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Research Article

Row spacing and nitrogen fertilization effect on red fescue (*Festuca rubra* L.) seed yield

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Abstract

Red fescue (*Festuca rubra* L.) is a grass that can grow in low-nutrient soils and can withstand extreme abiotic stresses like winter cold and summer drought. It is used in seed mixtures for lawns and pastures. Red fescue production is affected various factors: genotype, climate, and edaphic conditions. The purpose of this study was to measure red fescue seed yield of output in three successive years (2018-2020) under various sowing intervals and nitrogen fertilizer treatments, as well as to investigate the link between seed yield, N fertilization, and seeding rates. Weather conditions are crucial to the seed yield of red fescue. The average seed yield of red fescue per panicle was the highest in 2018, with a row spacing of 25 cm and the C2-b fertilization treatment. Grasses are productive due to their rapid growth response to high nitrogen (N) fertility rates. The results show that the effects of year, sowing spacing, fertilization method, and their interactions on seed yield per hectare were found to be highly significant. A consistent and highly significant correlation was observed between seed yield per panicle and seed yield per hectare across all years. A highly significant negative correlation was established between the weight of one thousand seeds and the number of seeds per gram.

Keywords: correlation coefficients; generative stems; seeds yield parameters; nitrogen treatment

Introduction

Red fescue is an excellent ground cover plant that is suitable for use in mixtures on sown pastures and meadows. It regenerates well after grazing and is commonly found in natural meadow and pasture communities

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(Tomić *et al*., 2011). It retains its green color until late autumn and can be successfully sown in various areas, such as the inter-row spaces of orchards, regions designated for noble game, for soil stabilization, protection against erosion, and even in the cultivation of ski slopes and canals. Additionally, it can be included in mixtures for establishing specialized lawns, including parks, sports fields, and home gardens. Red fescue is particularly effective in preventing erosion on ski slopes (Bjedov *et al*., 2011). With proper care, established grass areas can be used for many years in fodder production or as decorative surfaces due to the longevity of red fescue (Lakić *et al*., 2019). Its tolerance to low grazing and good hay quality makes it a valuable forage grass (Stanisavljević *et al*., 2014).

The yield and quality of legumes are primarily influenced by balanced mineral nutrition, which is affected by soil and climatic conditions of the region, along with other agroecological factors (Popović *et al*., 2022; 2024; Bojović *et al*., 2024). While red fescue thrives in warmer climates, it also exhibits good frost tolerance. Variations in traits among perennial grasses are often attributed to a combination of climatic conditions over the study years and the age of the crop (Biligetu *et al*., 2012; Amini *et al*., 2013; Terzić *et al*., 2019). Red fescue is both shade- and drought-tolerant and can be grown on different soil types; however, it reaches its full potential on fertile soils rich in nitrogen.

Bosnia and Herzegovina offer favorable conditions for red fescue seed production. Organizing seed production is crucial for any plant that reproduces generatively, as it serves as the foundation for lawns and quality fodder production (Žurek *et al*., 2017). During seed production, attention must be paid to inter-row spacing and fertilization with nitrogen, phosphorus, and potassium. After establishing a red fescue crop, it can be successfully maintained with low to moderate nitrogen amounts, typically less than or equal to 98 kg ha^{-1} (Braun *et al*., 2021). The productivity of grass seed crops is influenced not only by the genetic properties of species and varieties, but also by environmental conditions and applied agricultural techniques (Szczepanek *et al*., 2020). Increasing the spring rate of nitrogen fertilization may enhance panicle length and the number of spikelets per panicle, but it has no significant effect on the number of flowers, seeds per panicle, or the weight of one thousand seeds (Szczepanek *et al*., 2021). Nitrogen fertilization significantly impacts the yield of red fescue seeds, applied during seed production in both autumn and spring, either in a single dose or split into two applications. Autumn application of nitrogen encourages the formation of generative stems. Although red fescue can yield very high seed production, it often experiences low yields, resulting in significant variability for this plant species (Stanisavljević *et al*., 2013). The relationship between normally formed seeds, which exhibit high germination rates, and smaller seeds that do not germinate is also important for yield formation in forage grasses (Stanisavljević *et al*., 2007). The highest costs of grass seed production occur during the establishment of the seed crop and in the first year of production. Following establishment, it is essential to utilize the seed crop for several years to distribute costs evenly across all production years.

The aim of this study was to investigate the influence of different sowing distances and nitrogen fertilization methods on yield components and seed yield.

