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Plot size and number of replicates for ryegrass experiments sowed in rows

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Abstract – The objective of this work was to determine the optimal plot size and the number of replicates for the evaluation of the fresh weight of ryegrass sowed in rows. Seventy uniformity trials were performed with 'Barjumbo' ryegrass, in 16 basic experimental units (BEUs) of 0.51 m^2 each. The fresh weight of ryegrass in the BEUs of 18, 18, 6, 6, and 22 uniformity trials was determined, respectively, at 130, 131, 133, 134, and 137 days after sowing. The optimal plot size was determined through the method of the maximum curvature of the coefficient of variation. The number of replicates was determined in scenarios formed by combinations of treatments and differences between means to be detected as significant by Tukey's test, at 5% probability. The optimal plot size ranged from 1.73 to 3.18 m^2, and the variation coefficient in the optimal plot size from 7.58 to 13.96%. The number of replicates varied from 3.95 (~4) to 32.27 (~33), depending on the experimental design, the number of treatments, and the adopted minimum difference. The optimal plot size is 2.29 m^2, and, in experiments with up to 50 treatments, eight replicates are required to identify as significant the differences between treatment means of 20.24%.

Index terms: *Lolium multiflorum*, experiment planning, uniformity trials.

Tamanho de parcela e número de repetições para experimentos de azevém semeados em filas

Resumo – O objetivo deste trabalho foi determinar o tamanho ótimo de parcela e o número de repetições para avaliação da massa de matéria fresca de azevém semeado em filas. Setenta ensaios de uniformidade foram realizados com azevém 'Barjumbo', em 16 unidades experimentais básicas (UEBs) de 0,51 m^2 cada uma. A massa de matéria fresca do azevém nas UEBs de 18, 18, 6, 6 e 22 ensaios de uniformidade foi determinada, respectivamente, aos 130, 131, 133, 134 e 137 dias após a semeadura. O tamanho ótimo de parcela foi determinado pelo método da máxima curvatura do coeficiente de variação. O número de repetições foi determinado em cenários formados por combinações de número de tratamentos e de diferenças entre médias a serem detectadas como significativas pelo teste de Tukey, a 5% de probabilidade. O tamanho ótimo de parcela oscilou de 1,73 a 3,18 m^2, e o coeficiente de variação no tamanho ótimo de parcela de 7,58 a 13,96%. O número de repetições oscilou de 3,95 (~4) a 32,27 (~33), conforme o delineamento, o número de tratamentos e a diferença mínima adotada. O tamanho ótimo de parcela é de 2,29 m^2 e, em experimentos com até 50 tratamentos, são necessárias oito repetições para identificar como significativas as diferenças entre médias de tratamentos de 20,24%.

Termos para indexação: *Lolium multiflorum*, planejamento experimental, ensaios de uniformidade.

Introduction

Ryegrass (*Lolium multiflorum* L.) is one of the most cultivated forage species, and it can be used either alone or in mixtures to eliminate the lack of forage in southern Brazil, during the winter (Flores et al., 2008). The expansion of research on ryegrass is significant because of the importance of this crop to phytotechnology, soil conservation, phytosanitary defense, and animal nutrition (Flores et al., 2008; Agostinetto et al., 2017; Vizioli et al., 2018; Salazar et al., 2019; Toebe et al., 2020a). In this sense, it is important to define experimental protocols, to optimize the quality and reliability of research, as well as to minimize expenditures with financial, human, and time resources. Therefore, knowing the plot size and number of replicates for combinations of the number of treatments, experimental designs, and precision levels, is essential to consolidate the scientific development in agricultural crops.

Several studies have used the method of the maximum curvature of the coefficient of variation, proposed by Paranaiba et al. (2009), to scale plot size. The number of replicates has been defined in scenarios formed by combinations of differences among means to be detected as significant by Tukey's test and the number of treatments, in an iterative process until convergence, according to the method described by Cargnelutti Filho et al. (2014a). These two methods have been applied in experimental planning to determine the fresh weight of forage crops (Cargnelutti Filho et al., 2014a, 2015, 2017, 2020; Burin et al., 2015; Chaves et al., 2018; Lavezo et al., 2018; Toebe et al., 2020b). These methods have been also applied for the determination of plot size and the number of replicates, to evaluate the fresh weight of broadcast-seeded ryegrass (Toebe et al., 2020a).

The sowing systems and the spatial arrangement of plants interfere with the growth, development, and productivity of crops, and they are able to alter the recommendations of the number of replicates and plot size. In this sense, Cargnelutti Filho et al. (2014b) identified differences in the experimental design of turnip sowed both in rows and broadcasted. Thus, although the number of replicates and the plot size for ryegrass sowing by broadcast have already been carried out by Toebe et al. (2020a), this information for ryegrass experiments sowed in rows has not been reported in the literature yet.

