Ciência

Physiological and sanitary quality of ryegrass seeds submitted to different defoliation frequencies

Ricardo Pereira da Cunha¹¹⁰ Joice Fernanda Lübke Bonow^{2*}¹⁰ Andréa Mittelmann³¹⁰ Manoel de Souza Maia¹ Alberto Bohn¹¹⁰ Roberto Caetano de Oliveira¹¹⁰ Jéssica Dias Gomes da Silva¹¹⁰ Carlos Eduardo da Silva Pedroso¹¹⁰

¹Programa de Pós-graduação em Ciência e Tecnologia de Sementes, Departamento de Fitotecnia, Universidade Federal de Pelotas (UFPel), Capão do Leão, Rio Grande do Sul, Brasil.

²Programa de Pós-graduação em Sistemas de Produção Agrícola Familiar, Departamento de Fitotecnia, Universidade Federal de Pelotas (UFPel), 96010–900, Capão do Leão, RS, Brasil. E-mail: joicef.agronomia@gmail.com. *Corresponding author. ³Embrapa Gado de Leite/Embrapa Clima Temperado, Pelotas-RS, Brasil.

ABSTRACT: This study determined the effect of different defoliation frequencies in a long-cycle ryegrass cultivar on yield components and the physiological and sanitary quality of seeds. Four defoliation frequencies were applied (without, one, two, and three defoliations). The time between defoliations was 350 degree-days. At the first, second, and third defoliation time, the plants were 15 cm, 20 cm, and 35 cm, respectively, and were lowered to half their height. The yield components and plant structure were evaluated through the tillers, along with the physiological quality of the seeds, which was verified by germination tests, first germination count, accelerated aging, tetrazolium test, field emergence, and weight of a thousand seeds. For the sanitary quality analysis, the percentage (%) of fungi incidence in the seeds was verified. The second defoliation determined the seeds' high physiological and sanitary quality due to the higher amount of primary and secondary tillers produced concerning the other treatments. However, there was no significant difference for the third defoliation since the aerial tillers issued the same quality of seeds originated from primary and secondary tillers. The average incidence of fungi such as Colletotrichum sp., Fusarium sp., Phoma sp., and Dreshslera spp. was lower in ryegrass plants subjected to three defoliations.

Qualidade fisiológica e sanitária de sementes de azevém-anual submetidas a diferentes frequências de desfolha

RESUMO: O presente estudo teve como objetivo determinar o efeito de diferentes frequências de desfolha em uma cultivar de azevém de ciclo longo, sobre os componentes de rendimento e a qualidade fisiológica e sanitária das sementes. Foram aplicadas quatro frequências de desfolhas (sem, uma, duas e três). O tempo entre desfolhas foi de 350 graus-dia. No momento da primeira, segunda e terceira desfolha as plantas estavam com 15 cm, 20 cm e 35 cm, respectivamente e foram rebaixadas para metade de suas alturas. Avaliaram-se os componentes de rendimento e a estrutura das plantas por meio dos perfilhos, juntamente com a qualidade fisiológica das sementes, a qual foi verificada por testes de germinação, primeira contagem de germinação, envelhecimento acelerado, teste de tetrazólio, emergência a campo e peso de mil sementes. Para a análise da qualidade sanitária verificou-se a porcentagem (%) de incidência de fungos nas sementes. A segunda desfolha determinou alta qualidade fisiológica e sanitária das sementes devido a maior quantidade de perfilhos primários e secundários produzidos em relação aos demais tratamentos. No entanto não houve diferença significativa para a terceira desfolha, visto que nos perfilhos aéreos emitidos observou-se a mesma qualidade das sementes originadas de perfilhos primários e secundários. A incidência média de fungos como Colletorichum sp., Fusarium sp., Phoma sp. e Dreshsleras pp. foi menor em plantas de azevém submetidas a três desfolhas. **Palavras-chave:** germinação, fungos, Lolium multiflorum, perfilhos aéreos.

INTRODUCTION

The annual ryegrass *Lolium multiflorum* is a forage highly adapted to southern Brazil's climate, providing high levels of forage and seed production (CUNHA et al., 2016; BOHN et al., 2020). Despite having high adaptation and various purposes of use, the exploitation of this forage species varies according to the management of defoliation adopted and nitrogen fertilization in coverage (BOHN et al., 2020; BOLKE et al., 2019). Concerning defoliation, the exploitation of the high tillering capacity of this species is essential due to the better quality of light radiation that reaches the base of the clump; and

Received 08.03.20 Approved 07.13.21 Returned by the author 09.22.21 CR-2020-0719.R1 Editors: Leandro Souza da Silva consequently, activates the buds that will originate new basilar tillers (CANDIDO et al., 2006). However, the pruning should not be drastic, as annual plants like ryegrass have a minimum partition of assimilates for reserve formation purposes. Therefore, the residue of live leaves becomes necessary for the regrowth of these plants.