Materials and Methods

Experimental design

Field research was conducted at the experimental field of the Agricultural Institute of the Republic of Srpska in Banja Luka, Bosnia and Herzegovina. The experiment took place during the summer-autumn sowing period, utilizing a randomized block design with four replications. This research was carried out over three years, from 2018 to 2020. Each basic plot measured 10 m^2, and prior to sowing, standard agronomic practices were implemented. Basic fertilization involved applying 300 kg ha^{-1} of mineral fertilizer (NPK 15:15:15).

For the sowing process, seeds of the red fescue variety 'Buki' were used. During the establishment of the experiment, sowing was performed at two different intervals: 12.5 cm (B1) and 25.0 cm (B2). A sowing rate of

30 kg ha^{-1} was used for a row distance of 12.5 cm, whereas at a spacing of 25 cm, the sowing rate was reduced to 15 kg ha^{-1}. The distance between plots within a replication was 40 cm, while the distance between replications was 1 m.

Nitrogenous mineral fertilizer, CAN (27% N), was used for top dressing the crops. For each sowing interval, two fertilization treatments with nitrogen-based mineral fertilizers were applied:

C1-a = Nitrogen fertilizer was applied in mid-March at a rate of 54 kg ha^{-1} of N, followed by a second application 20 days later (beginning of April) with 27 kg ha^{-1} of N.

C2-b = Top dressing with nitrogen fertilizer CAN (27% N) was applied at the end of October with a dose of 27 kg ha^{-1} of N and during the onset of vegetation (coinciding with forsythia bloom) at a rate of 54 kg ha^{-1} of N.

Weed control was implemented on two occasions. The first treatment in all years was conducted at the beginning of April using the herbicide Sekator OD (active ingredients: iodosulfuron-methyl-sodium g l^{-1}, amidosulfuron 100 g l^{-1}, mefenpyr-diethyl 250 g l^{-1}) at a dose of 150 ml ha^{-1}. The second herbicide treatment was applied 20 days after the first with the herbicide Focus Ultra (active ingredient: cyloxidim 100 g l^{-1}) at a dose of 3 l ha^{-1}. This second treatment aimed to control problematic grass species in red fescue seed production, such as cat grass, English ryegrass, and Italian ryegrass.

During the experiment, the following parameters were monitored: number of generative stems/m^2 (BGS), mass of panicle with seeds (g), seed yield/panicle (g) (PSM), number of seeds/g (BSG), weight of one thousand seeds (g) (MHS), and seed yield/ha (kg ha^{-1}) (PSH). The number of seeds/g was determined using a Contador seed counter, while the mass of one thousand seeds was assessed from the pure seed fraction in the Seed Quality Control Laboratory of the Agricultural Institute of the Republic of Srpska in Banja Luka. A sample of one hundred seeds was taken in eight repetitions according to ISTA Rules, 2018. Seed yield was measured after harvesting the crops with a Hege 125C field combine and processing on a Kamas Westrup laboratory sorting machine.

The analysis of mean monthly temperature (°C) and total monthly precipitation (mm) was conducted using data obtained from the Republic of Srpska Hydrometeorological Institute for the Banja Luka area. These climate variables were compared to the multi-year averages for the region.

Chemical analyses of soil samples were carried out at the Laboratory for Soil Fertility Control of the Agricultural Institute of the Republic of Srpska in Banja Luka. The assessment of the arable soil layer included measuring soil fertility parameters such as soil acidity (pH, potentiometric method), humus content (calorimetric method), ammonia nitrogen content (spectrophotometric method), and readily available phosphorus and potassium content (AL method).

Soil analysis

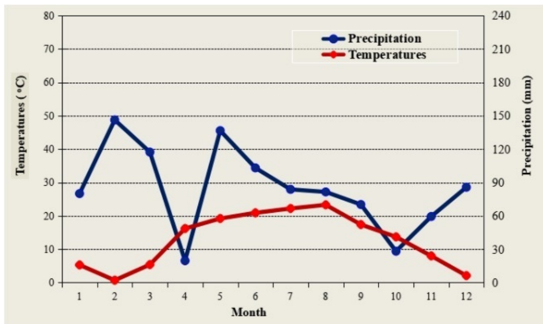
The soil used for the experimental tests is classified as valley-brown soil, located on the alluvial substrate of the Vrbas River. It is categorized as loamy clay based on its mechanical composition. The surface layer has a crumbly structure and exhibits moderate water capacity. Soil samples were collected from the arable layer, reaching a depth of up to 30 cm, and the results of the chemical analyses are presented in Table 1. The findings indicate that this is a neutral type of soil with a low humus content (<3.0%). The levels of easily accessible phosphorus and potassium were found to be adequate. The surface layer of the soil maintains a crumbly structure and demonstrates moderate water-holding capacity.

