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SAMPLING INTENSITY IN LOTS OF FORAGE SEEDS

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ABSTRACT: Forage seeds have economic importance, making it essential to obtain representative samples for evaluating seed lot quality. This study aimed to evaluate the effect of sampling intensity on the results of seed quality analyses. Seed lots of Italian ryegrass, cultivar TG Santa Júlia, red clover, cultivar Estanzuela 116, and Persian clover, cultivar Lightning, were evaluated using four sampling intensities, corresponding to 10, 20, 30, and 40 simple samples. The samples were collected from big bags and subsequently sent to the Flávio Farias da Rocha Seed Analysis Teaching Laboratory at the Federal University of Pelotas for purity, determination of other seeds, inert matter, germination, and tetrazolium analyses. The results obtained at the different sampling intensities were compared with the tolerance limits established by the Rules for Seed Testing. No differences exceeding the established tolerance limits were observed for the evaluated variables, indicating that reducing sampling intensity may be possible without compromising the results of the analyses under the conditions evaluated.

KEYWORDS: Sampling, Physiological quality, Physical purity, Germination, Viability.

INTRODUCTION

The production of seeds of forage species is economically relevant, especially species from tropical and subtropical climates (Bolke et al., 2019). Despite its importance for agriculture, the production chain still has obstacles that have been gradually overcome (Rech et al., 2022).

In this context, ryegrass stands out, a species of importance for Brazilian livestock due to its high adaptability to winter and to temperate and subtropical climate regions (Bohn et al., 2020). The search for greater efficiency and profitability has driven the launch of new ryegrass cultivars in the Brazilian market, expanding the options for genotypes adapted to different environments and production systems (Bolke et al., 2019).

Ryegrass seed production can vary significantly depending on the management adopted, the sowing time, the cultivar used, and climatic conditions (Bohn et al., 2020). In general, productivity can vary from 69.9 to 933 kg ha^{-1} (Pereira et al., 2008; IBGE, 2025). Clover is also an important forage option, with a yield of 295.7 kg ha^{-1} in Uruguayan seed production (INIA, 2024).

Among the different species of clover, two cultivars recently introduced in southern Brazil stand out due to their high productive potential. The red clover, cultivar Estanzuela 116, derived from New Zealand materials, is a diploid cultivar, with an upright to semi-erect size, with early flowering, biannual character and peak production in November, maintaining productivity for two years and providing additional forage in the third spring (Castro et al., 2025). In turn, the Persia clover, Lightning cultivar, has an upright growth habit, absence of hard seeds, expressive initial vigor, wide edaphic adaptation, tolerance to floods and saline soils, in addition to high forage production with excellent digestibility (Castro et al., 2025).

To ensure seed quality, it is essential to carry out adequate sampling, as established by Law No. 10,711/2003, in order to obtain representative samples for quality testing, certification and inspection. Sampling procedures follow standards established by the Ministry of Agriculture and Livestock (MAPA), including the Rules for Seed Analysis (RAS) and the guidelines of the International Seed Testing Association (ISTA) (BRASIL, 2025).

To obtain accurate results, it is essential to collect samples according to official methods, since the results obtained only represent the quality of the seeds present in the analyzed sample. Thus, it is essential to ensure the representativeness of the sample sent for analysis. The sampling intensity should consider criteria such as the number of containers that make up the lot, the number of simple samples to be collected, and the capacity of the containers, among other factors (BRASIL, 2025).

In view of the above, this study aimed to evaluate whether the reduction of sampling intensity affects the results of seed quality analyses.

MATERIAL AND METHODS

The work was carried out from February to December 2025, in two stages. The first stage consisted of sampling the lots, carried out with a Nobbe type liming, inserted to the center of big bags with a capacity of 1,000 kg, with the opening facing downwards and the tip facing upwards, forming an angle of 30° in relation to the horizontal. After insertion, the lime was rotated 180°, leaving the opening facing upwards, and removed at a progressively slower speed,

in order to increase the amount of seeds collected along the route, from the center to the periphery of the big bag.

