

## IMPACT OF MANAGEMENT PRACTICES ON ITALIAN RYEGRASS SEED QUALITY

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**Abstract:** Thousand-seed weight and total germination from the first production year and two seed harvests were measured in tetraploid Italian ryegrass (cv. Tetraflorum) to study variations in seed quality. Four-year field experiments were carried out (2002-2006) in western Serbia and Italian ryegrass was established with three row spacings (20, 40 and 60 cm), four seeding rates (5, 10, 15 and 20 kg ha<sup>-1</sup>) and using four spring nitrogen rates (0, 50, 100 and 150 kg ha<sup>-1</sup>). High seed quality was obtained, except for the first experimental year (2003) and the second seed harvests which yielded the seed of unsatisfactory quality and validity. Applied factors and treatments did not change seed quality significantly unlike environmental conditions of production years which had considerable influence on seed quality.

**Key words:** inter-row spacing, Italian ryegrass, nitrogen application, seeding rate, seed quality.

### Introduction

Italian ryegrass is one of the best forage grasses in Serbia, producing high-quality forage. According to Simić et al. (2009) excellent ryegrass seed yield was achieved in Serbia in the first year, but local production covers only 50% of forage production needs for this seed. Italian ryegrass was most severely affected by drought in terms of tillering, leaf extension and subsequent recovery (Norms and Thomas, 1982). Shortage of soil moisture, resulting from variations in rainfall and soil type, is the main cause of differences in seed yields from year to year and site to site in Serbia. This experiment was conducted to determine the differences among Italian ryegrass seed sets obtained by management practices of the seed

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crop in the first production year, using different sowing rates, row spacings and spring nitrogen (N) application.

Any seed lot when harvested includes seed of different sizes. This variation is due to differences between the seeds harvested from different plants, and differences among seeds borne on the same plant. Seed size, usually measured as thousand seed weight (TSW), may be an indicator of quality, as increasing TSW can result in improved seedling growth (Hill et al., 1998). It is, therefore, a useful character which can be rapidly estimated to give an assessment of seed quality. One of the first noticed characteristics of tetraploid ryegrasses was larger seeds than the corresponding values recorded for diploid strains (Roberts, 1971). Quality of Italian ryegrass seed on the same spike is not uniform. There is a slight fall in floret fertility in the upper spikelets, compared with those in the middle and at the base of the spike, and a marked reduction in fertility in the outer florets of each spikelet (Anslow, 1963). Bean (1973) pointed out that TSW was significantly and positively correlated with subsequent seedling dry weight for Italian ryegrass. Hampton (1986) reported that increasing seed weight from 3.2 g to 5 g, seedling performance was increased in field sowings in Italian ryegrass from 68% to 85%. Seed vigour (seedling weight and germination after accelerated ageing) could be positively and significantly related to increasing TSW (Rowarth et al., 1999). Otherwise, the large seeds are more susceptible to mechanical damage at harvest and therefore require lower drum speed. TSW can also be used as a cultivar descriptor where a standard is set for certified seed production (Hill et al., 1998).

Historically, grass seed quality has been synonymous with germination, i.e. the measurement of the percentage of seeds growing normally under standardised, controlled, optimum laboratory conditions, set so that seed is given every chance to germinate to its full potential (ISTA, 2010). Germination is the result of seed forming under environmental conditions and tetraploid ryegrass for seed attains commercially critical level of 90% germination early in the ripening process (Roberts, 1971). According to Raja Harun and Bean (1979), germination values had reached a maximum 27 days after anthesis. The application of different nitrogen rates and seeding techniques affected Italian ryegrass seed yield in Serbia (Vučković et al., 2003; Simić et al., 2009), but it is not clear whether management practice influenced ryegrass seed quality. Vučković et al. (1998) achieved higher germination rate of Italian ryegrass seed grown at row distance of 50 cm and applying seeding rate of 4 kg ha<sup>-1</sup> than at the distance of 20 cm and applying higher seeding rates. Simić et al. (2005) reported that three Italian ryegrass cultivars produced seed of an excellent quality, with total germination above 90%.