Table 1. Results of chemical analyzes of the arable soil layer

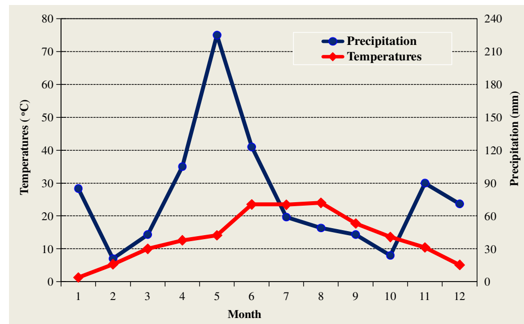
Depth (cm)	Hummus (%)	pH		mg 100g soil^{-1}	
		H_2O	KCl	P_2O_5	K_2O
0-30	2.0	7.10	6.40	16.1	22.9

Meteorological conditions

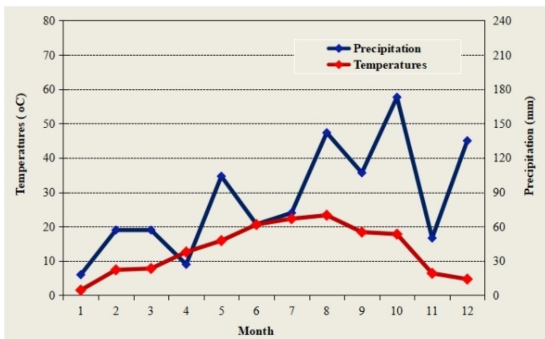
Weather conditions around the experimental site were monitored during the field surveys. For this study, data from the Banja Luka Hydrometeorological Station were used. Average monthly air temperatures and total monthly precipitation from 2018 to 2020, as well as the ten-year average from 2011 to 2020, are presented in Figures 1a, 1b, 1c, and 1d.



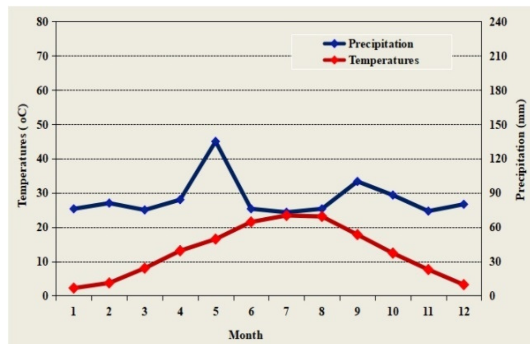
a.



b.



c.



d.

Figure 1. Precipitation and temperature in 2018, Banja Luka, B&H (a); Precipitation and temperature 2019, Banja Luka, B&H (b); Temperatures (°C) and precipitation (mm) in 2020 (c) and in long term period, 2011-2020, Banja Luka, B&H (d)

During the 2018 production year, a significant amount of precipitation was recorded from January to the end of March, surpassing the ten-year average. However, a dry period in the first half of April negatively impacted vegetation growth, with drought conditions lasting until mid-April. The total rainfall in April 2018 was only 19.7 mm, making it difficult for plants to emerge and grow. From May until the end of the growing season, there was sufficient rainfall, which positively affected the seed yield of the red fescue crop.

The average temperature in April and May 2018 was higher than that in the second and third years of the study, as well as above the multi-year average. In June, however, the average temperature dropped to 20.9 °C, which was lower than the long-term average. This reduction in temperature at the end of the vegetation period positively affected the seed yield.

During the second year of the survey (2019), from March to the end of June, there was a higher amount of precipitation recorded, which was above the ten-year average (Figure 1d). The highest rainfall occurred in May, totaling 225 mm, which was 112 mm more than the ten-year average for that month. Despite the increase in precipitation during the growing season of red fescue, the yield of the seeds remained unaffected.

After the vegetation growth period ended in March, temperatures in April and May were slightly lower compared to the perennial harvest averages. By June, the average monthly temperature reached 23.5 °C, higher than the averages of previous years in the study. The weather conditions in 2019 had a positive impact on the yield of red fescue seeds.

In contrast, from January to the harvest in early July 2020, precipitation levels were significantly lower compared to both the first and second years of the research as well as the multi-year average. A drought in April, with only 27.0 mm of rainfall, significantly reduced the seed crop. Additionally, only 62.0 mm of precipitation was recorded in June, which adversely impacted the seed yield that year. During the growing season in 2020, temperatures were slightly below the multi-year average for that period.

Statistical analysis

The results of the research were analyzed using the statistical method of Analysis of Variance (ANOVA). When significant differences were identified, the Least Significant Difference (LSD) test was applied. The coefficient of variation (CV %) was calculated for the proportion of seeds and plant residues in the total mass of the red fescue panicle. To determine the relationship between the studied properties of red fescue, the correlation coefficient (r) was calculated.