Lots of ryegrass seeds, cultivar TG Santa Júlia, red clover, cultivar Estanzuela 116, and Persia clover, cultivar Lightning, were sampled at different sampling intensities. 40, 30, 20, and 10 simple samples were collected, corresponding to 100, 75, 50, and 25% of the sampling intensity, respectively. The lots belonged to the 2023/2024 harvest, were in the S2 category, and were awaiting the results of the official analysis. Evaluations were carried out within each lot.

After collection, the seeds were submitted to three passes in a conical soil divider, packed in boxes with a capacity of 500 g and sent to the Flávio Farias da Rocha Didactic Laboratory of Seed Analysis, of the Eliseu Maciel Faculty of Agronomy, Federal University of Pelotas, in the municipality of Capão do Leão, RS. In the laboratory, the samples were homogenized and divided again in a soil divider to obtain the work samples, according to the recommendations of the Rules for Seed Analysis (RAS).

Table 1. Information on lots, species, weight, number of packages per lot and recommended sampling intensity. Capão do Leão, 2025.

Species	Plot	Batch weight (kg)	No. of packages per batch	Recommended No. of Samples
Ryegrass – Santa Júlia	1	20	10	40
Ryegrass – Santa Júlia	2	20	10	40
Ryegrass – Santa Júlia	3	20	10	40
Ryegrass – Santa Júlia	4	20	10	40
Persian Clover – Lightning	1	20	8	40
Persian Clover – Lightning	2	20	8	40
Persian Clover – Lightning	3	20	8	40
Persian Clover – Lightning	4	20	8	40
Red clover – Estanzuela 116	1	20	8	40
Red clover – Estanzuela 116	2	20	8	40
Red clover – Estanzuela 116	3	20	8	40
Red clover – Estanzuela 116	4	20	8	40

Physical purity

The physical purity analysis was carried out according to the Rules for Seed Analysis (BRASIL, 2025). The sample was separated into three fractions: pure seeds, other seeds and inert material, and the results were expressed as a percentage of the mass of the working sample. For ryegrass, a 6 g working sample was used, while for clovers, a 2 g sample was used.

Germination test

For the germination test, 400 seeds were used, distributed in four replicates of 100 seeds, on paper moistened with water in an amount equivalent to 2.5 times the mass of the dry paper. The seeds were kept in a germinator at 25 °C. The evaluations were carried out according to the recommendations of the Rules for Seed Analysis (RAS), between the fifth and fourteenth day, and the results were expressed as a percentage of normal seedlings for ryegrass, red clover and Persian clover seeds.

Tetrazolium test

For the tetrazolium test on ryegrass seeds, 100 seeds were used, distributed in two samples of 50 seeds per repeat. The seeds were subjected to imbibition for 18 hours in BOD, at 20 °C. After imbibition, a longitudinal section was made through the embryo, and the seeds were placed in a solution of 2,3,5-triphenyl tetrazolium chloride, at a concentration of 0.5%, for four hours, at 30 °C, in BOD. After staining, the seeds were washed with distilled water and evaluated under an illuminated stereoscopic microscope, according to the methodology described in the Rules for Seed Analysis (RAS).

For red clover and Persian clover seeds, the methodology was adapted, since the imbibition period described in the Rules for Seed Analysis did not present satisfactory results. 100 seeds of each cultivar were used, distributed in two samples of 50 seeds per repeat, submitted to imbibition for eight hours in BOD, at 20 °C. After imbibition, an incision was made to remove the integument, and the seeds were placed in a solution of 2,3,5-triphenyl tetrazolium chloride, at a concentration of 1%, for 10 hours, at 30 °C, in BOD. After staining, the seeds were washed with distilled water and evaluated under a stereoscopic microscope with illumination.

Analysis of the results

The results obtained at the different sampling intensities were compared with each other, considering the tolerance limits established in the Rules for Seed Analysis (RAS) (BRASIL, 2025). For each variable evaluated, the means of the results were calculated and compared with the tolerance values presented in the RAS tables. The difference between the results was considered acceptable when it did not exceed the tolerance limit corresponding to the mean and the probability of 5%.