Defoliation management must prioritize the quality of the seed produced, as excessively defoliated plants tend to accumulate fewer reserves for seed formation (CASSOL et al., 2011). The time when this defoliation occurs will influence the arrangement of yield components since the quality is directly and positively correlated with the leaves' dry matter production, cob length, and crude protein content (MÜLLER et al., 2012). The thousand seeds' weight greatly influences the desired color and seed vigor (PASLAUSKI et al., 2014). In the ryegrass of the cultivar Comum, seed vigor reaches its maximum when the plants are subjected to two defoliations during the vegetative period (PASLAUSKI et al., 2014). Similarly, germination also has favorable results when defoliation on the plant is performed during tillering, that is, at the beginning of the vegetative period, as the restriction of sugars in this period does not alter the seeds' formation. (RODOLFO et al., 2017).

In addition to the physiological aspect, the sanitary quality of seeds can also be affected by defoliation management. PIESANTI (2019) claimed the period in which defoliation occurs directly influences the time of seed harvest after anthesis. Temperature and humidity conditions may fluctuate at this stage, favoring the incidence of fungi, bacteria, and other microorganisms associated with the seed (PIESANTI, 2019). SILVA et al. (2014) detected the presence of several fungi in ryegrass seeds such as Alternaria alternata, Bipolaris sorokiniana, Drechslera spp., D. siccans, Fusarium graminearum, Fusarium spp., Aspergillus spp. and Penicillium sp., and the incidence of most of these fungi had already been dated by LUCCA-FILHO et al. (1999), which partially demonstrates the low sanitary quality of commercialized seeds.

The physiological and sanitary quality of the seed is of utmost importance to guarantee the satisfactory establishment of the plants in the production fields. Defoliation can change the source/ drain ratio in the plant and harm the quality and establishment of these (PEREIRA et al., 2012). Therefore, there is cost-effectiveness in defoliation. Therefore it is relevant to perform germination, vigor, and performance tests of produced seeds as a function of the number of defoliations. Thus, the present study determined the effect of different defoliation frequencies in a longcycle ryegrass cultivar on the yield components and the physiological and sanitary quality of the seeds.

MATERIALS AND METHODS

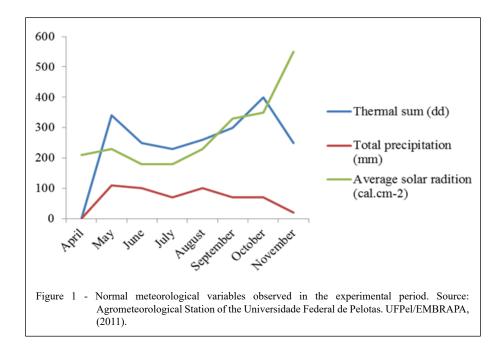
The experiment was conducted in an experimental area located in the municipality of Capão do Leão-RS (31° 80' S and 52° 40' W, 13 m above sea level), belonging to Embrapa Clima Temperado/Experimental Station Terras Baixas.

Annual ryegrass, cultivar BRS Ponteio, was used. Sowing was done in a row, using a no-tillage seeder, model Semina 3. The density used was 25 kg ha⁻¹ of viable pure seeds, with a spacing between rows of 20 cm. The soil in the experimental area is classified as Solodic Dystrophic Haplic Plano soil (SANTOS et al., 2018), which is submitted to conventional preparation, with liming and fertilization according to soil analysis. The experiment had four treatments arranged in a randomized block experimental design, with four replications. The area of each plot was 88 m².

The defoliation frequency factor evaluations used the following treatments: D0 = Zero Defoliation; D1 = One defoliation, performed when the forage mass reached approximately 1500 kg DM ha⁻¹. The plants with an average height of 14 cm and a thermal sum of 864 degree-days; D2 =Two Defoliations performed with a thermal sum of 420 degrees-day after the first defoliation, when the plants reached around 20 cm, on 23/09; D3 = Three Defoliations, performed with a thermal accumulation of 357 degrees-day, after the second, with approximately 35 cm in height. The post-defoliation condition for all managements was at half the height of the pasture at the time of pre-defoliation.

The calculation of daily thermal accumulation was performed according to CONFORTIN et al. (2010) through the equation: $[(t^{\circ} Mx + t^{\circ} Mn)/2] - 5$, where to Mx = maximumtemperature, to Mn = minimum temperature and 5°C = the base temperature (Tb) of ryegrass. The normal data of meteorological variables were observed during the experimental period and collected at the Agrometeorological Station of the Universidade Federal de Pelotas (UFPel), located next to the experimental area (Figure 1).