The objective of this work was to determine the optimal plot size and the number of replicates for the evaluation of the fresh weight of ryegrass sowed in rows.

Materials and Methods

Seventy uniformity trials with ryegrass were carried out in the experimental area of Universidade Federal do Pampa, Itaqui Campus, located in the municipality of Itaqui, in the state of Rio Grande do Sul, Brazil (29º09'25"S, 56º33'16"W, at 74 m altitude). The region shows a Cfa climate, according to the Köppen-Geiger's classification. The soil is classified as Plintossolo Háplico according to the Brazilian soil classification system (Santos et al., 2018), which corresponds to the Ultisol classification (Soil Survey Staff, 1999). 'Barjumbo' ryegrass seed were sowed with 25 kg ha^{-1}, on June 8, 2015, in rows spaced at 0.17 m between rows, in 1,800 m^2 area. The fertilization was carried out following the recommendations for the crop (Tedesco et al., 2004), and the cultural practices were performed evenly within the experimental area.

Seventy uniformity trials were delimited in the central area of the experiment. Each uniformity trial of 4×2.04 m (8.16 m^2) was divided into 16 basic experimental units (BEUs) of 1.0×0.51 m (0.51 m^2, 1.0 m × three rows), forming a matrix of four rows and four columns. The plant collection for determining fresh weight was performed as follows: 18 trials were collected 130 days after sowing (DAS); 18 trials, 131 DAS; 6 trials, 133 DAS; 6 trials, 134 DAS; and 22 trials, 137 DAS, in each BEU. Plants were cut near the soil surface, and the fresh weight was determined (g 0.51 m^{-2}).

Based on the fresh weight in the 16 BEUs of each uniform trial, the first-order spatial autocorrelation coefficient (ρ), variance (s^2), mean (m, g), coefficient of variation of the trial (CV_{trial}, %), optimal plot size (Xo, in BEU and m^2), and the coefficient of variation in the optimal plot size (CV_{Xo}, %) were determined. The estimate of ρ was obtained in the direction of the rows, and the statistics Xo and CV_{Xo} were obtained using the equations described by Paranaiba et al. (2009) and applied by Cargnelutti Filho et al. (2014a) and Toebe et al. (2020a):

$$Xo = \left(10\sqrt[3]{2(1-\rho^2)s^2m}\right)\Big/m, \text{ and}$$

$$CV_{Xo} = \left(\sqrt{(1-\rho^2)s^2 / m^2}\right) \Big/ \sqrt{Xo} \times 100.$$

The comparisons of means of the statistics ρ, s^2, m, CV_{trial}, Xo, and CV_{Xo} among the evaluation days were performed using Tukey's test via bootstrap with 10,000 resamplings, at 5% probability, as detailed and applied by Toebe et al. (2020a).

The number of replicates was defined from the least significant difference (d) of Tukey's test (in percentage of the overall experimental mean), estimated through the following equation:

$$D = \left(q_{\alpha(i;DFE)} \sqrt{EMS / r}\right) \Big/ m \times 100,$$

where $q_{\alpha(i;DFE)}$ is the critical value of Tukey's test at level α of probability error (α=0.05, in the present study); i is the number of treatments; DFE is the number of degrees of freedom of error [i(r-1)], for the completely randomized design, and [(i-1) (r-1)] for the randomized block design; EMS is the error mean square; r is the number of replicates; and m is the mean of the experiment. By replacing the expression of the experimental coefficient of variation $(CV = \sqrt{EMS} / m \times 100)$, in percentage, in the expression for the calculation of d, and isolating r, the following expression is obtained:

$$r = \left(q_{\alpha(i;DFE)} CV / d\right)^2.$$

In this study, the CV, expressed as a percentage, corresponds to the CV_{Xo}, since this is the CV expected for an experiment with the optimal plot size (Xo) determined. The number of replicates (r) was determined for experiments with completely randomized design and randomized block design, by an iterative process until convergence, using the mean of CV_{Xo} among the evaluation days. The r was determined in scenarios formed by combinations of i treatments (i = 3, 4, ..., 50) and d minimum differences among means of treatments to be detected as significant by Tukey's test at 5% probability, expressed as a percentage of the experimental mean (d = 10%, 11%, ..., 20%), according to Cargnelutti Filho et al. (2014a) and Toebe et al. (2020a), in ryegrass sown to haul. Statistical analyses were performed with Microsoft Office Excel, R (R Core Team, 2020), and Sisvar softwares (Ferreira, 2014).