RESULTS

The results of the purity analyses, determination of other seeds and inert material, germination and tetrazolium test of the lots of ryegrass seeds, cultivar TG Santa Júlia, submitted to different sampling intensities (Table 2).

Table 2. Purity percentages, determination of other seeds/inert material, germination and viability by tetrazolium test in lots of ryegrass seeds, cultivar TG Santa Júlia, submitted to different sampling intensities. Capão do Leão, 2025.

Tests	Lots	A40	A30	A20	A10
Purity	L1	95,8	94.8 ^{ns}	94.6 ^{ns}	93.6 ^{ns}
	L2	96,7	96.7 ^{ns}	95.8 ^{ns}	95.6 ^{ns}
	L3	96,6	94.9 ^{ns}	94.7 ^{ns}	94.7 ^{ns}
	L4	95,8	95.6 ^{ns}	94.9 ^{ns}	94.9 ^{ns}
D.O.S/M. I	L1	0,0	0.0 ^{ns}	0.0 ^{ns}	0.0 ^{ns}
	L2	0,0	0.0 ^{ns}	0.0 ^{ns}	0.0 ^{ns}
	L3	0,0	0.0 ^{ns}	0.0 ^{ns}	0.0 ^{ns}
	L4	0,0	0.0 ^{ns}	0.0 ^{ns}	0.0 ^{ns}
Germination	L1	76	74 ^{ns}	75 ^{ns}	70 ^{ns}
	L2	78	75 ^{ns}	76 ^{ns}	70 ^{ns}
	L3	80	78 ^{ns}	76 ^{ns}	76 ^{ns}
	L4	77	76 ^{ns}	72 ^{ns}	71 ^{ns}
tetrazolium	L1	77	74 ^{ns}	74 ^{ns}	72 ^{ns}
	L2	79	76 ^{ns}	75 ^{ns}	71 ^{ns}
	L3	80	77 ^{ns}	76 ^{ns}	70 ^{ns}
	L4	78	76 ^{ns}	72 ^{ns}	72 ^{ns}

D.O.S./M.I. = determination of other seeds/inert material. A40, A30, A20 and A10 correspond to 40, 30, 20 and 10 simple samples, respectively. Source: adapted from Madruga (2025).

In the analysis of ryegrass seed purity, the values ranged from 93.6 to 96.7% between lots and sampling intensities. Lot 1 showed 95.8% purity at intensity A40, reducing to 93.6% at A10. In lot 2, the values ranged from 95.6 to 96.7%, while lots 3 and 4 showed variations from 94.7 to 96.6% and from 94.9 to 95.8%, respectively. Although numerical variations were observed between the intensities, the differences remained within the tolerance limits established by the Rules for Seed Analysis (RAS), indicating that the reduction in sampling intensity did not compromise the evaluation of the purity of the lots under the conditions studied.

The results of the purity analyses, determination of other seeds and inert material, germination and tetrazolium test of the lots of Persia clover, cultivar Lightning, and red clover, cultivar Estanzuela 116, submitted to different sampling intensities, are presented in Table 3.

Table 3. Purity percentages, determination of other seeds/inert material, germination and viability by tetrazolium test in lots of Persia clover, cultivar Lightning, and red clover, cultivar Estanzuela 116, submitted to different sampling intensities. Capão do Leão, 2025.