Results and Discussion

The results of the analysis regarding individual yield components and the seed yield of red fescue over three production years are presented in Table 2. The highest average number of generative stems/m^2 was observed with a sowing distance of 25 cm and the C1-a fertilization treatment. Conversely, the lowest average number of generative stems per m^2 occurred with an inter-row sowing distance of 12.5 cm and the C2-b nitrogen fertilization treatment. Throughout these tests, the number of generative stems per m^2 ranged from 189.0 in 2018 at the B1 sowing spacing with the C2-b fertilization treatment to 348.5 in 2019 at the B2 sowing spacing with the C1-a fertilization treatment.

Table 2. Number of generative stems/m^2, weight of seeds/panicle, number of seeds/g, the one thousand seed weight and seed yield/ha

Treatment		Year/ average (A)	Number of generative stems/m^2	Seed mass/ panicle (g)	Number of seeds/g	The one thousand seed weight (g)	Seed yield/ha (kg ha^{-1})
Sowing distance (B)	Method of nitrogen fertilization (C)						
12.5 cm (B1)	C1-a	2018	273.8	0.28	763.0	1.413	636.7
		2019	331.5	0.24	805.3	1.363	722.2
		2020	265.0	0.14	806.3	1.350	347.6
		Average	**291.1**	**0.22**	**791.5**	**1.375**	**568.8**
25 cm (B2)	C2-b	2018	189.0	0.26	661.8	1.538	389.7
		2019	259.8	0.27	686.0	1.488	558.8
		2020	217.8	0.14	789.9	1.375	270.8
		Average	**222.2**	**0.22**	**712.4**	**1.467**	**406.4**
12.5 cm (B1)	C1-a	2018	270.0	0.26	775.5	1.450	499.9
		2019	348.5	0.24	882.3	1.293	654.8
		2020	312.8	0.12	841.0	1.273	334.9
		Average	**310.4**	**0.21**	**832.9**	**1.339**	**496.5**
25 cm (B2)	C2-b	2018	189.8	0.33	725.5	1.488	572.0
		2019	307.8	0.26	819.8	1.475	845.1
		2020	262.8	0.14	789.8	1.395	348.1
		Average	**253.5**	**0.24**	**778.4**	**1.453**	**588.4**

The average seed mass in the panicle ranged from 0.21 g, observed with a 12.5 cm inter-row spacing under the C1-a fertilization treatment, to 0.24 g, found with B2 inter-row spacing and C2-b fertilization treatment. The highest seed yield/panicle occurred in 2018, specifically with B2 inter-row spacing and C2-b fertilization treatment. Conversely, the lowest seed mass per panicle across all sowing intervals and fertilization treatments was recorded in the third year of the study, ranging from 0.12 g to 0.14 g.

Table 3 presents the average number of seeds/g, varying by the year of the study, sowing distance, and nitrogen fertilization method. Over the three-year period, the average number of seeds/g varied from 661.8 in 2018 to 882.3 in 2019, influenced by these factors. The highest average number of seeds/g was recorded in 2019, with the B2 inter-row spacing and C1-a fertilization treatment.

Table 3. ANOVA parameters number of generative stems/m^2, mass of seeds/panicle, number of seeds/g, the one thousand seed weight and seed yield/ha

Parameters	F test/ LSD	Year (A)	Sowing distance (B)	Fertilization (C)	AB	AC	BC	ABC
Number of generative stems/m^2	F exp	37.39**	11.20**	65.70**	3.41*	1.77^{ns}	0.50^{ns}	0.44^{ns}
	0.05	19.2	15.6	15.6	27.1	27.1	22.1	38.3
	0.01	27.5	21.0	21.0	36.4	36.4	29.7	51.4
Seed mass/ panicle (g)	F exp	232.49**	0.40^{ns}	10.03**	2.85^{ns}	0.71^{ns}	6.78*	5.49**
	0.05	0.01	0.01	0.01	0.02	0.02	0.02	0.03
	0.01	0.02	0.02	0.02	0.03	0.03	0.03	0.04
Number of seeds/g	F exp	6.90**	8.79**	13.63**	2.14^{ns}	0.88^{ns}	0.46^{ns}	0.66^{ns}
	0.05	45.0	36.8	36.8	63.7	63.7	52.0	90.1
	0.01	60.4	49.3	49.3	85.5	85.5	69.8	120.9
The one thousand seed weight (g)	F exp	7.34**	0.84^{ns}	14.78**	0.12^{ns}	0.84^{ns}	0.22^{ns}	1.16^{ns}
	0.05	0.066	0.054	0.054	0.093	0.093	0.076	0.131
	0.01	0.088	0.072	0.072	0.125	0.125	0.102	0.176
Seed yield/ha (kg ha^{-1})	F exp	295.33**	19.44**	8,04**	4,86**	5.51**	104.52**	11.07**
	0.05	30.9	25.3	25.3	43.7	43.7	35.7	61.9
	0.01	41.5	33.9	33.9	58.7	58.7	47.9	83.0