Results and Discussion

There were no significant differences among the evaluation time in days for ρ and s^2 (Tables 1 and 2). For the mean of fresh weight per BEU, differences were found among evaluation days, with means ranging between 1281 g and 1534 g per BEU. Among all the 70 uniformity trials, the mean productivity of fresh weight was 1449 g per BEU of 0.51 m^2 (28,412 kg ha^{-1}), ranging from 1179 g per BEU (23,118 kg ha^{-1} in the trial 2, at 134 DAS) to 1751 g per BEU (34,333 kg ha^{-1} in the trial 1, at 131 DAS). The variation of the fresh weight is important for the experimental design, as it contemplates real situations of variability that occur in experimental areas. The mean productivity of fresh weight was higher than the one obtained by Toebe et al. (2020a) for the broadcast-seeded 'BRS Ponteio' ryegrass, in the same experimental area and year. This difference is possibly due to the cultivar and the type of sowing.

The overall coefficient of variation was 22.96% (Table 2), which is considered high according to Pimentel-Gomes (2009). Among the 70 uniformity trials, the CV_{trial} oscillated between 14.09 and 39.80%, respectively, for trials 11 (at 131 DAS) and 5 (at 130 DAS), that is, the CV fluctuated from medium to very high values, according to the classification by Pimentel-Gomes (2009). However, no significant differences were verified among the evaluation days ($18.86\% \leq CV \leq 25.59\%$).

The optimal plot size based on the 70 trials was 4.49 BEUs or 2.29 m^2, and no significant differences occurred among the evaluation days ($2.06\ m^2 \leq Xo \leq 2.41\ m^2$ – Tables 1 and 2). The optimal plot size calculated for each uniform trial ranged between 1.73 and 3.18 m^2, respectively for trial 11, at 131 DAS, and trial 5, at 130 DAS. These two trials showed the lowest and highest CV values, respectively, as previously discussed. The coefficient of variation in the optimal plot size ranged between 7.58% and 13.96%, with an overall mean of 10.04%, which is considered to be at the limit between the low and medium percentages according to Pimentel-Gomes (2009). As for the ρ, s^2, CV_{trial}, and Xo statistics, no significant differences were identified for CV_{Xo} between the evaluation dates ($9.03\% \leq CV_{Xo} \leq 10.57\%$). Therefore, the means of Xo and CV_{Xo} statistics based on the 70 uniformity trials were used for the calculation of the number of replicates.

The number of replicates varied between 3.95 and 32.27 (Tables 3 and 4), depending on the experimental design, the number of treatments (i), and the minimum differences (d). As already described [Cargnelutti Filho et al. (2014a, 2015, 2017, 2020); Burin et al. (2015); Chaves et al. (2018); Lavezo et al. (2018); and Toebe et al. (2020a, 2020b)], the greater is the term i, and the lower is the d, the greater will be the number of replicates. In this sense, the use of five, six, seven, and eight replicates is sufficient to identify differences

Table 1. First order spatial autocorrelation coefficient (ρ), variance (s^2), mean (m), coefficient of variation of the trial (CV_{trial}, %), optimal plot size (Xo, in BEUs of 0.51 m^2 and in m^2), and the coefficient of variation of the optimal plot size (CV_{Xo}, %) for the fresh weight of 'Barjumbo' ryegrass (*Lolium multiflorum*), sowed in rows and evaluated in uniformity trials at 130 and 131 days after sowing (DAS).