Tests	Cultivars	Lots	A40	A30	A20	A10
Purity	Shamrock of Persia-Lightning	L1	96	96 ns	95 ns	94 ns
		L2	95	96 ns	94 ns	90 ns
		L3	96	94 ns	94 ns	91 ns
		L4	95	95 ns	94 ns	92 ns
	Red Clover 116	L1	96	96 ns	95 ns	94 ns
		L2	95	96 ns	94 ns	90 ns
		L3	94	94 ns	93 ns	90 ns
		L4	96	94 ns	94 ns	91 ns
D.O.S/ M. I	Shamrock of Persia-Lightning	L1	0	0 night sentry	0 night sentry	0 night sentry
		L2	0	0 night sentry	0 night sentry	0 night sentry
		L3	0	0 night sentry	0 night sentry	0 night sentry
		L4	0	0 night sentry	0 night sentry	0 night sentry
	Red Clover 116	L1	0	0 night sentry	0 night sentry	0 night sentry
		L2	0	0 night sentry	0 night sentry	0 night sentry
		L3	0	0 night sentry	0 night sentry	0 night sentry
		L4	0	0 night sentry	0 night sentry	0 night sentry
Germination	Shamrock of Persia-Lightning	L1	80	78 ns	76 ns	76 ns
		L2	77	76 ns	72 ns	71 ns
		L3	80	79 ns	78 ns	79 ns
		L4	79	79 ns	77 ns	78 ns
	Red Clover 116	L1	79	78 ns	78 ns	78 ns
		L2	80	79 ns	78 ns	78 ns
		L3	79	79 ns	77 ns	78 ns

		L4	81	80 ns	78 ns	78 ns
tetrazolium	Shamrock of Persia-Lightning	L1	82	80 ns	80 ns	79 ns
		L2	81	81 ns	80 ns	78 ns
		L3	79	77 ns	76 ns	70 ns
		L4	78	76 ns	72 ns	72 ns
	Red Clover 116	L1	79	80 ns	79 ns	77 ns
		L2	80	80 ns	80 ns	80 ns
		L3	81	80 ns	80 ns	80 ns
		L4	81	81 ns	80 ns	80 ns

D.O.S./M.I. = determination of other seeds/inert material. A40, A30, A20 and A10 correspond to 40, 30, 20 and 10 simple samples, respectively. The differences were evaluated considering the tolerance limits established in the RAS. Source: adapted from Madruga (2025).

In the purity analysis, the lots of Persian clover, cultivar Lightning, presented values between 90 and 96%, while the lots of red clover, cultivar Estanzuela 116, presented values between 90 and 96%. In Persia clover, lot 2 presented 95% purity at intensity A40 and 90% at A10, while lot 3 varied from 96% to 91% between these same intensities. For red clover, lots 2 and 3 presented 90% purity at intensity A10, compared to 95 and 94% at A40, respectively. Despite the variations observed between the sampling intensities, the results remained within the tolerance limits considered in the Rules for Seed Analysis (RAS), indicating that the reduction in sampling intensity did not compromise the evaluation of the purity of the evaluated lots.

DISCUSSION

For the determination of other seeds and inert material, no occurrences were observed in any of the ryegrass lots, regardless of the sampling intensity used. In all the intensities evaluated, the results were equal to 0.0%, indicating that the reduction in the number of simple samples did not alter the identification of these fractions in the analyzed lots.

In the germination test, the values ranged from 70 to 80% between lots and sampling intensities. Lot 3 presented the highest result in intensity A40, with 80% germination, while the lowest values were observed in lots 1 and 2, both with 70% in intensity A10. In lot 4, germination ranged from 71 to 77%, while in lots 1 and 2, reductions from 76 to 70% and from 78 to 70%, respectively, were observed between intensities A40 and A10. Despite the numerical variations observed, the results remained within the tolerance limits established by the Rules

for Seed Analysis (RAS), indicating that the different sampling intensities did not compromise the evaluation of the germination of the analyzed lots.

Regarding the tetrazolium test, the results ranged from 70 to 80% of viable seeds between lots and sampling intensities. The highest result was observed in lot 3, with 80% viability at intensity A40, while the lowest value was 70%, observed in the same lot at intensity A10. In lots 1, 2 and 4, the results also showed a reduction with the decrease in sampling intensity, ranging from 77 to 72%, from 79 to 71% and from 78 to 72%, respectively. Despite the numerical variations, the differences between the intensities remained within the tolerance limits established by the Rules for Seed Analysis (RAS), indicating that the reduction in sampling intensity did not compromise the evaluation of the viability of ryegrass seeds under the conditions evaluated.

The results obtained are similar to those found by Nunes et al. (2024) for the tetrazolium test on ryegrass seeds. Similarly, Silva et al. (2025) found equivalent results for the purity and germination analyses of these seeds. These results reinforce the importance of seed quality control and the use of appropriate methods for the evaluation of physical and physiological quality.