To determine the yield components, before each cut, ten plants were collected per plot, which evaluated: number of tillers per plant (main, secondary, and aerial); the number of fertile tillers per plant; tiller length (TL); cobs length (CL); the number



of spikelets per cob (NSC); the number of seeds per spikelet (NSS) and weight of a thousand seeds (WTS). In addition to these evaluations, the number of visible or palpable nodes (NVP) of each tiller was quantified and the plant population by counting in four 50 cm linear samples per plot.

The researchers harvested the seeds manually when they presented approximately 35% moisture. Regarding monitoring, samples were collected daily to determine moisture by the oven method at $105 \,^{\circ}C$ (BRASIL, 2009) and the expedited oil distillation method with a Gehaka apparatus. The visual verification of the beginning of senescence of the oldest leaves, the change in consistency of the endosperm (up to "sebum point"), the days after maximum anthesis (EICHELBERGER et al., 2003), and the changes in cob coloring (from green to greenish-brown) represented parameters for the determinations beginning.

Given the desired moisture content of the seeds, the harvest was performed by cutting eight samples per plot (50x50 cm) close to the ground. Drying was carried out in the shade for the first 12 hours on a covered concrete floor and then completed in an oven with forced air circulation until reaching a water content between 10% and 13% to avoid loss of seed quality. During the entire drying process, the seeds remained inside the spikelets.

Pre-cooling at 5 °C for seven days determined the seeds' physiological quality, verifying

that they were not dormant. The following tests were applied according to BRASIL (2009): Germination conducted with four subsamples of 50 seeds, at 25 °C, in the presence of light and on blotting paper, moistened with a volume of distilled water 2.5 times the substrate weight. Two counts were made, at 5 and 14 days, respectively, after assembly. The first count - performed together with the germination test, computing the average percentage of normal seedlings four days after sowing. Tetrazolium - to determine the viability of seeds: four samples of 100 seeds, cut longitudinally through the embryo and 3/4 of the endosperm; the concentration of the tetrazolium solution was 0.5%, and the coloration was at 30 °C for four hours. Accelerated aging: was performed using 1.0 g of seeds that were distributed on a screen in plastic boxes gerbox-type, with 40 mL of distilled water at the bottom and were kept in a BOD chamber at 40 °C and about 100% relative humidity, for 72 hours and then the germination test was conducted. The seedling evaluation was performed at five and 14 days. Field emergence: sowing performed in a seedbed with the same type of soil already mentioned. One hundred seeds (1 cm deep) were sown in 1 m long rows, with the averages of four rows composing one repetition. The assessment was performed once at 21 days after sowing.

For the health test, samples of seeds from different treatments were taken to the Laboratory of

Cunha et al.

Phytopathology in the Departamento de Fitossanitária of the Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas (UFPel). These were subjected to seed pathology analysis according to BRASIL (2009). The analysis used four replicates of 50 seeds per gerbox box, each containing two sheets of filter paper moistened with distilled water, previously disinfested with 1% hypochlorite. The gerbox boxes were incubated for seven days, under a 12-hour photoperiod and a temperature of \pm 23 °C. At the end of the incubation period, the seeds were analyzed individually by a stereomicroscope, assessing their fungi incidence.

The following year seeds produced under different treatments (D0; D1; D2; D3) were sown in beds on April 29th. The soil type was as described above, and seeds were sown at a maximum depth of 1 cm. The experimental design was randomized blocks with four replications. Each repetition consisted of four 1 m length rows, spaced 10 cm apart, containing 100 seeds each. After the plants had approximately four leaves, plants were assessed weekly for their morphogenic and structural characteristics using the marked tiller technique (CARRÈRE et al., 1997). The assessment studied the structural characteristics of tillers m², Number of Living Leaves (NLL), and Final Length of the Green Leaf Fraction (FLGLF). The morphogenic variables evaluated were: Leaf Appearance Rate, Phyllochron, Leaf Expansion Rate, Leaf Senescence Rate, Stem Elongation Rate, Tiller Rate, and Leaf Lifespan.

At the end of the establishment period, ten plants were collected close to the ground and dried in an oven with forced air circulation at 60 °C to determine the dry matter. Statistical analyzes were performed with the help of the SASM-Agri program (CANTERI et al., 2001), by comparing averages by the Scott-Knott Test at a 5% probability of error.

