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Effect of nitrogen fertilization and mowing on seed yield and germination of annual ryegrass (*Lolium multiflorum L.*)

Duygu YAMAN[1], Süleyman AVCI[1]*
[1]Department of Field Crops, Faculty of Agriculture, University of Osmangazi, 26160 Eskişehir
(orcid.org/0000-0002-8444-6602), (orcid.org/0000-0002-4653-5567)
*e-mail:savci@ogu.edu.tr

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Abstract: To determine how mowing and nitrogen applications affect the seed yield components and germination characteristics of annual ryegrass (*Lolium multiflorum* L.), an experiment was conducted in Eskişehir Ecological Conditions in 2018 with Split-Plot in the Randomized Complete Block. The primary factor was mowing or not mowing, whereas the subfactor was nitrogen dose (0, 5, 10, 15, 20, and 25 kg/da). To determine the effect of the applications on the seed production and quality of annual ryegrass, plant height, spike length, number of fertile tillers, number of spikelets, thousand seed weight, seed yield, germination percentage, second-day germination percentage, mean germination time, seedling length, seedling fresh, and dry weight were examined. Among the results, mowing did not significantly affect the seed production and quality of annual ryegrass, and no significant difference occurred between nitrogen doses of 10–20 kg/da in terms of fertile tiller number and TSW. The highest seed yield, 132 kg/da, was obtained with the application of 10 kg/da of nitrogen to the mowed plots, and loss in yield was inevitable after this dose. Although total germination percentage did not change according to nitrogen dose, the highest second-day germination percentage and mean germination time were achieved in the unmowed plots with 20 kg/da of nitrogen. As a result, fields for planting annual ryegrass for seed production can be mowed or grazed in early spring. Although the best nitrogen dose for seed production was determined to be 10 kg/da, that rate can be slightly increased for earlier germination and seed emergence.

Keywords: Annual ryegrass, nitrogen doses, mowing, seed, germination

Tek yıllık çimde (*Lolium multiflorum* L.) azot gübrelemesi ve biçmenin tohum verimi ve çimlenme üzerine etkisi

Öz: Bu araştırma, tek yıllık çimde (*Lolium multiflorum* L.) biçim şekli ve azot dozu uygulamalarının tohum verim öğeleri ile çimlenme özellikleri üzerine etkisini belirlemek amacıyla Tesadüf Bloklarında Bölünmüş Parseller deneme desenine göre Eskişehir Ekolojik Koşullarında 2018 yılında yapılmıştır. Ana faktörü biçim uygulamaları (biçilen ve biçilmeyen) ve alt faktörü ise azot dozları (0, 5, 10, 15, 20 ve 25 kg/da) oluşturmuştur. Tek yıllık çimde yapılan uygulamaların tohumluk üretimine ve kalitesine etkisini belirlemek amacıyla bitki boyu, başak boyu, fertil kardeş sayısı, başakçık sayısı, bin tane ağırlığı, tohum verimi, çimlenme yüzdesi, 2. gün çimlenme yüzdesi, ortalama çimlenme süresi, fide boyu, fide yaş ve kuru ağırlığı özellikleri incelenmiştir. Sonuçlara göre, biçim uygulamasının tek yıllık çimde tohum üretimi ve kalitesine etkisi önemsiz bulunmuştur ve fertil kardeş sayısı ve bin tane ağırlığı bakımından 10-20 kg/da azot dozları arasında istatistiki olarak bir fark oluşmamıştır. En yüksek tohum verimi 132 kg/da ile 10 kg/da saf N uygulanan biçilmiş parsellerden elde edilmiş ve bu doz sonrasında verim kayıpları kaçınılmaz olmuştur. Toplam çimlenme oranı azot dozlarına göre değişmezken, 2. gün çimlenme oranı ve ortalama çimlenme süresi bakımından en yüksek değerler biçilmeyen parsellere 20 kg/da N uygulaması sonucu elde edilmiştir. Sonuç olarak, tek yıllık çimde tohumluk üretimi için ekilen alanlar erken ilkbaharda biçilebilir veya otlatılabilir. Tohum üretimi için en uygun azot dozu 10 kg/da olarak belirlenmiştir. Bununla birlikte, tohumun daha hızlı çimlenme ve çıkış yapması için bu oran bir miktar arttırılabilir.