DAS	Trial[1]	Statistics[2]						
		ρ	s^2	Mean (g)	CV_{trial} (%)	Xo (BEU)	Xo (m^2)	CV_{Xo} (%)
130	1	0.33	165,739	1669	24.39	4.73	2.41	10.59
130	2	0.68	148,334	1645	23.41	3.90	1.99	8.72
130	3	0.73	321,524	1718	33.01	4.68	2.39	10.46
130	4	0.05	102,314	1531	20.90	4.43	2.26	9.91
130	5	0.48	352,434	1492	39.80	6.24	3.18	13.96
130	6	0.73	222,567	1588	29.70	4.37	2.23	9.76
130	7	-0.25	108,038	1681	19.56	4.15	2.12	9.29
130	8	0.19	64,116	1490	16.99	3.82	1.95	8.53
130	9	0.43	73,509	1603	16.91	3.60	1.84	8.05
130	10	-0.15	106,343	1621	20.11	4.29	2.19	9.60
130	11	0.50	108,856	1634	20.20	3.95	2.01	8.83
130	12	0.32	245,602	1593	31.11	5.58	2.85	12.48
130	13	0.47	112,923	1532	21.94	4.22	2.15	9.43
130	14	0.39	123,526	1441	24.39	4.66	2.38	10.42
130	15	0.31	194,855	1523	28.99	5.34	2.72	11.93
130	16	0.09	79,367	1322	21.31	4.48	2.29	10.02
130	17	0.14	79,558	1275	22.13	4.58	2.34	10.24
130	18	0.13	110,282	1248	26.62	5.19	2.64	11.59
Mean		0.31a	151,105a	1534a	24.53a	4.57a	2.33a	10.21a
131	1	0.44	186,204	1751	24.64	4.61	2.35	10.31
131	2	0.40	194,003	1713	25.71	4.80	2.45	10.73
131	3	0.43	219,903	1661	28.24	5.06	2.58	11.33
131	4	0.19	234,622	1680	28.84	5.43	2.77	12.14
131	5	0.16	89,087	1501	19.88	4.26	2.17	9.52
131	6	0.26	216,421	1479	31.45	5.70	2.90	12.74
131	7	0.22	82,401	1597	17.98	3.95	2.01	8.82
131	8	0.28	79,919	1566	18.05	3.91	2.00	8.75
131	9	0.49	158,284	1495	26.62	4.75	2.42	10.62
131	10	0.03	42,092	1380	14.87	3.54	1.80	7.91
131	11	0.14	39,757	1416	14.09	3.39	1.73	7.58
131	12	0.16	64,255	1606	15.78	3.65	1.86	8.15
131	13	0.25	70,792	1336	19.92	4.20	2.14	9.40
131	14	0.19	79,198	1356	20.75	4.36	2.22	9.75
131	15	0.20	88,628	1274	23.36	4.71	2.40	10.54
131	16	0.08	72,630	1231	21.90	4.57	2.33	10.21
131	17	0.31	49,195	1266	17.52	3.81	1.94	8.53
131	18	0.07	93,024	1392	21.91	4.57	2.33	10.22
Mean		0.24a	114,468a	1483a	21.75a	4.40a	2.25a	9.85a

[1]Each uniformity trial with 4×2.04 m (= 8.16 m^2) was divided into 16 basic experimental units (BEUs) of 1.0×0.51 m (= 0.51 m^2), forming a matrix of four rows and four columns. Ryegrass fresh weight was evaluated in grams per BEU of 1.0×0.51 m (= 0.51 m^2). [2]For the statistics ρ, s^2, m, CV_{trial}, Xo, and CV_{Xo}, the means followed by equal letters, in the columns (comparison of means between the evaluation days), do not differ by Tukey's test, at 5% probability, via bootstrap with 10,000 resamplings.

d=20% as significant between means, in experiments with up to 6, 13, 25, and 45 treatments, regardless of the experimental design.

Using the same methods of the present study to determine plot size and the number of replicates (in black oats, oat, forage pea, millet, rye, Sudan grass, triticale, and vetch), the plot size ranged from 1.66 to 7.95 m^2, and the number of replicates ranged from four to eight (Cargnelutti Filho et al., 2014a, 2015, 2017,

Table 2. First order spatial autocorrelation coefficient (ρ), variance (s^2), mean (Me), coefficient of variation of the trial (CV, in %), optimal plot size (Xo, m^2, in BEUs of 0.51 m^2), and the coefficient of variation in the optimal plot size (CV_{Xo}, %), for the fresh weight of 'Barjumbo' ryegrass (*Lolium multiflorum*) sowed in rows and evaluated in uniformity trials at 133, 134, and 137 days after sowing (DAS).

