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***Festulolium* field performance under fluctuating growing conditions in Lithuania**

V. KEMEŠYTĖ, K. JAŠKŪNĖ, and G. STATKEVIČIŪTĖ*

*Institute of Agriculture, Lithuanian Research Centre of Agriculture and Forestry,
LT-58344 Akademija, Kėdainiai distr., Lithuania*

Abstract

Festulolium cultivars are widely utilized in Lithuania because they are persistent under abiotic stresses and are high yielding. However, changing climate challenges the existing *Festulolium* cultivars to adapt to new growing conditions and still maintain the yield. In this study, we aimed at evaluating the yield stability of two *Festulolium* cultivars in field trials under fluctuating Lithuanian conditions. The mean total dry matter yield (DMY) of both *Festulolium* cultivars fluctuated greatly between the years and ANOVA analysis showed a significant effect of environment on total DMY as well as DMY of each cut, but the genotype \times environment interaction was not significant. There was a high difference between the total DMY of 1st year and 2nd year of use of plots in each year of observation. The highest DMYs were harvested in the years 2015 and 2016. Dry matter yield of the 1st cut was the largest component of the total DMY for most of the years. The plants overwintered the first winter after sowing very well over the whole study period, resulting in excellent spring growth. The winter survival scores of 2nd year of use of plots were lower than 1st year of use and strongly correlated with the 1st cut DMY of 2nd year of use ($r = 0.81$). Spring growth of plants at 2nd year of use was poorer, the correlation between winter survival and spring growth of 2nd year of use was 0.62. The scores of regrowth after the cuts of 1st and 2nd years of use were very similar for most of the experimental years and moderately correlated with the sum of DMYs after cuts ($r = 0.55$ and $r = 0.5$, respectively).

Additional key words: crown rust, dry matter yield, intergeneric hybrids, leaf spot diseases, winter survival.

Introduction

In recent years, the observed weather anomalies left no doubts about climate change and its effect on crop productivity in Europe and worldwide. Climate change trends, in particular rising temperatures, altered amounts and pattern of precipitation, prolonged growing season affects overwintering, growth and development of forage grasses at the cultivar or even species level resulting in frequent losses of yield (Kipling *et al.* 2016). Moreover, climate change may impact grassland composition and grassland quality, having consequences on feed production for livestock (Ergon *et al.* 2018). In order to meet the challenges that climate change will bring and to improve the production of perennial herbage plants, both breeders and farmers should carefully choose the cultivated species.

The breeders aim at combining the best traits of

different species and even genera resulted in a new grass genus - *Festulolium*. It is the hybrid between fescue (*Festuca*) species and ryegrass (*Lolium*) species offering the possibility of combining superior agricultural traits, such as excellent feeding quality of ryegrass with the persistency and stress tolerance of fescue into one single plant (Ghesquière *et al.* 2010a, Thomas *et al.* 2003). *Festulolium* is valued for quick establishment, weed suppression determined by its aggressive growth, as well as very good winter survival and regrowth capacity, thus high yields (Ghesquière *et al.* 2010b, Østrem *et al.* 2013, Jiao *et al.* 2017).

In Lithuania, *Festulolium* breeding program was initiated in 1979 when the first interspecific crosses were performed at LAMMC Institute of Agriculture, former Lithuanian Institute of Agriculture. Various crossing combinations have been used in breeding programs which

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Abbreviations: DMY1 - dry matter yield of the first cut; DMY2 - dry matter yield of the second cut; DMY3 - dry matter yield of the third cut; DMY4 - dry matter yield of the fourth cut; RG - regrowth after cuts; SG - spring growth; WS - winter survival; Y1 - first year of use; Y2 - second year of use.