Anahtar Kelimeler: Tek yıllık çim, azot dozları, biçim, tohum, çimlenme

1. Introduction

Mostly used for primary livestock feed, annual ryegrass (*Lolium multiflorum* L.) is always cultivated alone or with other grass species in order to improve the quality of pastures (Han et al., 2013; Pan et al., 2017). In

Turkey, sowing areas for annual ryegrass have increased 20-fold in the past 5 years (TÜİK, 2018), because its forage quality and yield have been good, and for that reason, demand for its seed has increased as well. In Turkey's coastal regions, annual ryegrass is grown as a winter crop for hay production or grazing, whereas the plant is also cultivated as the main summer crop in the Turkey's central regions in order to produce hay or seed (Kuşvuran and Tansı, 2005).

Due to its tiny seeds, the quality of annual ryegrass's seed material to be used in planting is critical (Avcıoğlu, 1997). Seed production and quality are affected by several factors, including irrigation, fertilization, plant growth regulators, mowing, disease control, harvesting, and especially climate and soil conditions (Rolston et al. 2018).

In past study, grazing or mowing grass fields in autumn or early spring did not adversely affect seed yield in areas that received regular rainfall or irrigation (Açıkgöz, 2001). However, the grazing of pastures in late spring did prevent fertile tiller development (Rolston et al. 2018). Because late mowing or grazing increases the removal of biomass, seed yield may decrease due to its removal from areas with the potential to generate emerging spikes. In that case, removing growth points in early tillers can help spikes to emerge more uniformly and can improve the estimation of growth stages for crop decisions concerning plant growth regulator (Rolston et al. 2010).

Among other nutrients, nitrogen is the top factor of seed yield in ryegrass, and accordingly, nitrogenous fertilizers are widely used for ryegrass seed production (Simić et al. 2012). Whereas nitrogen fertilization in autumn is generally not recommended for seed production in general (Hart et al. 2011; Bartholomew, 2015), the spring application of nitrogen can prove critical to the seed production of ryegrass and often increase seed yields (Youngberg, 1980; Young et al. 1996). However, nitrogen applied at the wrong time in the wrong quantities can decrease ryegrass seed production. In particular, excessive nitrogen application increases competitive vegetative tiller production, which can reduce seed yields (Young, 1988; Griffith, 1992). Although the amount of fertilizer to be applied on ryegrass varies by climate and soil conditions, a study conducted in Western Oregon recommended fertilizer applications for seed production in the range of 150–250 kg/ha per year (Davis et al., 2006). Simić et al. (2012) indicated that optimal seed production for annual ryegrass was obtained with a nitrogen application of 50 kg/ha, whereas nitrogen applications of 100 and 150 kg/ha were reported to cause decreases in yield.

Various seed yields were obtained under different ecological conditions depending on the nitrogenous fertilizers applied in the ryegrass. For this reason, it will be useful to make various yield experiments depending on different doses of nitrogenous fertilizers in different ecological regions. Also, there is very limited study on the effect of mowing on seed production of annual ryegrass in seed production sites, which are among the important sources of quality forage. In this study, the effects of different mowing and nitrogen doses on seed yield and quality characteristics of annual ryegrass were investigated in Eskişehir Ecological Conditions.

2. Material and Methods

The study was performed during 2018 in the Province of Eskisehir, Turkey, at an experimental site that received more rain in 2018 than the long-term average (Table 1), especially during the experiment's harvest time in July. However, average temperature and relative humidity values in 2018 did not significantly differ with the long-term averages. The soil at the site is clayey–loam (pH = 8.24), 1.37% organic matter, and extremely calcareous.

In the experiment, involving Split-Plot in the Randomized Complete Block Design with four replications, the primary plots received the mowing applications-mowing and not mowing- whereas the sub-plots received different applications of nitrogen (0, 5, 10, 15, 20, and 25 kg/da).

Table 1. Climatic data at the experimental fields in 2018 and long-term
Çizelge 1. *Deneme alanının 2018 ve uzun yıllar iklim verileri*

	Rainfall (mm)		Temperature (°C)		Relative humidity (%)	
	2018	**Long-term**	**2018**	**Long-term**	**2018**	**Long-term**
January	37.2	44.4	1.6	0.0	86.4	84.0
February	39.8	27.2	5.8	1.9	82.3	79.3
March	46.4	31.1	9.2	6.0	73.5	73.0
April	12.6	29.5	13.8	10.2	61.6	70.1
May	62.2	42.6	16.8	15.0	74.8	69.8
June	46.6	34.7	19.9	19.4	69.5	66.9
July	46.0	5.2	22.3	22.4	65.5	62.1
August	12.6	17.7	22.9	22.4	63.5	64.1
September	2.8	18.0	18.6	17.7	65.5	68.1
October	29.2	36.6	13.3	12.0	77.4	76.5
November	18.0	22.0	7.6	6.1	82.5	80.4
December	42.2	22.0	2.3	1.7	91.0	84.6
Mean/Total	395.6	331.0	12.84	11.2	74.45	73.2

All data were provided by Turkish State Meteorological Service.