DAS	Trial[(1)]	Statistics[(2)]						
		ρ	s^2	Me (g)	CV (%)	Xo (BEU)	Xo (m^2)	CV_{Xo} (%)
133	1	0.30	52,100	1308	17.46	3.81	1.95	8.53
133	2	0.27	38,796	1293	15.23	3.51	1.79	7.84
133	3	-0.24	100,114	1701	18.60	4.03	2.05	9.01
133	4	-0.09	109,321	1535	21.54	4.51	2.30	10.10
133	5	0.40	69,980	1337	19.79	4.04	2.06	9.04
133	6	0.18	83,526	1408	20.53	4.34	2.21	9.70
Mean		0.14a	75,639a	1430ab	18.86a	4.04a	2.06a	9.03a
134	1	0.45	189,668	1301	33.46	5.63	2.87	12.60
134	2	0.58	89,941	1179	25.43	4.42	2.25	9.88
134	3	0.06	62,784	1253	20.00	4.30	2.19	9.62
134	4	-0.46	75,094	1211	22.62	4.32	2.20	9.67
134	5	0.36	111,634	1504	22.22	4.42	2.25	9.87
134	6	0.42	135,997	1239	29.77	5.26	2.68	11.76
Mean		0.24a	110,853a	1281b	25.59a	4.73a	2.41a	10.57a
137	1	0.36	103,978	1488	21.68	4.34	2.21	9.70
137	2	0.14	104,107	1472	21.93	4.55	2.32	10.18
137	3	0.34	158,968	1336	29.85	5.40	2.76	12.08
137	4	0.33	65,419	1313	19.48	4.08	2.08	9.12
137	5	0.70	71,442	1297	20.61	3.52	1.79	7.87
137	6	0.15	247,706	1512	32.91	5.96	3.04	13.32
137	7	0.36	127,188	1213	29.40	5.32	2.71	11.90
137	8	0.33	101,556	1390	22.93	4.54	2.31	10.14
137	9	0.13	139,654	1354	27.60	5.31	2.71	11.87
137	10	0.22	78,547	1327	21.12	4.39	2.24	9.83
137	11	-0.11	154,597	1583	24.85	4.96	2.53	11.09
137	12	0.01	127,913	1423	25.14	5.02	2.56	11.22
137	13	0.40	107,704	1274	25.77	4.81	2.45	10.76
137	14	-0.09	47,197	1231	17.64	3.95	2.02	8.84
137	15	0.36	133,445	1432	25.51	4.84	2.47	10.83
137	16	0.21	72,168	1439	18.67	4.05	2.07	9.06
137	17	0.22	53,242	1347	17.13	3.82	1.95	8.54
137	18	0.18	123,891	1440	24.44	4.87	2.48	10.89
137	19	0.01	88,141	1407	21.09	4.46	2.28	9.98
137	20	0.61	124,593	1600	22.06	3.95	2.01	8.83
137	21	0.14	101,580	1655	19.26	4.17	2.13	9.33
137	22	0.36	57,476	1298	18.46	3.91	1.99	8.73
Mean		0.24a	108,660a	1401ab	23.07a	4.56a	2.32a	10.19a
Overall mean		0.25	118,425	1449	22.96	4.49	2.29	10.04

[(1)]Each uniformity trial of 4×2.04 m (= 8.16 m^2) was divided into 16 basic experimental units (BEUs) of 1.0×0.51 m (= 0.51 m^2), forming a matrix of four rows and four columns. The fresh weight was evaluated in grams per basic experimental unit of 1.0×0.51 m (= 0.51 m^2). [(2)]For the statistics ρ, s^2, Me, CV, Xo, and CV_{Xo}, the means followed by equal letters, in the columns (comparison of means between the evaluation days), do not differ by Tukey's test, at 5% probability, via bootstrap with 10,000 resamplings.

Table 3. Number of replicates of experiments in a completely randomized design, in scenarios formed by the combinations of i treatments (i = 3, 4, ..., 50) and d least differences between treatment means to be detected as significant by Tukey's test, at 5% probability, expressed as percentage of the overall experimental mean (d = 10, 11, ..., 20%), to evaluate the fresh weight of 'Barjumbo' ryegrass (*Lolium multiflorum*) sowed in rows of the optimal plot size (Xo = 2.29 m^2), and the coefficient of variation in the optimal plot size (CV_{Xo} = 10.04%).