Over the three years of testing and across both sowing intervals, the one thousand seed weight was consistently higher in the C2-b fertilization treatment compared to the C1-a nitrogen fertilization treatment. The average one thousand seed weight ranged from 1,273 g in 2020, recorded at the sowing spacing B2 with the C1-a treatment, to 1,528 g in 2018, observed at the sowing spacing B1 with the C2-b treatment. The highest three-year average one thousand seed weight of 1,467 g was noted at the sowing spacing B1 using the C2-b fertilization treatment. The analysis of variance results for the one thousand seed weight indicate that both the year of testing and the method of nitrogen fertilization have a highly significant impact on this measurement (F exp = 7.34**; F exp = 14.78**).

Table 3 illustrates the impact of year, sowing spacing, method of fertilization, and their interactions on seed yield/ha over the three-year research period. The average seed yield ranged from 406.4 kg ha^{-1} to 588.4 kg ha^{-1}, depending on these factors. The highest average seed yield was obtained with the inter-row spacing of sowing B2 and the C2-b fertilization stage.

The peak seed yields for all tested treatments occurred in the second year (2019), while the lowest yields were recorded in the third year (2020). The greatest seed yield of processed grains was achieved at the B2 sowing spacing and C2-b fertilization treatment in 2018, reaching 845.1 kg ha^{-1}. The lowest seed yields across all three years were associated with the B1 sowing spacing and C2-b fertilization treatment (Table 2). The influence of year, sowing spacing, method of fertilization, and their interactions on the seed yield/ha of red fescue during the three-year research period was found to be highly significant (Table 3).

After harvesting, the panicles from an area of one square meter were dried and weighed, after which the grains were separated. Following the threshing of the panicles, the remaining plant material and seeds were weighed. The percentage representation of the seeds and plant remains concerning the total panicle mass was then calculated. The average share of seeds, i.e. plant remains in the panicle, during the duration of these tests is shown in Table 4.

Table 4. Ratio of seeds/panicle remains (%) of red fescue by year

Parameter	Year	Sowing distance 12.5 cm				Sowing distance 25 cm			
		Methods of fertilization				Methods of fertilization			
		A		B		A		B	
		Seed	Panicle remains	Seed	Panicle remains	Seed	Panicle remains	Seed	Panicle remains
Ratio of seeds/plant residues in panicle (%)	2018	77.94	22.06	78.9	21.10	78.24	21.76	78.22	21.78
	2019	76.88	23.12	76.04	23.96	78.08	21.92	78.70	21.30
	2020	71.31	28.69	71.12	28.88	68.73	31.27	74.64	25.36
	Average ± $S_{\bar{x}}$	75.38 ±5.44	24.62 ±4.64	75.35 ±4.33	24.65 ±4.33	76.53 ±5.37	23.47 ±4.79	77.85 ±4.77	22.15 ±4.27
CV (%)		7.22	18.61	5.75	17.57	7.16	19.32	6.18	18.64

The percentage of seeds in the panicle ranged from 75.35% to 77.85%, influenced by factors such as the year, sowing distance, and nitrogen fertilization method. The coefficient of variation (CV) for the proportion of seeds in the mass of red fescue panicles was low. Conversely, the share of plant remains in the total panicle mass varied between 22.15% and 24.65%. The variability of plant residues in the total mass of the panicle was significantly greater than that of the seeds (Table 4). The correlation coefficients for the characteristics examined are presented in Table 5.

Table 5. Correlation coefficients for the analyzed traits, 2018-2020

Traits	BGS	PSM	BS	TGW	PSH
Sowing distance 12.5 cm (B1); fertilization treatment C1-a					
BGS	**1.000**	0.238^{ns}	0.031^{ns}	-0.035^{ns}	0.603*
PSM		**1.000**	-0.227^{ns}	0.230^{ns}	0.854**
BSG			**1.000**	-0.937**	-0.065^{ns}
TGW				**1.000**	0.111^{ns}
PSH					**1.000**
Sowing distance 12.5 cm (B1); fertilization treatment C2-b					
BGS	**1.000**	0.023^{ns}	0.105^{ns}	-0.273^{ns}	0.502^{ns}
PSM		**1.000**	-0.871**	0.648*	0.812**
BS			**1.000**	-0.631*	-0.636*
TGW				**1.000**	0.377^{ns}
PSH					**1.000**