Seeds of annual ryegrass (cv.Efe82) were sown on March 1, 2018, in an amount of 4 kg/da with a spacing between rows of 14 cm. Diammonium phosphate fertilizer (28 kg/da) was used with sowing, and sprinkler irrigation was applied to prompt and uniform emergence. Plotting was carried out when the plant leaves were 10-15 cm tall after emergence. After plotting, mowing and top-dressing with nitrogen applications containing urea (46%) were performed with grass cutting shears and by hand, respectively. For weed control, herbicide with 2,4-D active substance was used on May 16, 2018. After emergence, irrigation was applied twice with sprinkling, each time for 7 to 8 hour.

On July 15, 2018, approximately 1 month after flowering at caryopsis loosening stage, plots in an area of 0.25 m^2 were harvested by manual mowing. Plant length (cm), spike length (cm), fertile tiller number (number/m^2), spikelet number (number/spike), thousand seed weight (TSW, g), and seed yield (kg/da) was observed and recorded for those plants.

Germination was performed in an incubator under dark conditions at 20 °C for annual ryegrass, as described by ISTA (2018). Seeds of each application were germinated between two filter papers 20 × 20 cm in size with four replicates of 50 seeds for each**.** After 7 mL of water required for each filter paper was added, the prepared samples were placed in sealed bags to prevent evaporation. When the bags were opened for counting, the amount of water was checked, and bags whose water had decreased were refilled. Seeds were counted daily, and ones with a root length of 2 mm were considered to have germinated. After 8 days (d) of germination, as recommended for annual ryegrass by ISTA (2018), mean germination time was calculated by using the formula of Ellis and Roberts (1980).

The data were subjected to analysis of variance using SPSS version 16 and MSTAT-C. Arcsin transformation was applied to percentage values (Sokal and Rolf 1981), and Duncan's test was used to determine the significance of differences between means.

3. Results and Discussion

The effects of mowing or not mowing as well as various doses of nitrogen on seed yield, yield components, and germination characteristics of annual ryegrass were investigated during the 2018 growing period in Eskişehir ecological conditions.

The effect of mowing was significant only on the number of fertile tillers (Table 2), the increase in which can be expected to improve seed yields (Fairey and Lefkovitch, 2001). The effect of nitrogen doses on plant height, spike height, fertile tiller number, TSW, and seed yield were significant as well (Table 2). Nitrogen application increased the plant height of annual ryegrass, while the shortest heights were recorded for the control. The results of

İnce (2000) and Kesiktaş (2010) were thus confirmed, for they reported that annual ryegrass became taller as nitrogen doses increased. Although increasing nitrogen doses did increase spike length, no statistical difference occurred between doses of 5 to 25 kg/da. Against those findings, Uygun (1994), Kuşvuran and Tansı (2005), and Gültekin and Tansı (2009) found that the effects of nitrogen doses on the spike lengths of annual ryegrass were not significant.

Table 2. Analysis of variance and differences between mean values of yield components in annual ryegrass grown on various nitrogen and mowing application

Çizelge 2. *Tek yıllık çimin çeşitli azot dozları ve biçim uygulamalarında yetiştirilmesiyle elde edilen verim komponentlerine ait değerlerin varyans analiz sonuçları ve ortalama değerler arasındaki farklar.*

Factors	**Plant Height (cm)**	**Spike lenght (cm)**	**Fertile tiller number (No/m²)**	**Spikelet number (No/spikelet)**	**Tousand seed weight (gr)**	**Seed yield(kg/da)**
Mowing applications (MA)						
Mowing	91.95	19.66	900^{a}	19.37	2.50	109.3
Unmowing	91.78	18.69	878^{b}	17.79	2.64	107.3
Nitrogen doses (kg da^{-1})						
0	89.36^{c}†	17.48^{b}	836^{b}	18.75	2.49^{c}	106^{b}
5	92.64^{b}	19.13^{ab}	803^{b}	18.87	2.53^{bc}	108^{b}
10	91.86^{bc}	18.82^{ab}	952^{a}	18.00	2.61^{ab}	126^{a}
15	90.78^{bc}	19.55^{a}	1007^{a}	19.37	2.58^{abc}	112^{b}
20	91.13^{bc}	19.42^{a}	980^{a}	18.00	2.67^{a}	114^{b}
25	95.42^{a}	20.65^{a}	755^{b}	18.50	2.55^{bc}	84^{c}
Analysis of variance						
Mowing application (MA)	ns	ns	*	ns	ns	ns
Nitrogen doses (N)	**	**	**	ns	*	**
MAXN	ns	ns	ns	ns	**	**

***, **:** significant level of 5% and 1%, respectively, ns: non-significant. †: letters show different groups at 5% level.