i	10%	11%	12%	13%	14%	15%	16%	17%	18%	19%	20%
3	12.13	10.21	8.77	7.64	6.75	6.03	5.45	4.97	4.56	4.22	3.95
4	14.19	11.89	10.14	8.78	7.71	6.84	6.14	5.55	5.07	4.66	4.32
5	15.76	13.17	11.19	9.66	8.45	7.47	6.67	6.01	5.46	5.00	4.60
6	17.05	14.21	12.06	10.38	9.05	7.99	7.11	6.39	5.79	5.28	4.85
7	18.13	15.10	12.79	10.99	9.57	8.43	7.49	6.72	6.07	5.52	5.06
8	19.07	15.86	13.42	11.53	10.02	8.81	7.82	7.00	6.32	5.74	5.25
9	19.90	16.54	13.99	12.00	10.42	9.15	8.12	7.26	6.54	5.93	5.42
10	20.65	17.15	14.49	12.42	10.78	9.46	8.38	7.49	6.74	6.11	5.57
11	21.33	17.70	14.95	12.81	11.11	9.74	8.62	7.70	6.92	6.27	5.71
12	21.94	18.21	15.37	13.16	11.41	10.00	8.85	7.89	7.09	6.42	5.84
13	22.51	18.68	15.76	13.49	11.69	10.24	9.05	8.07	7.25	6.55	5.96
14	23.04	19.11	16.12	13.79	11.95	10.46	9.25	8.24	7.40	6.68	6.08
15	23.54	19.51	16.46	14.08	12.19	10.67	9.43	8.40	7.53	6.81	6.18
16	24.00	19.89	16.77	14.34	12.42	10.86	9.59	8.54	7.66	6.92	6.28
17	24.43	20.25	17.07	14.59	12.63	11.05	9.75	8.68	7.79	7.03	6.38
18	24.84	20.59	17.35	14.83	12.83	11.22	9.91	8.82	7.90	7.13	6.47
19	25.23	20.90	17.61	15.06	13.03	11.39	10.05	8.94	8.01	7.23	6.56
20	25.60	21.21	17.87	15.27	13.21	11.55	10.19	9.06	8.12	7.32	6.64
21	25.95	21.50	18.11	15.47	13.38	11.70	10.32	9.17	8.22	7.41	6.72
22	26.29	21.77	18.34	15.67	13.55	11.84	10.44	9.28	8.31	7.49	6.79
23	26.61	22.04	18.56	15.85	13.71	11.98	10.56	9.39	8.41	7.58	6.87
24	26.92	22.29	18.77	16.03	13.86	12.11	10.68	9.49	8.49	7.65	6.94
25	27.21	22.53	18.97	16.20	14.01	12.24	10.79	9.59	8.58	7.73	7.00
26	27.50	22.76	19.17	16.37	14.15	12.36	10.89	9.68	8.66	7.80	7.07
27	27.77	22.99	19.36	16.53	14.28	12.47	10.99	9.77	8.74	7.87	7.13
28	28.03	23.21	19.54	16.68	14.42	12.59	11.09	9.86	8.82	7.94	7.19
29	28.29	23.42	19.71	16.83	14.54	12.70	11.19	9.94	8.89	8.01	7.25
30	28.53	23.62	19.88	16.97	14.67	12.80	11.28	10.02	8.96	8.07	7.31
31	28.77	23.82	20.05	17.11	14.78	12.91	11.37	10.10	9.03	8.13	7.36
32	29.00	24.01	20.20	17.25	14.90	13.01	11.46	10.18	9.10	8.19	7.42
33	29.23	24.19	20.36	17.38	15.01	13.10	11.54	10.25	9.17	8.25	7.47
34	29.45	24.37	20.51	17.50	15.12	13.20	11.62	10.32	9.23	8.31	7.52
35	29.66	24.54	20.65	17.63	15.23	13.29	11.70	10.39	9.29	8.36	7.57
36	29.86	24.71	20.80	17.75	15.33	13.38	11.78	10.46	9.35	8.42	7.62
37	30.06	24.88	20.93	17.86	15.43	13.46	11.86	10.53	9.41	8.47	7.66
38	30.26	25.04	21.07	17.98	15.53	13.55	11.93	10.59	9.47	8.52	7.71
39	30.45	25.19	21.20	18.09	15.62	13.63	12.00	10.65	9.52	8.57	7.75
40	30.63	25.35	21.33	18.20	15.71	13.71	12.07	10.72	9.58	8.62	7.80
41	30.81	25.50	21.45	18.30	15.80	13.79	12.14	10.78	9.63	8.66	7.84
42	30.99	25.64	21.57	18.41	15.89	13.87	12.21	10.84	9.68	8.71	7.88
43	31.16	25.78	21.69	18.51	15.98	13.94	12.27	10.89	9.74	8.76	7.92
44	31.33	25.92	21.81	18.60	16.06	14.02	12.34	10.95	9.79	8.80	7.96
45	31.50	26.06	21.92	18.70	16.15	14.09	12.40	11.00	9.83	8.84	8.00
46	31.66	26.19	22.03	18.80	16.23	14.16	12.46	11.06	9.88	8.89	8.04
47	31.82	26.32	22.14	18.89	16.31	14.23	12.52	11.11	9.93	8.93	8.08
48	31.97	26.45	22.25	18.98	16.38	14.29	12.58	11.16	9.97	8.97	8.11
49	32.12	26.57	22.35	19.07	16.46	14.36	12.64	11.21	10.02	9.01	8.15
50	32.27	26.69	22.45	19.15	16.54	14.42	12.70	11.26	10.06	9.05	8.18