[1]BGS - number of generative stems/m^2; PSM - seed yield/panicle (g); BS - number of seeds/g; TGW - one thousand seed weight (g); PSH – seed yield/ha (kg ha^{-1}); [2]*significant at 0.05; **significant at 0.01;

Between 2018 and 2020, during experiments conducted with inter-row spacings B1 and C1 under nitrogen fertilization treatment, we found a highly significant positive correlation between seed yield/panicle and seed yield/ha (r = 0.854**). Conversely, there was a highly significant negative correlation between the number of seeds/g and the weight of one thousand seeds (r = -0.937**). Additionally, a significant positive relationship between the number of generative stems/m^2 and seed yield/ha (r = 0.603*) was observed.

On the other hand, it was identified negative correlations between seed yield/panicle and the number of seeds/g (r = -0.227), between the number of generative stems/m^2 and the weight of one thousand seeds (r = -0.035), and between the number of seeds/g and seed yield/ha (r = -0.065). Although these negative correlation values were not statistically significant, they indicate that, in these instances, an increase in one trait is associated with a decrease in another.

In the seed crop of red fescue grown with inter-row spacings B1 and C2-b under nitrogen fertilization, a highly significant positive correlation was found between seed yield/panicle and seed yield/ha (r = 0.812**). Conversely, a highly significant negative correlation was observed between seed yield/panicle and the number of seeds/g (r = -0.871**). There was also a significant positive correlation between seed yield/panicle and one thousand seed weight (r = 0.648*).

These significant correlations imply that as the value of one trait increases, the value of the other trait also tends to increase. Furthermore, a significant negative correlation was found between the number of seeds/g and one thousand seed weight (r = -0.631*), as well as between the number of seeds/g and seed yield/ha (r = -0.636*).

Additionally, a negative correlation that was not statistically significant was noted between the number of generative stems/m^2 and one thousand seed weight (r = -0.273). During the tests conducted by Stanisavljević *et al*. (2013), no significant correlation (r = 0.082) was found between the seed yield of red fescue per unit area and the one thousand seed weight.

A negative correlation that was not statistically significant was observed between the number of generative stems/m^2 and the one thousand seed weight (r = -0.273). In a study conducted by Stanisavljević et al. (2013), no significant correlation (r = 0.082) was found between the seed yield of red fescue per unit area and the one thousand seed weight.

Crop density affects the number of inflorescences, and densely sown seed crops often have a higher proportion of vegetative stems (Bitarafan *et al*., 2019). In cases where there are fewer generative stems, the length of the inflorescence, branching, and the number of seeds formed in the panicle branches can partially compensate for the reduced number of generative stems (Stanisavljević *et al*., 2007). Precipitation, nutrient supply in the soil, and their interaction during the autumn period are essential for the formation of an optimal number of generative stems (Vučković *et al*., 2003).

For both fodder and ornamental grasses, the development of generative stems typically occurs in the autumn. Key factors that influence the development of generative stems include crop density, nutrient availability, soil moisture, proper application of cultivation techniques, and overall crop health (Stanisavljević *et al*., 2010; Stanisavljević *et al*., 2012).

In fodder grass seed production, establishing crops with wider inter-row distances allows for the development of a greater number of stems, more seeds per stem, a more favorable balance between generative and vegetative stems, and often leads to higher seed yields (Stanisavljević *et al*., 2013).

The analysis of variance regarding the influence of sowing spacing, fertilization method, and year on the number of generative stems/m^2 is presented in Table 3. The results indicate a highly significant influence of year (F exp = 37.39**), sowing spacing (F exp = 11.20**), and method of fertilization (F exp = 65.70**) on this trait. Additionally, the interaction effect between year (A) and sowing distance (B) on the number of generative stems/m^2 in the red fescue seed crop was also significant (F exp = 3.41*).

Furthermore, the analysis demonstrated that both the year and the application of nitrogen fertilizer significantly affected the mass of seeds per panicle (F exp = 232.49**; F exp = 10.03**). The interaction between the various sowing distances and the method of fertilization during these tests showed a significant effect on seed mass per panicle. Additionally, the interaction involving year, sowing spacing, and fertilization method (ABC) had a highly significant impact on seed mass per panicle.

It was also established that the year, sowing distance, and method of nitrogen fertilization significantly influenced the number of seeds/g (Table 3). According to Erić et al. (2016), one gram of red fescue seeds contains between 850 and 960 seeds. The number of seeds in one kilogram of red fescue seeds ranges from 650,000 to 830,000 (Fairy and Lefkovitch, 1996), which means that there are about 650 to 830 seeds in one gram. This aligns with the results of this study.