In order to obtain a better understanding of the influence of environmental factors on the quality of Italian ryegrass seed grown in the field, the effect of plant density and application of nitrogenous fertiliser were studied. In most published papers on Italian ryegrass seed production in the world, seed yield obtained from

one and only harvest cut in the vegetation season was reported. Earlier researches (Radojević, 1975) were reserved about possibilities to produce the second harvest in the same vegetation season. According to Tomić and Sokolović (2000), Italian ryegrass in Serbian conditions reached seed yield of 130 kg ha<sup>-1</sup> (diploid K-13) and 171 kg ha<sup>-1</sup> (tetraploid K-29) in the second harvest of the first production year. Also, Simić et al. (2007) obtained two ryegrass seed harvests in the first production year using one introduced and two native cultivars, at the site in Western Serbia.

### Materials and Methods

The study was maintained for 4 consecutive years, between 2002 and 2006, near Šabac, Serbia (44°47' N, 19°35' E, 80 m asl) which is located in a semi-humid region (with very variable years). Seed from the primary and secondary growth of tetraploid Italian ryegrass cv. Tetraflorum was harvested in the first production year after the establishment. Italian ryegrass was planted (row spaces of 20, 40 and 60 cm) each autumn (the first to third decade of October) prior to the preceding summer seed harvest. The seeding rate was equivalent to 5, 10, 15 and 20 kg ha<sup>-1</sup>, providing 12 spatial plant treatments in combination with inter-row spacing. The plot for harvest was 10 m<sup>2</sup>, and it was replicated four times in a randomised complete block design. Phosphorus, potassium and a portion of nitrogen were applied in the autumn before each seed production year (250 kg ha<sup>-1</sup> of 8-16-24 fertiliser NPK), with additional application of N (0, 50, 100 and 150 kg ha<sup>-1</sup>) in the spring. Soil in the experimental area was humofluvisol (2.54% humus), with rinsed limestone. Rainfalls during experimental period were very variable.

All plots were harvested at the peak of seed ripeness and it was dried at room temperature. Two characters were measured to provide an estimate of seed quality: (1) 1000-seed weight and (2) germination. TSW was determined from samples allowed to dry at room temperature. Germination was assessed by placing four hundred seeds in replicates of 100 seeds on pads in a germination tank at 20°C (ISTA, 2010). Seeds with coleoptil were counted on the 14th day. TSW and germination were tested after two months of storage in the laboratory. Data were analysed by using appropriate statistical analyses and Statistica 8.0 software packages. Significance ( $P \geq 0.05$ ) level was determined by LSD test.

### Results and Discussion

#### Thousand seed weight

Average TSW varied during experimental years from 3.15 g to 5.12 g (Table 1), and that variation could be explained by temperature and other environmental changes during years, but seed quality can also be influenced by the

time at which the seed is harvested (Akpan and Bean, 1980). TSW coefficients of variation by year were slightly influenced by treatments (from 3.74% to 7.54%).

Table 1. Effect of inter-row spacing, seeding rate and spring nitrogen application on seed characteristics of Italian ryegrass first harvest 2003-2006.