RESULTS AND DISCUSSION

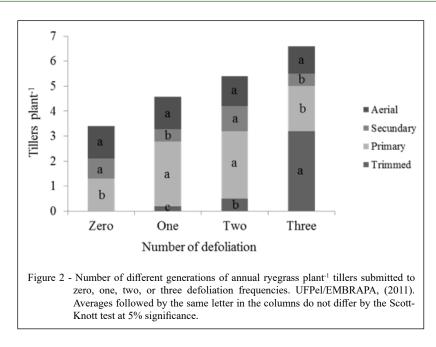
For the variable of yield components (Table 1), ryegrass plants displayed the same seed yield in treatments with zero, one, or two defoliations. After two defoliations, the number of defoliations significantly reduced the tiller length. The experiment also highlighted a tendency towards reducing the number of plants, cob length, and spikelets per cob, but the differences were not statistically significant. Plants subjected to two defoliations presented a greater number of fertile tillers at the time of seed harvesting, a determinant variable for the maintenance of seed yield (HEINECK et al., 2020), similar to that of plants not subjected to defoliation or submitted only once. Double defoliation is the most suitable for forming yield components, as the plants equaled the weight of a thousand seeds concerning the other treatments. Still, the plants that presented the lowest number of tillers per plant, shorter tiller, and cob lengths did not differ in the weight of a thousand seeds when the same number of defoliations was applied.

The third defoliation determined the death of fertile tillers and the appearance of smaller tillers with smaller cobs compared to the other treatments (Table 1). The main tillers number did not differ in the zero defoliation from the three defoliation treatments (Figure 2). For the control, the smaller number of main tillers was due to the closing of the plant's canopy and alteration of the

Table 1 - Yield components - plants per square meter (Pl m²), fertile tillers per plant (FT/Pl), tiller length (TL), cob length (CL), spikelets per cob (Sl/Co), seeds per spikelet (S/Sl) and weight of a thousand seeds (WTS) - of annual ryegrass plants subjected to different defoliation frequencies. UFPel/EMBRAPA, (2011).

Defoliation	Yield Components							
	Pl m ²	FT/Pl	TL	CL	Sl/Co	S/S1	WTS (g)	
Zero	141.3 a	3.4 b	85 a	23 a	16.7 b	5.1 a	2.191 a	
One	128.1 a	3.7 b	92 a	23 a	18.8 a	4.0 a	2.206 a	
Two	131.3 a	4.3 a	71 b	20 a	15.4 b	4.1 a	2.101 a	
Three	131.9 a	3.3 b	56 c	16 b	15.3 b	4.5 a	2.045 a	
CV (%)	11.2	9.5	7.7	9.2	7.9	17.2	8.38	

Averages followed by the same letter in the columns do not differ by the Scott-Knott test at 5% significance.



light transmitted to the base, decreasing the basilar buds activation. CUNHA et al. (2016) highlighted that the morphogenic rates of tillers submitted to third defoliation were minimal, especially tillering rates. Thus, due to the third defoliation, the same research pointed out a significant reduction in seed yield about the other treatments.

Seed yield is strongly related to the dynamics of the tiller population as a function of defoliation (HEINECK et al., 2020). Data reported by CUNHA (2016) showed seed production values (777.70, 736.63, 624.59, 234.41 Kg ha⁻¹) for zero, one, two, and three defoliations, respectively. Treatments submitted to up to two defoliations displayed the highest seed yields and the highest basilar tillers amount. In the present study, the third defoliation determined the highest mortality of the basilar tillers (primary and secondary). Thus, the seed produced with three defoliations is mainly originated from aerial tillers (Figure 2).

Defoliation with plants already in full flowering causes a strong disturbance in the plants since the allocation of reserves and nutrients at this stage generally are towards the formation of reproductive structures (FONTANELI, 2012). As cutting these parts, secondary and aerial tillers form due to the reduced reserves of the annual plant and the sensitivity to photoperiod right after defoliation. According to FONTANELI (2012), tiller's cut or defoliation activates the buds below due to an interruption and redirection in the assimilates supply to the reproductive structures.

However, the germination and vigor tests, except for the first germination count, indicated no variation in the physiological quality of the seed even when the pasture was submitted to up to three defoliations (Table 2). Seeds from different treatments had an average of 94% viability and an average of 89% germination. It was expected that plants subjected to defoliation during the full reproductive period had lower viability and germination, as already described by MEDEIROS & NABINGER (2001) on a common ryegrass cultivar. However, the values were numerically higher than the other treatments, 92% and 96% for viability and germination, respectively. For germination, this value is above the identity and quality parameters required by the Ministry of Agriculture for the commercialization of ryegrass seed (MAPA, 2016).