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* Corresponding author; e-mail: grazina.statkeviciute@lammc.lt

resulted in four cultivars, namely Punia DS, Puga, Vėtra, and Lina DS, registered in the Common European Variety List. Two of the registered cultivars, Punia DS and Puga, were obtained from crosses between *F. pratensis* and *L. multiflorum* ssp *italicum*, Vėtra was developed from crossing *F. arundinaceae* and *L. multiflorum* ssp *italicum*, and Lina DS resulted from crossing *F. pratensis* and *L. multiflorum* ssp *multiflorum* (Nekrošas and Kemešytė 2007). All cultivars are highly tolerant to diseases and have good overwintering, in particular Punia DS, where the maternal plant was *Festuca pratensis* (Lemežienė *et al.* 2004, Nekrošas and Kemešytė 2007). *Festuca* species are known for their persistence under unfavourable conditions (Sandve *et al.* 2011), thus *Festulolium* cultivars maintaining these agriculturally important traits become increasingly relevant with more extreme environmental conditions. In terms of Lithuanian climatic conditions, *Festulolium* cultivars are widely utilized because of their good winter survival over the harsh winters and still the production of high yields (Lemežienė *et al.* 2004). However, due to climate change, winter patterns have changed to milder requiring adaptation of plants to prolonged vegetation season, to cope with spells of freezing temperatures with less or without snow cover. Moreover, increased air temperature, water shortage and unpredictable precipitation distribution during the growing season challenge *Festulolium* cultivars to maintain the yield over the whole growing season.

In this study we have chosen two *Festulolium* cultivars of different cross combination and aimed at 1) evaluating their performance in field trials over the period of seven years and 2) assessing the yield stability under fluctuating weather conditions.

Materials and methods

Plants and field experiments: Two tetraploid loloid type *Festulolium* cultivars Punia DS and Lina DS were evaluated in the field experiments. The experiments were carried out at LAMMC Institute of Agriculture (55°23'N, 23°57'E) over the period of 2013 - 2019. One experimental cycle, which started in 2014 and comprised of a year of sowing and two years of use, was omitted. The experiment was set up in 7.5 - 13.25 m² test plots using a randomized complete block design with 3 replications.

The soil of the experimental fields was Endocalcari - Epihypogleyic Cambisols (CMg-p-w-can), characterized by a homogeneous texture, pH KCl 7.3 - 7.0, humus content 1.9 - 2.2 %, available P₂O₅ 206 - 270 mg kg⁻¹, and K₂O 101 - 154 mg kg⁻¹. In the autumn of each year of use 6 g (P₂O₅) m⁻² and 9 g (K₂O) m⁻² fertilizers were applied. Nitrogenous fertilizers were applied each year of herbage use in several applications: in spring 6 g (N) m⁻², and 4.5 g (N) m⁻² after each cut, except the last one.

To determine the dry matter yield (DMY), 0.5 kg of fresh biomass of each sample was oven-dried at 105 °C to constant mass. The plots were harvested 3 - 4 times per year depending on the meteorological conditions; herbage yield (kg m⁻²) was assessed as production per plot. At the beginning of heading stage plots were cut for the first

time, while the second, third, and fourth cuts were taken after the plants reached ≥ 20 cm height. Winter survival and spring growth, disease resistance, re-growth after cuts were assessed in scores, where 1 is very low, 3 - low, 5 - medium, 7 - high, 9 - very high value of the trait. The beginning of the heading was recorded when more than 50 % of plants emerged.

Meteorological data: According to the environmental stratification of Europe, Lithuania is assigned to nemoral zone with a cool temperate climate and quite short growing season of 190 - 195 d (Metzger *et al.* 2012). Daily meteorological data (mean, minimal, and maximal temperatures, precipitation and snow cover) over the period of 2013 - 2019, recorded at the meteorological station in Noreikiškės, Kaunas region (54°52'N, 23°50'E) were obtained from the *Ogimet* repository by using 'climate' package for R (Czernecki *et al.* 2020).

Winter period was considered to start since the first day when $T_{\min} \leq -10$ °C, cold days were considered when T_{\min} is ≤ -15 °C, while the warm days when $T_{\text{mean}} \geq 0$ °C (Bélanger *et al.* 2002, Thorsen and Höglind 2010). Degree days (DD) were calculated with the base temperature of 5 °C. The average annual precipitation at the experimental site is 550 - 600 mm and the average annual temperature is 6.0 - 6.5 °C (Fig. 1 Suppl.). The mean length of winter season over the experimental period was 110.3 d. The shortest winter season was in 2013/2014 lasting for 66 d, while the winter of 2013 and 2015 was the longest - 134 d. The winter of 2013 was distinguished by permanent snow cover and mean temperatures below 0 °C, however without extreme cold spells (Fig. 1 Suppl.). The winter season of 2018 was short, lasting 86 d, nevertheless, it was characterized by the highest number of cold days, when the minimum temperature dropped below -15 °C and with very low snow insulation. Cool and rainy spring was recorded in 2017, while in August 2015 and June 2016 the drought occurred, no precipitation was recorded for 25 and 20 d, respectively. The longest vegetation season was in summer of 2017, though this season was the coolest, characterized by the lowest value of DD throughout all the cuts (Table 1 Suppl.). The same trends were also observed for the summer of 2016. The shortest growing season was observed in 2015; moreover, it was one of the driest, with only 174.9 mm of rainfall, and one of the coolest (Table 1 Suppl.). Summers of 2018 and 2019 were characterized by the highest DDs over all periods from one cut to another, also as very dry seasons having the least amount and frequency of rainfall. In contrast, the vegetation season of 2017 is featured the highest percentage of rainy days (87 %) and the most intensive rainfall of 477.4 mm (Table 1 Suppl.).