The highest number of fertile tillers, a total of 1007, was obtained with a nitrogen dose of 15 kg/da, and no statistically significant difference surfaced between 10 and 20 kg/da doses (Table 2). The lowest number of fertile tillers, 755, was obtained with a nitrogen dose of 25 kg/da. Açıkgöz et al. (1996) observed the most fertile tillers from plots administered 12 kg/da of nitrogen. By comparison, Bahmani et al. (2001) found that the number of fertile tillers varied from 969 to 8,698 with different applications of nitrogen in perennial ryegrass. More recently, Nizam (2009) recorded the highest number of fertile tillers from plants in plots that received 24 kg/da of nitrogen in the first year and 36 kg/da in the second.

Among other results, TSW ranged from 2.49 to 2.67 g (Table 2). The highest values were obtained with 20 kg/da of nitrogen, the lowest from the control, and no statistically significant difference emerged between doses of 10 to 20 kg/da. Contrary to those findings, Rolston et al. (2005), and Nizam (2009) reported that different nitrogen applications exerted no significant effect on the TSW of perennial ryegrass.

Seed yields varied between 84 and 126 kg/da according to nitrogen doses (Table 2). The highest seed yield was obtained with the nitrogen application of 10 kg/da, whereas the lowest values were measured with a dose of 25 kg/da. Nizam (2009) reported that nitrogen fertilizer applications positively affected seed yield, which was highest with a nitrogen dose of 24 kg/da. Simic et al. (2012) found that applying 50 kg/ha of nitrogen was optimal for the seed production of annual ryegrass in Western Serbia. Higher application rates of nitrogen (100–150 kg/ha) did not affect seed yield, or else yield decreased after seed loss. Hampton (1987) and Rowarth et al. (1998) both showed that 100 kg/da of nitrogen was sufficient for optimum seed production in perennial ryegrass.

According to all of those results, seed yields seem to be affected by different nitrogen doses under various ecological conditions.

The interaction of mowing application and nitrogen dose exerted a significant effect on TSW and seed yield only (Table 2). The highest and lowest TSWs were obtained with 25 kg/da of nitrogen in unmowed and mowed plots, respectively (Table 3). Furthermore, no statistical difference emerged between nitrogen applications of 10 to 25 kg/da in the unmowed condition. The highest seed yield was 132 kg/da with 10 kg/da of nitrogen in mowing plots, while the lowest was 83 kg/da with 25 kg/da of nitrogen in unmowing plots. By comparison, Kuşvuran and Tansı (2005) obtained the highest seed yields from annual ryegrass grown in plots applied 15 kg/da of nitrogen and mowed twice.

Table 3. Yield components of annual ryegrass as affected by the mowing and nitrogen applications
Çizelge 3. *Biçme ve azot uygulamalarından etkilenen tek yıllık çimin verim bileşenleri*

Mowing application	**Nitrogen doses (kg/da)**					
	0	5	10	15	20	25
Tousand seed weight (gr)						
Mowing	2.45[cd*]	2.54[bc]	2.50[bc]	2.55[bc]	2.64[ab]	2.35[d]
Unmowing	2.54[bc]	2.52[bc]	2.72[a]	2.62[ab]	2.70[a]	2.75[a]
Seed yield(kg/da)						
Mowing	100[e]	101[de]	132[a]	125[ab]	113[c]	85[f]
Unmowing	112[cd]	116[bc]	120[bc]	99[e]	114[bc]	83[f]

***:** Means followed by same letter(s) in the same row are not significant at 1%.

Table 4. Analysis of variance and differences between mean values of germination and seedling properties in annual ryegrass grown on various nitrogen and mowing application

Çizelge 4. *Tek yıllık çimin çeşitli azot dozları ve biçim uygulamalarında yetiştirilmesiyle elde edilen tohumların çimlenme ve fide özelliklerine ait değerlerin varyans analiz sonuçları ve ortalama değerler arasındaki farklar*