Inter-row spacing A	Thousand seed weight (g)				Total germination (%)			
	2003	2004	2005	2006	2003	2004	2005	2006
20 cm	3.208 <sup>a*</sup>	4.774 <sup>a</sup>	5.057 <sup>a</sup>	4.284 <sup>a</sup>	75.2 <sup>a</sup>	95.5 <sup>a</sup>	92.4 <sup>a</sup>	95.0 <sup>a</sup>
40 cm	3.155 <sup>ab</sup>	4.800 <sup>a</sup>	5.198 <sup>b</sup>	4.223 <sup>a</sup>	33.3 <sup>b</sup>	95.9 <sup>a</sup>	94.6 <sup>b</sup>	92.3 <sup>b</sup>
60 cm	3.097 <sup>b</sup>	4.736 <sup>a</sup>	5.098 <sup>a</sup>	4.431 <sup>b</sup>	56.3 <sup>c</sup>	95.9 <sup>a</sup>	95.9 <sup>b</sup>	94.5 <sup>a</sup>
LSD 0.05	0.073	0.082	0.058	0.101	7.26	0.66	1.16	1.20
Seeding rate B								
5 kg ha <sup>-1</sup>	3.030 <sup>a</sup>	4.777 <sup>a</sup>	5.173 <sup>a</sup>	4.157 <sup>a</sup>	50.4 <sup>a</sup>	96.0 <sup>a</sup>	95.1 <sup>a</sup>	91.0 <sup>a</sup>
10 kg ha <sup>-1</sup>	3.199 <sup>b</sup>	4.788 <sup>a</sup>	5.137 <sup>ab</sup>	4.462 <sup>b</sup>	53.3 <sup>a</sup>	95.7 <sup>a</sup>	94.9 <sup>ab</sup>	95.0 <sup>b</sup>
15 kg ha <sup>-1</sup>	3.182 <sup>b</sup>	4.722 <sup>a</sup>	5.092 <sup>b</sup>	4.344 <sup>c</sup>	54.6 <sup>a</sup>	95.6 <sup>a</sup>	93.7 <sup>b</sup>	95.3 <sup>b</sup>
20 kg ha <sup>-1</sup>	3.203 <sup>b</sup>	4.794 <sup>a</sup>	5.070 <sup>b</sup>	4.287 <sup>c</sup>	61.3 <sup>b</sup>	95.7 <sup>a</sup>	93.6 <sup>b</sup>	94.6 <sup>b</sup>
LSD 0.05	0.084	0.095	0.067	0.116	8.38	0.76	1.34	1.39
Nitrogen rate C								
0 kg ha <sup>-1</sup>	3.156 <sup>ab</sup>	4.792 <sup>a</sup>	5.095 <sup>a</sup>	4.253 <sup>a</sup>	54.8 <sup>a</sup>	96.0 <sup>a</sup>	94.6 <sup>a</sup>	93.3 <sup>a</sup>
50 kg ha <sup>-1</sup>	3.117 <sup>a</sup>	4.780 <sup>a</sup>	5.110 <sup>a</sup>	4.284 <sup>a</sup>	57.9 <sup>a</sup>	96.0 <sup>a</sup>	93.3 <sup>a</sup>	93.5 <sup>a</sup>
100 kg ha <sup>-1</sup>	3.112 <sup>a</sup>	4.738 <sup>a</sup>	5.115 <sup>a</sup>	4.325 <sup>a</sup>	57.7 <sup>a</sup>	95.2 <sup>a</sup>	94.8 <sup>a</sup>	94.6 <sup>a</sup>
150 kg ha <sup>-1</sup>	3.228 <sup>b</sup>	4.750 <sup>a</sup>	5.152 <sup>a</sup>	4.388 <sup>a</sup>	55.1 <sup>a</sup>	95.7 <sup>a</sup>	94.5 <sup>a</sup>	94.4 <sup>a</sup>
LSD 0.05	0.084	0.095	0.067	0.116	8.38	0.76	1.34	1.39
Average	3.153	4.770	5.118	4.312	53.9	95.8	94.3	93.9
CV (%)	7.54	4.90	3.74	7.19	53.2	2.02	3.83	4.04

\*Means in columns followed by the same letter are not significantly different by Fisher's protected LSD values (P=0.05).

The 2003 season was notable for its severe drought and high temperatures and although moisture stress may have affected seed development, the results obtained in the present investigation suggest that all factors may also have had important effects, but in the year of 2004, favourable for seed production, none of investigated factors had a significant influence (Table 3). Generally, this seed quality indicator was conditioned by the factors of vegetation area in crop establishment, whereas nitrogen had an influence only in extremely unfavourable years.