Statistical analysis was implemented in the open-source R statistical environment v. 4.0.2 (R Core Team 2020, R Foundation for Statistical Computing, Vienna 2020). Basic descriptive statistics was calculated using R package 'metan' function *desc_stat* (Olivoto and Lúcio 2020), analysis of variance and post-hoc tests were conducted using the R package 'agricolae' (De Mendiburu and Yaseen 2020). To estimate genotype and environment interaction

and effect on DMY, *ANOVA* and subsequently post hoc Tukey HSD test was applied. The Kruskal-Wallis test was used for analyzing the influence of year on qualitative traits and Fisher's least significant difference with Bonferroni correction was applied to test for significant differences between the trait means.

Results

The mean total DMY of both *Festulolium* cultivars fluctuated greatly between the years, ranging from 0.67 to 1.22 kg m⁻² in 1st year of use (Y1) and from 0.19 to 0.64 kg m⁻² in 2nd year of use (Y2) (Fig. 1). *ANOVA* analysis showed a significant effect ($P < 0.001$) of the environment on total DMY as well as DMY of each cut. However, the genotype and environment interactions were not significant; therefore, only means over the two cultivars are reported. The lowest total DMY of Y1 was obtained in 2013, whereas the highest total DMY of Y1 was obtained in 2016. The highest total DMY of Y2 was recorded in 2015, the lowest in 2018. The dry matter yield of the 1st cut (DMY1) was the largest component of the total DMY except for 2013 and 2014, when it did not differ from the 2nd cut (DMY2) in the Y1 plots and was the smallest part of total DMY of the Y2. In 2016 and 2017 it was possible to harvest the 4th cut in Y1, and in both Y1 and Y2 in 2019. DMY3 was the most variable DMY part in the Y1, cv = 48 %; DMY1 was the most variable yield component in the Y2 (cv = 69 %). Total DMY of Y2 plots was significantly lower than that of Y1 plots (considered 100 %), with a reduction of 43.5 % (2019) to 79 % (2018) and an average reduction of 61.4 % over all years. The highest yield difference was estimated in 2014 between the DMY1 (84.5 %) and the lowest one was in DMY3 of 2017, only 0.4 %.

The Y1 plots survived the first winter of the cycle very well, mean winter survival (WS) was 8.3 to 9, with the only exception being 2014 (mean WS score 6.4) (Fig. 2). The WS of Y1 was the only trait with significant cultivar effect: the differences between Punia DS and Lina DS were not statistically significant in each year, however, Lina DS scored steadily lower, resulting in lower mean winter survival ($P < 0.05$) over the whole period of investigation. The WS scores of Y2 plots were lower than Y1 but still rather high, the mean scores of 2015 - 2019 varied from 7.8 to 8.8. The plots suffered a greater winter damage only in 2013 and 2014 (WS score 4.7 to 5) (Fig. 2B). Strong correlation was estimated between WS of Y2 and DMY1 of Y2 ($r = 0.81$, $P < 0.001$, $n = 12$). Spring growth (SG) score of Y1 plants was high in all years, ranging from 7.9 to 9 (SD = 0.40). Y2 plots exhibited poorer SG and higher variation of this trait (SD = 1.36), with minimum SG score 4.8 (2013) and maximum 8.8 (2015 and 2017). The correlation between WS and SG of Y2 was 0.62 ($P < 0.001$). Regrowth after cuts (RG) score ranged from 6.7 to 8.8 in Y1 and from 4 to 7.5 in Y2. RG scores of Y1 and Y2 were very similar in 2013 and 2019, the highest RG score difference was reported in 2018 (RG of Y1 score was 7.1, RG of Y2 score was 4). A moderate correlation was estimated between RG and sum of DMYs after cuts in both Y1 and Y2 plots ($r = 0.55$ and $r = 0.5$, respectively, $P < 0.01$).