For the vigor tests (Table 2), only the first germination count was sensitive to the advance of the number of cuts (at four days after sowing). The plants with zero defoliation treatment and those submitted to only one defoliation showed similar values and higher than the others. The plants submitted to three defoliation originated seeds with a lower germination percentage in the first count. RODOLFO et al. (2017) claimed similar results, as they reported variations in wheat seeds' response to accelerated aging when plants were subjected to

Defoliation			Seed Quality (%)		
	Germination	FGC	Acc. Aging	Tetrazolium	Field Emerg.
Zero	86 a	14 a	84 a	94 a	79 a
One	88 a	14 a	81 a	94 a	74 a
Two	88 a	11 b	84 a	92 a	79 a
Three	92 a	9 c	81 a	96 a	78 a
CV (%)	6.5	9.6	4.6	2.3	4.6

Table 2 - Germination, first germination count (FGC), accelerated aging, tetrazolium, and field emergence of annual ryegrass managed under different defoliation frequencies. UFPel/EMBRAPA, (2011).

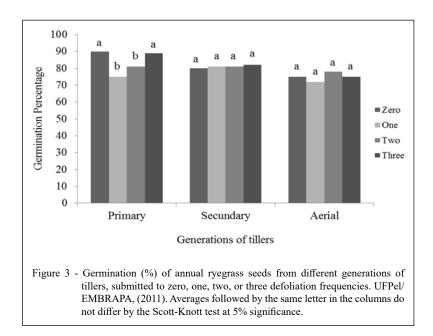
Averages followed by the same letter in the columns do not differ by the Scott-Knott test at 5% significance.

three defoliations, suggesting an adverse treatment effect on vigor rather than seed germination.

When submitted to vigor tests, the seeds, originating from all treatments, such as accelerated aging (high temperature and humidity), presented similar germination. Under the vigor test that demonstrates higher practical application, field emergence, results were also similar for all treatments. The similar weight of seeds produced in all treatments (between 2 and 2.2 g) (Table 1), in agreement with the adequate weight of ryegrass seed according to BRASIL (2009), was another indication of the high quality of produced seeds independently defoliation management.

The similarity of the seed physiological quality can be better explained by analyzing the seed produced in the different generations of tillers (Figure 3). Seeds from aerial tillers have good physiological quality and do not differ in the applied defoliation treatment and secondary tillers. Conversely, the germination percentage of seeds from the main tillers is higher in plants without defoliation and those submitted to three defoliations.

It was verified, regardless of the defoliation management, a high proportion of aerial tillers in this cultivar. RODOLFO et al. (2017) also suggested that there is a different adaptation response for each cultivar about the seed physiological quality after the



Ciência Rural, v.52, n.6, 2022.

defoliation action, which explains the fact that some studies present contradictory results. The assertion that ryegrass seeds of cultivar BRS Ponteio produced by plants subjected to three defoliations have high quality can be confirmed by the results of the variables obtained in the field seedling germination experiment carried out in the following year. These tests are necessary because those performed in the laboratory need to be validated in the field. Only then will be enough to provide helpful information for seed quality control. Although, the first germination count was different, this was not replicated for the seedbed, as the seedlings developed with equal vigor. There were no differences in structural characteristics and dry matter (Table 3 and 4).

The initial performance evaluations of the plants conducted in the following year by seeds originating from plants submitted to zero, one, two, and three defoliations were similar. Regardless of the number of defoliations imposed in the seedbed in the previous year, up to 60 days after sowing, the plants displayed the same leaf appearance rates (average value), phyllochron (average value) and leaf expansion rate (average value). At the end of the establishment period, the plants had the same number of live leaves (average value), the same final length of live leaf fraction (average value), and seedling dry matter yield (average value). Therefore, seedling originating from seeds of plants submitted to two defoliations developed more tillers, but the forage masses produced were similar amongst different defoliation treatments.

In this sense, considering the yield components and the quality of the seed produced, the execution of two defoliations becomes more attractive due to the more significant number of tillers plant⁻¹, forage harvest, and seed production (CUNHA et al., 2016), relating treatments with fewer defoliations. The imposition of three defoliations becomes attractive, especially when the main objectives are forage harvesting and natural reseeding. According to MAIA (2002), of the total annual production of ryegrass seeds, only 17% will be present in the Soil Seed Bank (SSB) at the end of March. Thus, with the execution of three defoliations, there was a harvest of 234 kg ha⁻¹ of seeds. Therefore, there was undoubtedly some loss, and the amount in the soil was higher than as performing two defoliations. However, considering only this production and germination of 92%, there would be at least 215 kg ha⁻¹ of seeds deposited in the soil; According to MAIA (2002), 37 kg ha⁻¹ of highquality seed at the end of March in the SSB would be enjoyable as the recommended sowing density for ryegrass is 20 kg ha⁻¹. In the same experimental area of the current study, BOHN et al. (2020) verified that after the ryegrass productive cycle and subsequent soybean cultivation, a ryegrass mass of approximately 1,500 kg ha⁻¹ from the ryegrass seed bank submitted to three defoliations in the previous year.