In their examination of various populations of red fescue, Stukonis *et al*. (2015) found that the mass of one thousand seeds from Latvia ranged from 0.97 to 1.58 grams, while seeds collected in Ukraine ranged from 1.22 to 1.43 grams. The average mass of the red fescue populations studied in both Latvia and Ukraine was 1.33 grams. Erić *et al*. (2016) reported that the weight of one thousand red fescue seeds was between 1.2 and 1.4 grams. Braun *et al*. (2023) observed that the weight of one thousand seeds from seven varieties of red fescue varied from 1.11 to 1.37 grams. In contrast, Szczepanek et al. (2021) conducted multi-year studies in Poland on two varieties of red fescue and found that the weight of one thousand seeds ranged from 0.87 to 1.03 grams, which is lower than the results of the previous studies.

A three-year trial with the red fescue variety 'Maxima' was conducted at the University of Copenhagen's research station in Denmark on clay soil, using various treatments. The seed yields achieved ranged from 1,573 to 2,176 kg ha^{-1} (Bitarafan *et al*., 2019). Nitrogen has been shown to stimulate the growth of generative stems, which leads to the accumulation of assimilates used for seed development (Chynoweth and Moot, 2016). Depending on the method and timing of nitrogen application, seed yields for red fescue varied between 535 and 597 kg ha^{-1} (Yoder, 2000). In the first year of the study, the average seed yield of red fescue grown with different sowing intervals was 720 kg ha^{-1}, while in the second year, it dropped to 290 kg ha^{-1} (Stanisavljević *et al*., 2013). The same authors noted that the Yemen variety's yield during the second year of testing was 60 kg ha^{-1} higher with an inter-row sowing spacing of 25 cm. For a sowing spacing of 30 cm, the seed yield achieved in the first year was 549 kg ha^{-1}, decreasing to 344 kg ha^{-1} in the second year (Fairey *et al*., 2006). Fairey *et al*. (2006) also reported that with an inter-row spacing of 15 cm, the yield during the first year (1999) was 586 kg ha^{-1}, while in the second year, it dropped to 221 kg ha^{-1}. In contrast, Szczepanek et al. (2020) reported significantly higher seed yields, ranging from 920 to 1,190 kg ha^{-1}, in experiments conducted with the application of growth regulators and varying doses of nitrogen compared to the yields in the 2021 studies.

The determined correlation coefficients from these experiments, which analyzed various traits of red fescue grown in the B2 sowing spacing under different nitrogen fertilization treatments, exhibited both positive and negative values, as shown in Table 6.

Table 6. Correlation coefficients for the analyzed traits, 2018-2020

Traits	BGS	PSM	BS	MHS	PSH
Sowing distance 25 cm (B2); fertilization treatment C1-a					
BGS	1.000	-0.142^{ns}	0.530^{ns}	-0.308^{ns}	0.387^{ns}
PSM		1.000	-0.210^{ns}	0.463^{ns}	0.710**
BS			1.000	-0.827**	0.311^{ns}
MHS				1.000	0.024^{ns}
PSH					1.000
Sowing distance 25 cm (B2); fertilization treatment C2-b					
BGS	1.000	-0.425^{ns}	0.830**	-0.174^{ns}	0.420^{ns}
PSM		1.000	-0.362^{ns}	0.454^{ns}	0.564*
BS			1.000	-0.298^{ns}	0.293^{ns}
MHS				1.000	0.383^{ns}
PSH					1.000

[1]BGS - number of generative stems/m^2; PSM - seed yield/panicle (g); BS - number of seeds/g; TGW - one thousand seed weight (g); PSH – seed yield/ha (kg ha^{-1}); 2*significant at 0.05; **significant at 0.01;

In the seed crop of red fescue sown at a B2 row spacing and fertilized with the C1-a nitrogen treatment, a highly significant positive correlation was established between seed yield/panicle and seed yield/ha (r = 0.710**). A highly significant negative correlation was found between the number of seeds/g and the weight of one thousand seeds (r = -0.827**). There was a negative relationship, which was not statistically significant, between the number of generative stems/m^2 and both the seed yield/panicle (r = -0.142) and the weight of one thousand seeds (r = -0.308). Additionally, there was a negative relationship between seed yield/panicle and the number of seeds/g (r = -0.210).

At the same inter-row spacing with the C2-b nitrogen fertilization treatment, a highly significant positive relationship was found between the number of generative stems/m^2 and the number of seeds/g (r = 0.830**). There was also a significant positive correlation between seed yield/panicle and seed yield/ha (r = 0.564*). Negative correlations were observed between the number of generative stems/m^2 and both the seed yield/panicle (r = -0.425) and the weight of one thousand seeds (r = -0.174). Furthermore, a negative relationship that was not statistically significant was noted between seed yield/panicle and the number of seeds/g (r = -0.362), as well as between the number of seeds/g and the weight of one thousand seeds (r = -0.298).