Plant height fluctuated between 53 to 107 cm (Y1) and 44 to 112 cm (Y2) during 2013 - 2019 period (Fig. 3A). In general, Y1 plots tended to be taller than Y2 plants except for 2014. The greatest height difference was measured in 2018, when Y2 plots were shorter than Y1 plots by 47 %. There was a moderate correlation between plant height and total DMY for the Y1 trials ($r = 0.49$, $P < 0.05$).

In all years of investigation, the heading was recorded in May, ranging from 21st to 31st (Fig. 3B). The latest

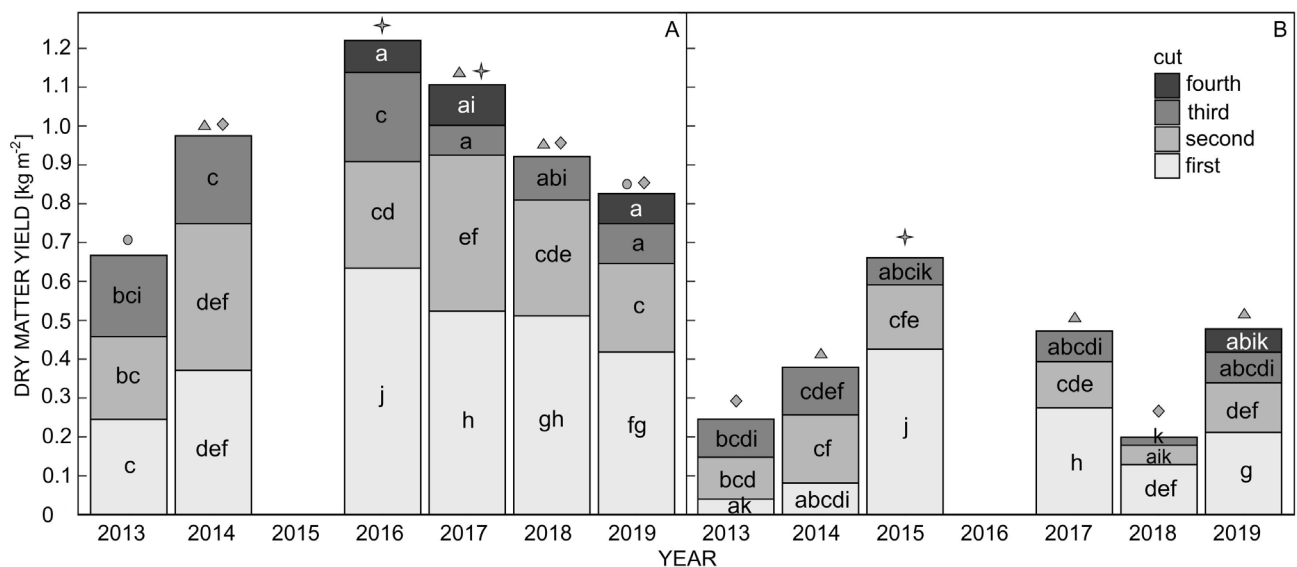


Fig. 1. The mean dry matter yield (DMY) of two *Festulolium* cultivars during the period of seven years. Means \pm SDs, $n = 6$. Within either the first year of use (A) or the second year of use (B), individual cuts denoted with the same letter, and total annual DMY columns topped with the same symbol, are not significantly different (Tukey's honestly significant difference test, $P > 0.05$).