Differently from what was observed for physiological quality, there was an effect of defoliation management on the sanitary quality of the seed produced (Table 5). The average pathogenic species incidence percentage, such as *Alternaria* sp. (29.9), *Epicoccum* sp. (18.8), *Cladosporium* sp. (10.1), and *Dreshslera* spp. (10.4) were the ones with the highest values, considering all defoliation managements. However, only the latter is considered potentially pathogenic for ryegrass seeds (LUCCA-FILHO et al., 1999). The other pathogens had an incidence always lower than 5%, regardless of the defoliation management performed. Fungi of the genus *Penicillium* sp., which are storage pathogens, were not reported in seeds. MENEGAES et al. (2019)

Table 3 - Tillers m² (T m²), live leaf number (LLN), the final length of the live leaf fraction (FLLLF), and dry matter (DM) of seedlings originating from annual ryegrass seeds submitted to field emergence. UFPel/EMBRAPA, (2011).

Defoliation	T m ²	LLN	FLLLF	DM
Zero	1905.12 b	2.33 a	33.09 a	0.15 a
One	1600.98 b	3.01 a	34.12 a	0.14 a
Two	2497.71 a	2.16 a	28.48 a	0.16 a
Three	1784.96 b	2.25 a	29.24 a	0.16 a
CV (%)	15.4	17.1	18.1	18.8

Averages followed by the same letter in the columns do not differ by the Scott-Knott test at 5% significance.

Table 4 - Leaf appearance rate (LAR), phyllochron (PIL), leaf expansion rate (LER), leaf senescence rate (LSR), stem appearance rate (SAR), tillering rate (TR) and leaf life duration (LLD) of seedlings originated from annual ryegrass seeds submitted to field emergence. UFPel/EMBRAPA, (2011).

Defoliation	LAR	PIL	LER	LSR	SAR	TR	LLD
Zero	0.08 a	24.73 a	0.73 a	0.14 a	0.04 a	0.05 b	80.47 a
One	0.09 a	18.67 a	0.72 a	0.14 a	0.03 a	0.05 b	67.72 a
Two	0.09 a	25.41 a	0.68 a	0.12 a	0.04 a	0.07 a	83.22 a
Three	0.08 a	22.54 a	0.69 a	0.11 a	0.04 a	0.05 b	76.22 a
CV (%)	4.8	16.5	9.1	10.3	16.2	5.6	12.1

Averages followed by the same letter in the columns do not differ by the Scott-Knott test at 5% significance.

stated that if seeds are harvested at the right time, with maximum physiological quality, and stored in an adequate low humidity environment, there will be no problems related to germination and vigor after the storage period, reducing the incidence of field fungi like *Fusarium* spp.

The pathogens incidence increased among the treatments as increasing the number of defoliations, but this did not occur regularly for all treatments. For example, the zero defoliation treatment had a total incidence of 4.7%, for one defoliation (6.6%), two defoliations 99.9%, and with three defoliations 14.2%. However, considering only the fungi potentially pathogenic to ryegrass, only *Curvularia* sp. and *Bipolaris* sp. followed this trend. *Colletotrichum* sp., *Fusarium* sp., *Phoma* sp. and *Dreshslera* spp. presented lower average incidence in plants subjected to three defoliations than in other treatments (Table 5). However, seeds from plants subjected to three defoliations have a higher incidence of non-pathogenic fungi for ryegrass and in plants subjected to zero defoliation or minimally defoliated treatment. Those that decreased their incidence with the progression of defoliation did not affect germination and establishment of ryegrass seedlings.

			Defoliation	
Fungi	Zero	One	Two	Three
Dreshslera spp.*	6.4	8.6	20.0	7.4
Phoma sp.*	4.4	4.9	4.4	4.0
<i>Bipolaris</i> sp.*	0.3	0.8	0.7	1.3
Fusarium spp.*	2.0	1.3	0.8	0.3
Colletotrichum sp.*	0.8	4.9	3.8	0.9
Alternaria sp.	20.4	24.6	30.0	44.9
Curvularia sp.*	0.6	1.1	1.0	2.3
Epicoccum sp.	4.1	8.8	19.4	43.1
Cladosporium sp.	3.8	2.9	9.5	24.0
Average	4.7	6.6	9.9	14.2

Table 5 - Percentage of incidence of different species of fungi in annual ryegrass seeds from plants subjected to different defoliation frequencies. UFPel/EMBRAPA, (2011).