Seed quality is directly related to obtaining lots with physical and physiological characteristics suitable for commercialization and use by producers. In this sense, measures to avoid contamination and preserve quality should be adopted during the different stages of production, contributing to obtaining seeds with desirable characteristics for the farmer (Nunes et al., 2024).

Advances are still needed, especially in relation to the laboratory tests used to evaluate the quality of ryegrass seeds. The germination test may have a longer duration in freshly harvested seeds due to dormancy, while the tetrazolium test allows a faster evaluation of viability. For this reason, the tetrazolium test is also important in the evaluation of ryegrass seeds.

For the determination of other seeds and inert material, no occurrences were observed in any of the lots of Persian clover, cultivar Lightning, or red clover, cultivar Estanzuela 116, regardless of the sampling intensity used. In all the intensities evaluated, the results were equal to 0%, indicating that the reduction in the number of simple samples did not alter the results of this analysis in the lots evaluated.

In the germination test, the results of Persia clover, cultivar Lightning, ranged from 71 to 80%. Lots 1 and 3 showed 80% germination at intensity A40, while lot 2 presented the lowest result, 71%, at intensity A10. For red clover, cultivar Estanzuela 116, the values ranged from

77 to 81%, with the highest result observed in lot 4, with 81% at intensity A40. In lots 2 and 4, germination went from 80 and 81%, respectively, at intensity A40, to 78% at A10. Despite the numerical variations between the sampling intensities, the results remained within the tolerance limits established by the Rules for Seed Analysis (RAS), indicating that the reduction in sampling intensity did not compromise the evaluation of germination of lots of Persian clover and red clover under the conditions evaluated (BRASIL, 2025).

In the tetrazolium test, the results of the Persian clover, cultivar Lightning, ranged from 70 to 82%. Lot 1 presented the highest result, with 82% viability at intensity A40, while lot 3 presented the greatest reduction among the intensities, going from 79% at A40 to 70% at A10. In lot 4, the values varied from 78% at A40 to 72% at A10. For red clover, cultivar Estanzuela 116, the results were more stable between the sampling intensities. Lot 1 presented values between 77 and 80%, while lots 2, 3 and 4 presented values of 80% at A40, A30, A20 and A10, with small variations in intermediate intensities. The observed differences remained within the tolerance limits established by the Rules for Seed Analysis (RAS), indicating that the reduction in sampling intensity did not compromise the evaluation of seed viability under the conditions evaluated (BRASIL, 2025).

The results of this study are consistent with Egan et al. (2021), who reported germination results in clover seeds, and with Braga et al. (2015), Freitas et al. (2018), and Radke et al. (2016), who evaluated the vigor of red clover, arugula, and coriander seeds, respectively.

According to Peske et al. (2019), seed vigor is related to the ability to germinate quickly, emerge uniformly, and develop under different conditions. Lots with higher vigor provide faster and more uniform stands, contributing to crop establishment. The germination test allows the evaluation of the maximum germination potential of seeds and helps in the comparison between lots and in the estimation of the value for sowing (BRASIL, 2022).

Like the germination test, the tetrazolium test is a method used to evaluate seed quality. It is a biochemical test that makes it possible to quickly assess viability, and is particularly useful in freshly harvested seeds, dormant seeds, or situations in which there is an occurrence of abnormal seedlings. The test can also help in the evaluation of vigor and in the identification of damage related to drying, insects, moisture, and management (BRASIL, 2025).

CONCLUSION

The reduction of the sampling intensity from 40 to 30, 20 and 10 simple samples did not compromise the results of the analysis of purity, determination of other seeds and inert material, germination and viability by the tetrazolium test in the lots of ryegrass, Persian clover and red

clover evaluated. Under the conditions of this study, the reduction of the sampling intensity was possible without exceeding the tolerance limits established by the Rules for Seed Analysis for the lots evaluated.

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Conflicts of interest

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Data Availability Statement

All data generated or analyzed during this study are included in this published article.

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.