Factors	**2nd day germination (%)**	**Total germination (%)**	**Mean germination time (day)**	**Seedling length (cm)**	**Seedling fresh weight (mg/seedling)**	**Seedling fresh weight (mg/seedling)**
Mowing applications (MA)						
Mowing	50.95	91.00	2.71	13.93	17.05	2.11
Unmowing	60.08	93.08	2.59	13.35	17.34	2.10
Nitrogen doses (kg da^{-1})						
0	50.25[b]†	93.37	2.68[a]	14.17	17.93	2.19
5	51.12[b]	92.00	2.65[a]	14.12	17.46	2.12
10	50.75[b]	89.50	2.78[a]	13.73	16.91	2.09
15	56.87[ab]	92.87	2.67[a]	13.29	17.91	2.09
20	63.75[a]	92.37	2.47[b]	13.45	17.15	2.16
25	60.37[a]	92.12	2.65[a]	13.11	15.81	2.00
Analysis of variance						
Mowing application (MA)	ns	ns	ns	ns	ns	ns
Nitrogen doses (N)	**	ns	**	ns	ns	ns
MAXN	**	ns	**	*	ns	ns

***, **:** significant level of 5% and 1%, respectively, ns: non-significant. †: letters show different groups at 5% level.

The effect of mowing on characteristics of seed quality was not significant (Table 4). Because annual ryegrass seeds are large and exceptionally strong, mowing and using nitrogen fertilizer do not affect seed germination percentages (Herron, 1975). The highest second-day germination and mean germination time were obtained with 20 kg/da of nitrogen.

Contrary to those findings, Hampton (1987) indicated that increasing the nitrogen rate increased the germination percentage of perennial grass. By contrast, DeFilippi et al. (1996) as well as Cookson et al. (2000) observed that varying nitrogen doses in perennial grass did not affect total germination rates.

Table 5. Annual ryegrass seed quality as affected by the mowing and nitrogen applications
Çizelge 5. *Biçme ve azot uygulamalarından etkilenen tek yıllık çimin çimlenme ve fide özellikleri*

Mowing application	Nitrogen doses (kg/da)					
	0	5	10	15	20	25
			2nd day germination (%)			
Mowing	35.50^{e*}	57.75^{bc}	52.25^{cd}	55.75^{bcd}	55.50^{bcd}	49.00^{cd}
Unmowing	65.00^{ab}	44.50^{de}	49.25^{cd}	58.00^{bc}	72.00^{a}	71.75^{a}
			Mean germination time (day)			
Mowing	2.90^{a}	2.58^{bc}	2.73^{ab}	2.74^{ab}	2.61^{bc}	2.72^{ab}
Unmowing	2.47^{cd}	2.73^{ab}	2.83^{a}	2.60^{bc}	2.34^{d}	2.59^{bc}
			Seedling length (cm)			
Mowing	14.16^{a-c}	13.50^{a-d}	14.36^{a-c}	13.17^{a-d}	14.98^{a}	13.46^{a-d}
Unmowing	14.18^{a-c}	14.75^{ab}	13.10^{b-d}	13.41^{a-d}	11.93^{d}	12.77^{cd}

***:** Means followed by same letter(s) in the same row for second day germination and mean germination time and seedling height are not significant at 1% and 5%, respectively.

The interaction of mowing and nitrogen dose affected second-day germination, mean germination time, and seedling length to a significant degree (Table 5). Second-day germination percentages ranged from 35.5% to 72.0% with 20 kg/da of nitrogen in unmowed plots, while nitrogen was not applied in the mowed plots. Mean germination time varied between 2.34–2.90 d. While the most slowly germinating seeds were obtained when no nitrogen was applied to the mowed plots, the most quickly germinating seeds occurred with the application of 20 kg/da of nitrogen in the unmowed plots. Seedling length ranged from 11.93 to 14.98 cm, and the effect of mowing on that characteristic was thus arguably positive. Schuurman and Knot (1974) found that increasing nitrogen doses increased shoot length in annual ryegrass, while Hara and Toriyama (1998) reported that increasing the amount of nitrogen in the seed positively affected shoot length and, in turn, accelerated the emergence of seedlings on the soil's surface.

4. Conclusions

Despite highly limited research on mowing applications with annual ryegrass, studies on using various nitrogen doses have produced divergent results in different ecological and soil conditions. In the investigation into the effects of mowing and nitrogen on the seed yield and quality of annual ryegrass described here, mowing and nitrogen fertilizer applications on annual ryegrass generated results similar to those reported in previous studies. Mowing in early spring did not adversely affect seed production, and the highest seed yield was obtained with mown plots by applying 10 or 15 kg of nitrogen. Moreover, no difference emerged between the total germination percentages of seeds obtained as a result of the applications on annual ryegrass. Overall, the best results in terms of second-day germination percentage and mean germination time were obtained with 20 kg/da of nitrogen in unmowed plots.

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