Table 4. Number of replicates for experiments in a randomized block design, in scenarios formed by combinations of i treatments (i = 3, 4, ..., 50) and d least differences between treatment means to be detected as significant by Tukey's test, at 5% probability, expressed in percentage of the overall experimental mean (d = 10, 11, ..., 20%), to evaluate the fresh weight of 'Barjumbo' ryegrass (*Lolium multiflorum*) sowed in rows in the optimal plot size (Xo = 2.29 m^2), and coefficient of variation in the optimal plot size (CV_{Xo} = 10.04%).

i	10%	11%	12%	13%	14%	15%	16%	17%	18%	19%	20%
3	12.62	10.71	9.26	8.13	7.24	6.52	5.94	5.44	5.03	4.69	4.37
4	14.47	12.17	10.42	9.06	7.99	7.13	6.42	5.83	5.35	4.93	4.59
5	15.95	13.35	11.38	9.85	8.63	7.66	6.86	6.20	5.64	5.18	4.79
6	17.18	14.34	12.19	10.51	9.19	8.12	7.25	6.52	5.92	5.41	4.98
7	18.23	15.20	12.89	11.09	9.67	8.53	7.59	6.82	6.17	5.63	5.16
8	19.15	15.94	13.50	11.60	10.10	8.89	7.90	7.08	6.40	5.82	5.33
9	19.97	16.61	14.05	12.06	10.49	9.22	8.18	7.32	6.60	6.00	5.48
10	20.70	17.20	14.54	12.48	10.84	9.51	8.43	7.54	6.79	6.16	5.62
11	21.37	17.75	14.99	12.85	11.16	9.79	8.67	7.74	6.97	6.31	5.76
12	21.98	18.25	15.41	13.20	11.45	10.04	8.88	7.93	7.13	6.46	5.88
13	22.55	18.71	15.79	13.52	11.72	10.27	9.09	8.10	7.28	6.59	6.00
14	23.07	19.14	16.15	13.82	11.98	10.49	9.27	8.27	7.43	6.71	6.11
15	23.56	19.54	16.48	14.10	12.22	10.70	9.45	8.42	7.56	6.83	6.21
16	24.02	19.91	16.79	14.37	12.44	10.89	9.62	8.57	7.69	6.94	6.31
17	24.45	20.27	17.09	14.61	12.65	11.07	9.78	8.70	7.81	7.05	6.40
18	24.86	20.60	17.37	14.85	12.85	11.24	9.92	8.83	7.92	7.15	6.49
19	25.25	20.92	17.63	15.07	13.04	11.41	10.07	8.96	8.03	7.24	6.57
20	25.61	21.22	17.88	15.28	13.22	11.56	10.20	9.08	8.13	7.34	6.66
21	25.96	21.51	18.12	15.49	13.40	11.71	10.33	9.19	8.23	7.42	6.73
22	26.30	21.78	18.35	15.68	13.56	11.85	10.45	9.30	8.33	7.51	6.81
23	26.62	22.05	18.57	15.86	13.72	11.99	10.57	9.40	8.42	7.59	6.88
24	26.93	22.30	18.78	16.04	13.87	12.12	10.69	9.50	8.51	7.67	6.95
25	27.22	22.54	18.98	16.21	14.02	12.25	10.80	9.60	8.59	7.74	7.02
26	27.50	22.77	19.18	16.38	14.16	12.37	10.90	9.69	8.67	7.81	7.08
27	27.78	23.00	19.36	16.54	14.29	12.48	11.00	9.78	8.75	7.88	7.14
28	28.04	23.21	19.54	16.69	14.42	12.60	11.10	9.86	8.83	7.95	7.20
29	28.30	23.42	19.72	16.84	14.55	12.71	11.20	9.95	8.90	8.01	7.26
30	28.54	23.63	19.89	16.98	14.67	12.81	11.29	10.03	8.97	8.08	7.32
31	28.78	23.82	20.05	17.12	14.79	12.91	11.38	10.11	9.04	8.14	7.37
32	29.01	24.01	20.21	17.25	14.91	13.01	11.46	10.18	9.11	8.20	7.42
33	29.23	24.20	20.36	17.38	15.02	13.11	11.55	10.26	9.17	8.26	7.47
34	29.45	24.37	20.51	17.51	15.13	13.20	11.63	10.33	9.24	8.31	7.53
35	29.66	24.55	20.66	17.63	15.23	13.29	11.71	10.40	9.30	8.37	7.57
36	29.87	24.72	20.80	17.75	15.33	13.38	11.79	10.47	9.36	8.42	7.62
37	30.07	24.88	20.94	17.87	15.43	13.47	11.86	10.53	9.42	8.47	7.67
38	30.26	25.04	21.07	17.98	15.53	13.55	11.94	10.60	9.47	8.52	7.71
39	30.45	25.20	21.20	18.09	15.63	13.64	12.01	10.66	9.53	8.57	7.76
40	30.64	25.35	21.33	18.20	15.72	13.72	12.08	10.72	9.58	8.62	7.80
41	30.82	25.50	21.45	18.31	15.81	13.79	12.15	10.78	9.64	8.67	7.84
42	30.99	25.64	21.58	18.41	15.90	13.87	12.21	10.84	9.69	8.72	7.88
43	31.17	25.79	21.69	18.51	15.98	13.95	12.28	10.90	9.74	8.76	7.93
44	31.34	25.92	21.81	18.61	16.07	14.02	12.34	10.95	9.79	8.80	7.96
45	31.50	26.06	21.92	18.70	16.15	14.09	12.40	11.01	9.84	8.85	8.00
46	31.66	26.19	22.03	18.80	16.23	14.16	12.47	11.06	9.89	8.89	8.04
47	31.82	26.32	22.14	18.89	16.31	14.23	12.53	11.11	9.93	8.93	8.08
48	31.97	26.45	22.25	18.98	16.39	14.30	12.58	11.17	9.98	8.97	8.11
49	32.12	26.57	22.35	19.07	16.46	14.36	12.64	11.22	10.02	9.01	8.15
50	32.27	26.70	22.46	19.16	16.54	14.43	12.70	11.27	10.07	9.05	8.19