In an aging red fescue crop, there is likely to be a lower production of inflorescences, which results in reduced seed yield (Meijer *et al*., 1984; Fairy and Lefkovitch, 1996; Deleuran *et al*., 1997; 2013). As the stand matures, crop density primarily influences the number of inflorescences produced (Meijer *et al*., 1984). Additionally, the extent of lodging negatively impacts seed yield (Bitarafan *et al*., 2019). Mathiassen *et al*. (2007) reported significant responses in seed yield following both early and late applications of Moddus M in red fescue cultivar 'Maxima'.

The rapid growth response of grasses to high nitrogen (N) fertility rates increases their susceptibility to lodging. Seed yield responses to nitrogen are often polynomial, showing an optimal yield followed by a decline at higher nitrogen rates (Rowarth *et al*., 1998; Gislum *et al*., 2007). Lodging occurs when fertile tillers lack the stem strength to support their weight, which can negatively affect pollination, seed development, and overall seed yield (Hebblethwaite *et al*., 1978; Stanisavljević *et al*., 2010).

Trinexapac-ethyl is a plant growth regulator (PGR) that should be applied when the first node becomes visible, typically in late April or May (Anonymous *et al*., 2018; Zapiola *et al*., 2006). Moddus M (containing 250 g L^{-1} trinexapac-ethyl, produced by Syngenta Nordic A/S) has an off-label registration for certain grass species in Denmark, including Festuca rubra. Generally, the application of Moddus M in Denmark results in an increase in seed yield that is lower than those reported from other countries (Mathiassen *et al*., 2007).

In grass species, this sterol biosynthesis inhibitor reduces the levels of endogenous gibberellins, leading to internode compression (Rademacher *et al*., 2000; Young *et al*., 2007). It effectively controls stem elongation, reduces lodging, and has significantly increased seed yields in cold-season grasses (Silberstein *et al*., 1999a-b; 2000a-b; 2001a-b).

Conclusions

The herbicide Focus Ultra (active ingredient: 100 g l^{-1} cyloxidim) effectively controlled various wild grass species, including cat grass, English ryegrass, and Italian ryegrass, which pose a significant challenge in red fescue seed production. A three-year field experiment conducted from 2018 to 2020 revealed that weather conditions, particularly the amount and distribution of precipitation during the growing season, had a considerable impact on the results.

Key factors influencing outcomes included the year of study, inter-row spacing, and nitrogen fertilization methods, all of which significantly affected the number of generative stems/m^2, seeds/g, and seed yield/ha. However, seed yield/panicle and the weight of a thousand seeds were not influenced by sowing spacing. Instead, the year and fertilization method had a highly significant impact.

A substantial interaction effect among year, sowing spacing, and fertilization method was observed regarding seed yield/ha across all combinations. The highest average number of generative stems/m^2 over three years was recorded with an inter-row spacing of B2 and nitrogen fertilization treatment C1-a. The average seed yield/panicle was highest at sowing spacing B2 with fertilization treatment C2-b. Additionally, the average weight of a thousand seeds was greatest at sowing spacing B1 with treatment C2-b.

Overall, the highest average seed yield was achieved when growing red fescue at row spacing B2 and applying nitrogen fertilization C2-b. The proportion of seeds in the total mass of the panicle ranged from 75.35% to 77.85%, exhibiting a low coefficient of variation. A highly significant relationship was found between seed yield/panicle and seed yield/ha in both fertilization treatments at sowing spacing B1. At inter-row spacing B2 with fertilization treatment C1-a, there was a highly significant relationship between seed yield/panicle and seed yield/ha, while treatment C2-b showed a significant but less pronounced relationship.

This study underscores the importance of ongoing research into the interactions between weather patterns and agricultural practices to achieve more consistent and higher seed yields. Future research will aim to optimize seed yield by refining inter-row spacing, nitrogen fertilization methods, and understanding the impact of environmental factors, such as weather conditions. Moreover, further studies will investigate the effectiveness of herbicide treatments and enhance fertilization practices to improve wild grass control and seed production in red fescue.

Authors' Contributions

ŽL has designed, supervised and written the paper; VP, DR, MA, IĐ, ŽL and BL have participated in the experimentation and sample collection; Conceptualization: VP, DR, IĐ and MA have analyzed the data obtained; VP and BL have overseen the project and revised the manuscript. All authors have read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article

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