Akpan and Bean (1980) reported that the seed from spaced plants had a higher TSW and seedling dry weight than the seed from narrow drills. They reported that

an increase in temperature from a 15°/10°C regime to a constant 25°C environment reduced TSW and seedling dry weight, but increased germination rate. The highest TSW changed from year to year and was the highest at the smallest inter-row spacing (2003) at medium spacing (2005), or inter-row spacing did not have any influence (2004). In 2006 TSW increased as inter-row space was raised, which is in agreement with findings of Choi et al. (2002) who noted the TSW increase when the inter-row space was raised from 15 cm to 45 cm (2.65 g and 2.73 g, respectively).

Table 2. Effect of inter-row spacing, seeding rate and spring nitrogen application on seed characteristics of Italian ryegrass second harvest 2003-2006.

Inter-row spacing A	Thousand seed weight (g)				Total germination (%)			
	2003	2004	2005	2006	2003	2004	2005	2006
20 cm	-	3.351 <sup>a*</sup>	3.678 <sup>a</sup>	2.822 <sup>a</sup>	-	94.3 <sup>a</sup>	67.8 <sup>a</sup>	80.4 <sup>a</sup>
40 cm	-	3.467 <sup>b</sup>	3.707 <sup>a</sup>	2.734 <sup>b</sup>	-	94.4 <sup>a</sup>	72.3 <sup>a</sup>	73.5 <sup>b</sup>
60 cm	-	3.458 <sup>b</sup>	3.638 <sup>a</sup>	2.596 <sup>c</sup>	-	95.5 <sup>b</sup>	71.9 <sup>a</sup>	79.3 <sup>a</sup>
LSD 0.05		0.086	0.071	0.078		0.82	4.59	2.21
Seeding rate B								
5 kg ha <sup>-1</sup>	-	3.474 <sup>a</sup>	3.653 <sup>a</sup>	2.711 <sup>a</sup>	-	94.9 <sup>a</sup>	71.3 <sup>a</sup>	76.8 <sup>a</sup>
10 kg ha <sup>-1</sup>	-	3.432 <sup>a</sup>	3.676 <sup>a</sup>	2.777 <sup>a</sup>	-	94.8 <sup>a</sup>	71.5 <sup>a</sup>	79.1 <sup>a</sup>
15 kg ha <sup>-1</sup>	-	3.388 <sup>a</sup>	3.688 <sup>a</sup>	2.751 <sup>a</sup>	-	94.5 <sup>a</sup>	72.6 <sup>a</sup>	77.0 <sup>a</sup>
20 kg ha <sup>-1</sup>	-	3.407 <sup>a</sup>	3.680 <sup>a</sup>	2.729 <sup>a</sup>	-	94.8 <sup>a</sup>	67.4 <sup>a</sup>	78.0 <sup>a</sup>
LSD 0.05		0.099	0.070	0.090		0.95	5.30	2.55
Nitrogen rate C								
0 kg ha <sup>-1</sup>	-	3.441 <sup>a</sup>	3.663 <sup>a</sup>	2.634 <sup>b</sup>	-	94.7 <sup>a</sup>	71.7 <sup>a</sup>	76.9 <sup>a</sup>
50 kg ha <sup>-1</sup>	-	3.431 <sup>a</sup>	3.682 <sup>a</sup>	2.739 <sup>a</sup>	-	94.5 <sup>a</sup>	69.3 <sup>a</sup>	77.4 <sup>a</sup>
100 kg ha <sup>-1</sup>	-	3.429 <sup>a</sup>	3.692 <sup>a</sup>	2.723 <sup>ab</sup>	-	94.5 <sup>a</sup>	71.0 <sup>a</sup>	78.6 <sup>a</sup>
150 kg ha <sup>-1</sup>	-	3.400 <sup>a</sup>	3.659 <sup>a</sup>	2.773 <sup>a</sup>	-	95.3 <sup>a</sup>	70.7 <sup>a</sup>	78.0 <sup>a</sup>
LSD 0.05		0.099	0.070	0.090		0.95	5.30	2.55
Average		3.425	3.674	2.717	-	94.7	70.7	77.7
CV (%)		6.96	4.87	8.57		2.63	18.9	8.05

\*Means in columns followed by the same letter are not significantly different by Fisher's protected LSD values (P=0.05).