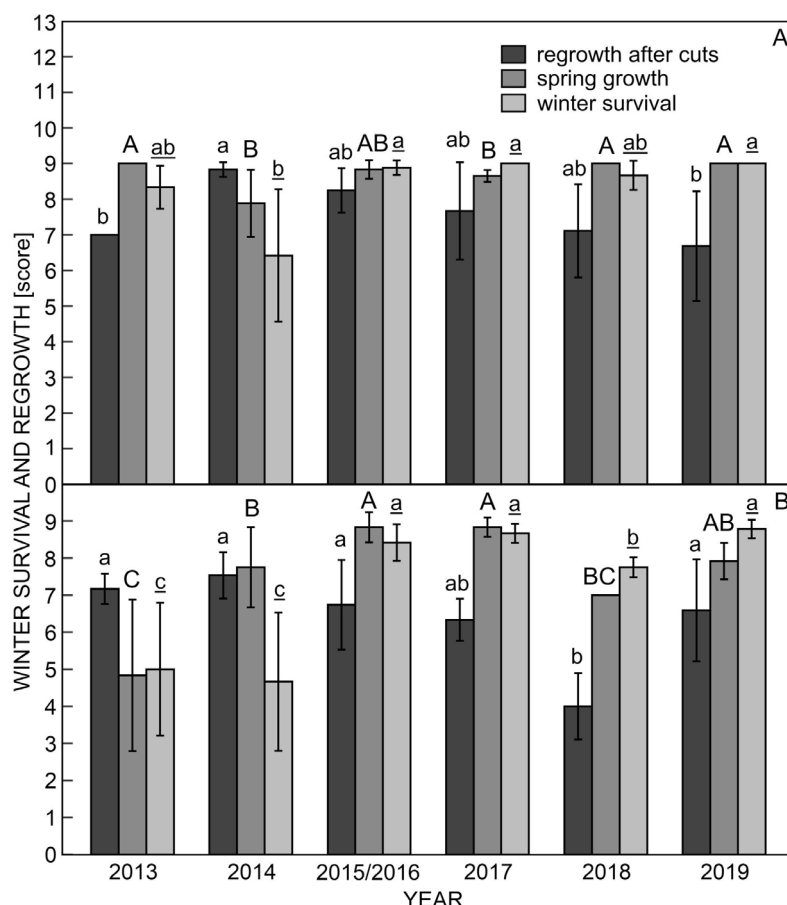


Fig. 2. The mean score of winter survival, re-growth in spring, and after the cuts of two *Festulolium* cultivars over the period of 2013-2019. The scores of the first year of use are presented in A and the scores of the second year of use in B. Means \pm SDs, $n = 6$. The letters indicate difference among years, where winter survival is specified with a lowercase letter, while regrowth in spring, and after cuts are represented by uppercase and underlined letters, respectively (Fisher's least significant difference test with Bonferroni correction, $P < 0.05$).

heading date of both Y1 and Y2 trials was recorded in 2017. Punia DS and Lina DS reached the heading stage at the same time or differed by only 1 d.

The mean crown rust and leaf spot disease infection scores ranged from 1 to 5.5 (Fig. 4). Both diseases were most prevalent in 2013 (mean crown rust score 5.2, mean leaf spot score 5.4) and least prevalent in 2019. The two cultivars did not differ in their disease resistance, the only significant environmental effect was confirmed by analysis of variance.

Discussion

Festulolium grasses, the interspecific hybrids between *Lolium* and *Festuca*, have been developed as an alternative to existing *Lolium* and *Festuca* cultivars as they featured superior resilience against abiotic stresses compared to perennial ryegrass (Ghesquière *et al.* 2010a,b, Humphreys *et al.* 2010). *Festulolium* cultivars are of similar forage quality as ryegrass (Humphreys *et al.* 2003), but their winter hardiness is typically lower than their parental *Festuca* species (Brink *et al.* 2010). The main reason limiting

the cultivation of ryegrasses in the Baltic region is their susceptibility to harsh winter conditions and subsequently low persistence (Kemesyte *et al.* 2017). It was demonstrated in the previous 13 years-long study, that perennial ryegrass is the least winter-hardy, compared to other forage grass species (Lemežienė *et al.* 2004), including *Festulolium*, under Lithuanian conditions. *Festulolium* cultivars, used in this study, offered high winter survival in the first year of use and excellent spring growth, resulting in high dry matter yields of the first cut. The record of low yield in 2013 was most likely determined by a short spring growth period rather than harsh winter. In general, autumn periods became longer and warmer than the long-term average in the recent decade in Lithuania as well as elsewhere in the Northern-Baltic region (Ergon *et al.* 2018). This phenomenon allowed to harvest the 4th cut in the first year of use in 2016, 2017 and 2019. Furthermore, long warm autumns allow successful cultivation of southern-adapted forage grass populations (Dalmannsdóttir *et al.* 2015), thus expanding the choice of cultivar for farmers. However, high autumn temperatures delay the start of the hardening period, which can negatively impact plant resistance to stress factors during winter (Ergon *et al.* 2018). Insufficient