*Fungi considered potentially pathogenic in annual ryegrass seeds (Lolium multiflorum Lam.) according to LUCCA-FILHO et al. (1999).

8

CONCLUSION

Ryegrass plants subjected to up to three defoliations produce seeds of high physiological quality. The high proportion of aerial tillers emitted by cultivar BRS Ponteio and the seeds' quality produced by them determine the equality in the physiological and sanitary quality of the seeds. Furthermore, the study also observed a lower average incidence of potentially pathogenic fungi such as *Colletotrichum* sp., *Fusarium* sp., *Phoma sp.*, and *Dreshslera* spp. in ryegrass plants defoliated three times.

ACKNOWLEDGEMENTS

The T.R.T. wa spartial funded by the Brazilian Federal Agencies: "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES), Brazil – Finance Code 001, and by the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), Brazil.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

REFERENCES

BOHN, A. et al. Nitrogen fertilization of self-seeding Italian ryegrass: effects on plant structure, forage and seed yield. **Ciência Rural**, Santa Maria, v.50, n.6, e20190510, 2020. Epub 04-Jun-2020. Available from: https://www.scielo.br/pdf/cr/v50n6/1678-4596-cr-50-06-e20190510.pdf>. Accessed: Jun. 22, 2020. doi: 10.1590/0103-8478cr20190510.

BOLKE, D. R. et al. Production of annual ryegrass with different doses of nitrogen fertilization in topdressing. **Semina: Ciências Agrárias**, Londrina, v.40, n.3, p.1329-1338, jun. 2019. Available from: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/207926/1/SeminaCiAg-Mittelmann-Production.pdf. Accessed: Jan. 12, 2020. doi: 10.5433/1679-0359.2019v40n3p1329.

BRASIL. **Regras para análise de sementes**. 1 ed. Ministério da Agricultura, Pecuária e Abastecimento: Secretaria de Defesa Agropecuária, 2009. 399p.

CANDIDO, M. J. D. et al. Biomass flow in tanzaniagrass pasture under three resting periods grazed by sheep. Revista **Brasileira de Zootecnia**, Viçosa, v.35, n.6, p.2234-2242, jul. 2006. Available from: https://www.scielo.br/pdf/rbz/v35n6/06.pdf. Accessed: May, 12, 2020. doi: 10.1590/S1516-35982006000800006. CANTERI, M. G. et al. SASM-AGRI: system for analysis and mean separation in agricultural assays using Scott-Knott, Tuney and Duncan methods. **Revista Brasileira de Agrocomputação**, v.1, n.2, p.18-24, 2001. Available from: http://www.agrocomputação, v.1, Accessed: Feb. 14, 2011.

CARRÉRE, P. et al. Tissue tumover within grass-clover mixed swards grazed by sheep. Methodology for calculating growth, senescence and intake fluxes. **Journal of Applied Ecology**, Ferrand, v.34, p.333-348, 1997. Available from: https://www.jstor.org/preview-page/10.2307/2404880?seq=1. Accessed: May, 12, 2020.doi: 10.2307/2404880.

CASSOL, L. C. et al. Yield and structural composition of oat and ryegrass subjected to differentperiods of cutting and nitrogen fertilization. **Revista Ceres**, Viçosa, v.58, n.4, p.438-443, jul. 2011. Available from: https://www.scielo.br/pdf/rceres/v58n4/ a06v58n4.pdf>. Accessed: Dec. 19, 2019. doi: 10.1590/S0034-737X2011000400006.

CONFORTIN, A. C. C. et al. Morphogenesis and structure of Italian ryegrass submitted to three grazing intensities. Acta Scientiarum. Animal Sciences, Maringá, v.32, n.4, p.385-391, ago. 2010. Available from: https://www.redalyc.org/pdf/3031/303126502004.pdf>. Accessed: Dec. 12, 2019. doi: 10.4025/actascianimsci.v32i4.8657.

CUNHA, R. P. et al. Relationship between the morphogenesis of Italian ryegrass cv. 'BRS Ponteio' with forage and seed production. **Ciência Rural**,Santa Maria, v.46, n.1, p.53-59, jan. 2016. Available from: https://www.scielo.br/scielo.php?script=sci_artt ext&pid=S0103-84782016000100053>. Accessed: Oct. 18, 2019. doi: 10.1590/0103-8478cr20150296.