Although the high crop density causes crop lodging, leading to unfavourable conditions for seed development, in 2005 seed took reserve assimilates from lodged tillers and achieved average seed weight greater than 5 mg. This result was in agreement with findings of Griffith's (2000), where Italian ryegrass obtained

higher TSW from lodged plants in the first harvest, whereas in the second year it was vice versa.

Seeding rate had a different influence year over year (Table 3), so the smallest TSW was achieved in 2003 when the sowing was sparser, that is 3.030 g, and in 2005 and 2006 TSW was the greatest although the smaller amounts of seed were used for sowing. N fertiliser application had a significant ( $P < 0.05$ ) and positive effect on TSW in 2003 with the highest application rate ( $150 \text{ kg ha}^{-1} \text{ N}$ ), whilst in the other experimental years a noticeable positive effect was identified on TSW but without significance. The N impact was sometimes noticed in diploid forms, and usually TSW decreases when N is applied (Choi et al., 2002).

Table 3. Statistical summary of seed characteristics responses to stand density and nitrogen application.

Main factors	2003	2004	2005	2006	2003	2004	2005	2006
	First harvest				Second harvest			
	TSW							
Inter-row spacing A	**	NS	**	**	-	*	NS	**
Seeding rate B	**	NS	*	**	-	NS	NS	NS
Nitrogen rate C	*	NS	NS	NS	-	NS	NS	*
A x B	*	NS	NS	NS	-	NS	NS	NS
A x C	*	NS	*	NS	-	NS	NS	NS
B x C	NS	NS	NS	NS	-	NS	*	NS
A x B x C	*	NS	NS	*	-	NS	NS	*
Total germination								
Inter-row spacing A	**	NS	**	**	-	**	NS	**
Seeding rate B	*	NS	*	**	-	NS	NS	NS
Nitrogen rate C	NS	NS	NS	NS	-	NS	NS	NS
A x B	**	NS	NS	**	-	NS	NS	*
A x C	NS	NS	NS	NS	-	NS	NS	NS
B x C	NS	NS	NS	NS	-	NS	NS	NS
A x B x C	NS	NS	NS	NS	-	NS	NS	NS

NS = not significant P value 0.05, \* = P value < 0.05, \*\* = P value < 0.01.

The second cut of Italian ryegrass seed in the first harvest year failed to occur in arid year of 2003 (Table 2), and the largest seed was obtained in 2005. The smaller seed weight at the second cut was expected, caused by both low rainfall and poor reserve assimilates supply, which is similar to TSW decreasing if in early spring one ryegrass cut is planned for forage and another one is intended for seed

production (Ahrens and Oliveira, 1997). The second cut TSW was not influenced by sowing density nor by spring N application, except for 2006, when this indicator increased as N applied amount was raised.

#### Total germination

Total seed germination of *Tetraflorum* was high, except for 2003 (Table 1), when a severe drought and unfavourable conditions were noticed during seed ripening, which resulted in great fluctuations of treatments (53.2%). Regarding other years, germination was very high and uniform, with few fluctuations. In the year of 2003 atypical branching occurred, but the cause for those unusual inflorescences, spikes shaped like panicle etc. was unknown. Griffith and Burr (1990) reported some possible factors: herbicide induction, environmental stress, recessive gene expression, or a combination of one or more of these or other factors. Our data suggest that assimilate supply may have limited seeds from achieving their potential final weight, and that was a possible cause of the low germination rate in 2003.