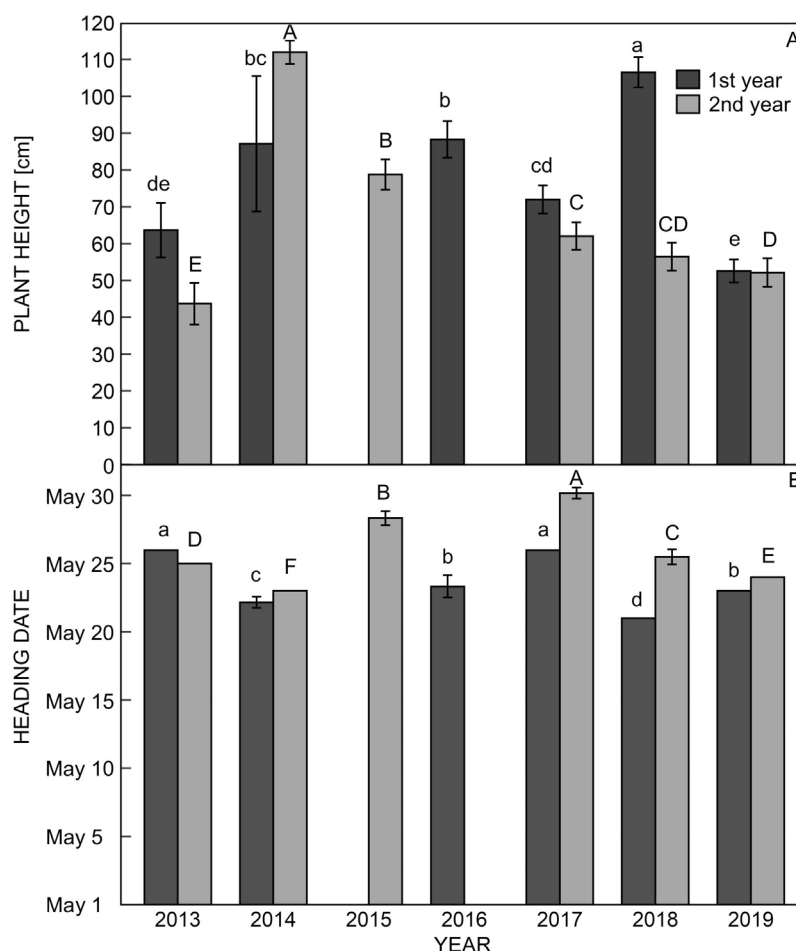


Fig. 3. The mean values of plant height and heading date of two *Festulolium* cultivars over the period of 2013–2019. Plant height is shown in A, where the difference between the first and second years of use are specified with lowercase and uppercase letters, respectively (Tukey's honestly significant difference test, $P < 0.05$). Heading date is given in B, where lowercase letters denote differences between years of the first year of use, and uppercase of the second year of use (Fisher's least significant difference test with Bonferroni correction, $P < 0.05$). Means \pm SDs, $n = 6$.

acclimation most likely reduced winter survival in 2014: most of the winter period was much warmer than 2013, but a sudden drop of mean temperatures below -12°C for 2 weeks with thin snow cover led to lower winter survival scores. The similar winter weather pattern was observed in 2016, but plants were most likely protected by the thicker snow cover. The highest total dry matter yield was obtained in the rather cool and rainy growing season of 2016, whereas heatwaves and drought spells of 2018 and 2019 significantly reduced the yields. *Festulolium* of loloid type is less resistant to drought than fescues, especially tall fescue (Fariaszewska *et al.* 2016), but more resistant than perennial ryegrass, most likely due to the deeper, better-developed root system (Humphreys *et al.* 2018), making it a more suitable high-quality forage source in the future. Another concern related to climate change is the possibility of increased incidence and severity of plant diseases in the Northern regions (Ergon *et al.* 2018). Crown rust and leaf spots were observed in all years of this study, however, the infection was not severe except in 2013, indicating that at least for the nearest future forage grass breeders do not have to shift their efforts in this direction. However, the sward

persistence was still the major issue. Both *Festulolium* cultivars suffered high yield reduction in the second year of use, especially in the growing season of 2018, which was the hottest and driest throughout the span of seven years of study, indicating that long-lasting grasslands can only be achieved with significant improvement of plant drought resistance. However, breeding for winter hardiness should not be abandoned either. Even though winters are predicted to get warmer in Northern Europe, the lack of adequate acclimation together with extreme weather events during winter can still lead to substantial yield losses.