2020; Burin et al., 2015; Chaves et al., 2018; Lavezo et al., 2018; Toebe et al., 2020b).

The combination of these plot sizes and numbers of replicates allowed to identify as significant the differences between treatment means ranging between 20.00 and 44.75% of the experimental mean, by Tukey's test, at 5% probability. Such differences for plot sizes and number of replicates between surveys are due to the intrinsic variability of each crop, between cultivars, the local of experimental conduction and crop year, among other factors.

In broadcast-seeded ryegrass , the optimal plot size to determine the fresh weight was 2.19 m^2, with a coefficient of variation of 9.79% (Toebe et al., 2020a). According to these authors, the use of eight replicates is recommended to identify differences of 20% between treatment means as significant, in experiments with up to 50 treatments. In the study developed by Toebe et al. (2020a), the CV values of the uniformity trials, optimal plot size, CV in the optimal plot size, and the number of replicates were similar to those of the present study. This fact shows the consistency of these statistics for ryegrass, even considering different cultivation systems and cultivars. This may not be the case for other crops, such as turnip, for which Cargnelutti Filho et al. (2014b) recommended a greater plot size to evaluate the fresh weight in broadcasted-seeding experiments than the plot size of experiments sowed in rows. This can be explained by differences of soil, agricultural years, and, especially, by the different response of crops to the spatial arrangement. Other researchers – Flores et al. (2008) and Vizioli et al. (2018) – have used larger plot sizes than the one recommended in the present work for ryegrass, that is, 3–100 m^2 plots. However, the useful area of evaluation and the number of replicates were generally lower than the values obtained in the present study. Thus, this work contributes to the definition of experimental protocols for ryegrass cultivation, with the use optimization of experimental resources and the quality guarantee of inferential data analysis.

In the present study, for the combination of a higher number of treatments (50 treatments) and lower precision (d = 20%), the number of replicates was 8.19 (Tables 3 and 4), which cannot be used in practice.

To calculate d as a function of the two designs with 50 treatments, the following equations are used: $d = (q_{5\%(50;350)} \times 10.04)/\sqrt{8} = (5.699681 \times 10.04)/\sqrt{8}) = 20.23\%$, for completely randomized designs, and $d=(q_{5\%(50;343)} \times 10.04)/\sqrt{8}=(5.700775 \times 10.04)/\sqrt{8})=20.24\%$, for randomized block designs.

Thus, differences between treatment means of 20.24% of the experimental mean are identified as significant, by Tukey's test, at 5% probability, in experiments with up to 50 treatments and eight replicates. It is noteworthy, however, that very large agricultural experiments (with many treatments) occupy large areas and, therefore, they have usually greater variability, which sometimes makes it difficult to detect significant differences between treatments. Hence, experiments with more than 50 treatments and more than eight replicates should be avoided, to minimize the experimental variability.

Conclusions

1. The optimal plot size to evaluate the fresh weight of ryegrass (*Lolium multiflorum*) sowed in rows is 2.29 m^2.
2. In experiments with up to 50 treatments, eight replicates are required to identify as significant the differences between treatment means of 20.24%.

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