The influence of ecological factors of the year as well as of applied treatments on total ryegrass germination was not uniform. Whilst a significant germination decrease was noticed in 2003, the impact of crop density in favourable production years having high rainfalls (for example in the year of 2004) was not remarkable. The high ryegrass germination in favourable conditions was confirmed by Choi et al. (2002) in Korean conditions, where the germination was not changed by different inter-row spaces at seed crop establishment. Conversely, Akpan and Bean (1980) reported that spaced plants seed had a slower germination rate than seed sown in narrow drills. In 2005 and 2006, besides a statistical importance, the influence of treatment, was practically slight on germination, which was in all treatments above 90% (Table 1). Seed germination was not affected by N fertiliser rate in any investigated year, which is in agreement with results of Cookson et al. (2000) and Choi et al. (2002). Vegetation area influenced total germination, more precisely both factors (row spacing and seeding rate) were noticed in 2005 and 2006, whilst an inter-row spacing was identified in 2003.

#### The second harvest

Based on the obtained results according to investigated years the highest germination rate of the second year Italian ryegrass seed was noticed in 2004 (Table 2), which was almost equivalent to the first cut germination, having few fluctuations among treatments. Except for a minimal impact of inter-row spacing, the effect of other investigated factors was not identified in the second harvest. Lower TSW in the second harvest, with presence of sclerotia *Claviceps purpurea* makes this seed unfavourable for commercial purposes.

TSW was not correlated with germination rate (except for the first harvest in 2006) in both harvests during experimental years (Table 4). Akpan and Bean (1980) suggested that sometimes smaller tetraploid ryegrass seeds had a faster rate of germination than the larger seeds. The year-to-year differences in seed quality were beyond the control of applied treatments. Analysing factors and their influence on TSW, it could be concluded that TSW was reduced by aerial competition among plants (considered to be competition for light, made by different seeding rates and inter-row spacing), but not by edaphic competition (used nitrogen as indicator), except where aerial competition also occurred.

Table 4. Correlation coefficients between thousand seed weight and total germination (I and II harvest).

Years	I harvest	II harvest
2003	0.21	-
2004	0.43	0.40
2005	0.01	-0.01
2006	0.63	0.30

### Conclusion

It is concluded that year-to-year variation in Italian ryegrass seed characters will occur because of temperature and other climatic changes. Average TSW varied during experimental years from 3.15 g to 5.12 g. TSW was influenced by factors forming crop density, whereas nitrogen had a strong influence only during the extremely unfavourable years.

The first harvest germination rate of Italian ryegrass produced in the first harvest year was high and exceeded 90% in investigated years, except for the year of 2003 when the seed had an average germination of 54%. The dry May/June period in that year might have increased the problem. Greater vegetation area positively influenced Italian ryegrass seed germination in other years, whilst N fertilisation did not have any impact on ryegrass germination rate.

Of the four years used to determine influence of management practise to Italian ryegrass quality, the first and fourth years are most influenced by different sowing techniques. The high tetraploid Italian ryegrass seed quality was confirmed and it is slightly influenced by different variants of establishment or by N spring application, having in mind that weather conditions can drastically decrease it.

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### UTICAJ AGROTEHNIČKIH MERA NA KVALITET SEMENA ITALIJANSKOG LJULJA

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#### R e z i m e

Ispitivana je masa 1000 semena i ukupna klijavost tetraploidnog italijanskog ljujla (sorte Tetraflorum) iz prve proizvodne godine i dve semenske žetve radi utvrđivanja razlika u kvalitetu semena. Četvorogodišnji poljski eksperiment je izveden (2002-2006) u zapadnoj Srbiji i italijanski ljuj je zasnovan na 3 međuredna rastojanja (20, 40 i 60 cm), četiri setvene norme (5, 10, 15 i 20 kg ha<sup>-1</sup>) i primenom četiri doze prihrane azotom (0, 50, 100 i 150 kg ha<sup>-1</sup>). Ostvaren je visok kvalitet semena, izuzev prve godine ispitivanja (2003), a ostvarena je i druga semenska žetva sa semenom nezadovoljavajućeg kvaliteta i upotrebljivosti. Primenjeni faktori i nivoi ispitivanja nisu značajno menjali kvalitet semena za razliku od vremenskih uslova u proizvodnim godinama koji su značajno uticali na kvalitet semena.

**Ključne reči:** međuredno rastojanje, italijanski ljuj, prihrana azotom, setvena norma, kvalitet semena.

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