Festulolium breeding, even though rather complicated due to the nature of interspecific crossing, can potentially be highly rewarding as the resulting hybrids can comprise the best traits of parental species, far exceeding the trait variation boundaries of parental species. The vast majority of *Festulolium* cultivars were developed by crossing *Lolium perenne* or *L. multiflorum* on the *Lolium* genus side and *Festuca pratensis* or to the lesser extent *F. arundinacea* on *Festuca* genus side (Kopecký *et al.* 2008). However, *Festuca* is a large genus, its complex phylogeny is being explored to this day (Cheng *et al.* 2016,

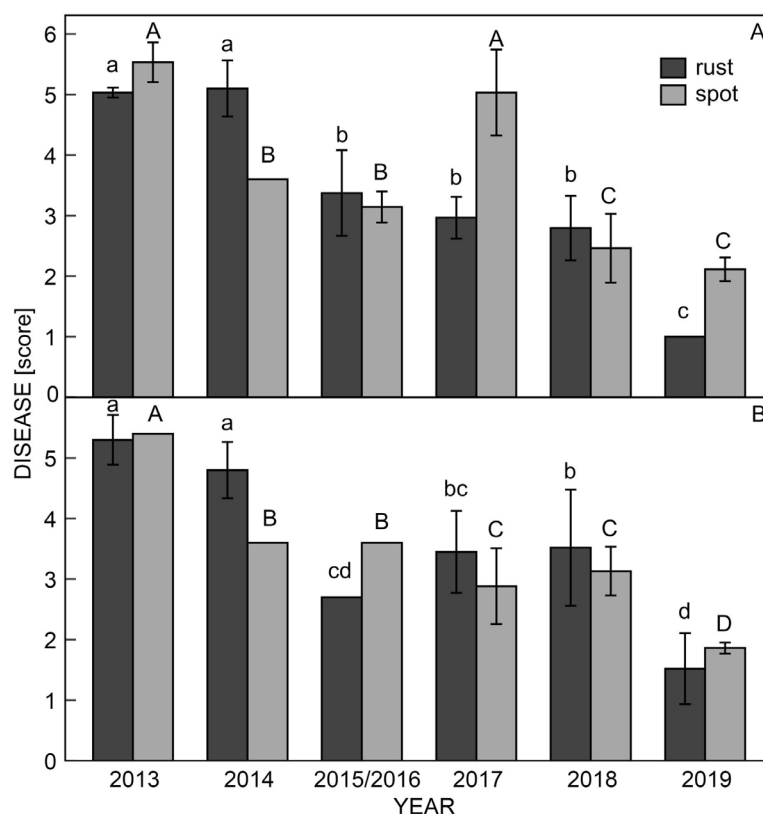


Fig. 4. The mean score of crown rust and leaf spot diseases of two *Festulolium* cultivars over the period of seven years. A and B show the mean scores of the first and second years of use, respectively. Means \pm SDs, $n = 6$. Differences among years are denoted with lowercase letters for crown rust and uppercase letters for leaf spot diseases (Fisher's least significant difference test with Bonferroni correction, $P < 0.05$).

Qiu *et al.* 2019), so the possibilities for the breeders to experiment are nearly limitless. Both *Festulolium* cultivars in this study came from the crosses with *F. pratensis*. However, this fescue species might not be the best choice as it is less resistant to drought and has a lower root mass and diameter than *F. arundinacea* (Wilman *et al.* 1998, Cougnon *et al.* 2017). Other fescue species, successfully applied in the development of highly productive hybrids, are *F. arundinacea* var. *glaucescens* and *F. mairei* (Ghesquière and Bourgoin, 2010, Humphreys *et al.* 2014). *F. arundinacea* and *F. rubra* has been included in the recently re-opened *Festulolium* breeding programme in Lithuania as well, however, further investigation is needed to evaluate whether these new hybrids have superior stress tolerance and persistence compared to currently grown cultivars.

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