also in the Norwegian programme. The differences in LSR between entries are also expressed in DMY. Although overall first-cut DMY was significantly higher in MS 3 than in MS 2, ×*Festulolium* cv. Hykor, cv. Felopa and Lp^{Fp}-D-UK did not benefit from further growth once MS 2 was reached, owing to their high LSR and energy transfer mainly to the leaves. There is a general contradiction between breeding for forage DDMY and seed production. Current breeding of ×*Festulolium* is aiming to increase the amount of inflorescences in the first cut to secure a satisfactory seed yield, which might be achieved without reducing the forage quality too much, and leafy regrowth.

Elongation of the growing season owing to climate change will increase the use of more leafy species, so amphiploid cultivars of loloid type ×*Festulolium* may constitute valuable alternatives to perennial ryegrass. Due to their high forage quality and DMY, such cultivars are surely interesting as companion species to increase the DDMY in timothy-based seed mixtures. Of the ×*Festulolium* candivars investigated here, LpFp-T may meet the requirements for such a mixture due to its relatively late heading and valuable attributes concerning DMY and DDM, as well as increasing the regrowth which is a necessity in a longer growing season. Amphiploid cultivars might also be a valuable substitute for meadow fescue in regions where the winter stress is not too harsh, since the winter survival of meadow fescue exceeds that of loloid type ×*Festulolium* in most regions. Using natural selection to generate more persistent loloid types of ×*Festulolium* might expand the growing area of ×*Festulolium* cultivars at high latitudes. Combining valuable attributes of ryegrasses and fescues is an innovative approach for grassland farming, but achieving full expression of the attributes of this interspecies combination is still truly a challenge.

Acknowledgements

This study was carried out at the regional departments of Bioforsk - Norwegian Institute for Agricultural and Environmental Research, in Fureneset and Vågønes. The technical staff at both locations are thanked for skilful work. This study was made possible by a grant from the Research Council of Norway (project no. 173319/I10: *Festulolium* with improved forage quality and winter survival for Norwegian farming). The authors are thankful to Odd-Arne Rognli for critical reading of the manuscript and for valuable comments on the paper of two anonymous referees.

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