EICHELBERGER, L. et al.Drying delay effect on physiological quality of stored annual ryegrass seeds. **Revista Agropecuária Brasileira**, Brasília, v.22, n.2, p.268-278, maio 2003. Available from: https://www.scielo.br/pdf/pab/v38n5/18178.pdf. Accessed: Oct. 19, 2019. doi: 10.1590/S0100-204X2003000500013.

FONTANELI, R. S. Qualidade e Valor Nutritivo de Forragem. In: Forrageiras para ILPF - integração lavoura pecuária-floresta na região Sul-brasileira. Brasília: Embrapa Trigo, 2012. Cap. 1, p. 27-129.

HEINECK, G. C. et al. Relationships and influence of yield components on spaced-plant and sward seed yield in perennial ryegrass. **Grass and Forage Science**, v.00, p.1-14, jul. 2020. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/gfs.12499. Accessed: Nov. 11, 2020. doi: 10.1111/gfs.12499.

LUCCA-FILHO, O. A. et al.Fungi in annual-reygrass (*Lolium multiflorum* Lam.) seeds and their effects on pasture stablishment. **Revista Brasileira de Sementes**, Londrina, vol. 21, n. 2, p. 142-147, dez. 1999. Available from: https://docplayer.com.br/30503752-Fungos-em-sementes-de-azevem-anual-lolium-multiflorum-lam-e-seus-effeitos-no-establecimento-da-pastagem-1.html. Accessed: Jan. 29, 2020.

MAIA, F. C. Padrões de variação do banco de sementes do solo em função dos fatores edáficos e da vegetação de um campo natural. 2002. 186f. Dissertação (Mestrado em Ecologia) – Programa de Pós-graduação em Ecologia, Universidade Federal do Rio Grande do Sul.

MAPA - Ministério da Agricultura Pecuária e Abastecimento. Instrução normativa no – 44, Brasília, n. 230, p.8-11, 2016. Available from: http://www.gov.br/agricultura/pt-br>. Accessed: Nov.12, 2020.

MEDEIROS, R. B.; NABINGER, C. Annual ryegrass seeds and forage yield responses to nitrogen levels and clipping regimes. **Revista Brasileira de Sementes**, Londrina, v.23, n.2, p.245-254, 2001. Available from: https://www.scielo.br/scielo.php?script=sci_nlinks&ref=000080&pid=S0103-8478200800030003200011&lng=pt. Accessed: Jan. 21, 2020. doi: 10.17801/0101-3122/rbs.v23n2p245-254.

MENEGAES, J. F. et al. Physiological and sanitary quality of cockscomb seeds stored for different periods. **Ornamental Horticulture**, Viçosa, v.25, n.1, p.34-41, abr. 2019. Available from: https://www.scielo.br/pdf/oh/v25n1/2447-536X-oh-25-01-0034. pdf>. Accessed from: Jan. 12, 2020. doi: 10.14295/ohv25i1.1228.

MÜLLER, L. et al. Pearson's simple and canonical correlations between components of forage dry matter and seeds of ryegrass. **Revista Brasileira de Sementes**, Londrina, v.34, n.1, p.86-93, jun. 2012. Available from: https://www.scielo.br/pdf/rbs/v34n1/allv34n1.pdf>. Accessed: Mar. 15, 2020. doi: 10.1590/S0101-31222012000100011.

PASLAUSKI, B. M. da C. et al. Productions and physiological seeds quality of ryegrass submited to cuts and harvest times. **Revista Trópica: Ciências Agrárias e Biológicas**, Chapadinha, v.09, n.1, p.01-13, 2014. Available from: http://www.periodicoseletronicos.

ufma.br/index.php/ccaatropica/article/view/1142/2775>. Accessed: Jan. 19, 2020.

PEREIRA, M. J. R. et al.Morphoagronomic characteristics of maize in response to different levels of defoliation. **Revista Ceres**, Viçosa, v.59, n.2, p.200-205, mar. 2012. Available from: https://www.scielo.br/scielo.php?script=sci_arttext&pid=S0034-737X2012000200008>. Accessed: Jan. 19, 2020. doi: 10.1590/S0034-737X2012000200008.

PIESANTI, S. R. Rendimento e qualidade fisiológica de sementes de azevém em distintas épocas de colheita. 2019. 60f. Dissertação (Mestrado em Agronomia) – Curso de Ciência e Tecnologia de Sementes, Universidade Federal de Pelotas.

RODOLFO, G. R. et al. Physiological quality of dual-purpose wheat seeds from plants subjected to artificial defoliation. **Ciência Rural**, Santa Maria, v.47, n.1, e20151582, 2017. Epub 10-Nov-2016. Available from: https://www.scielo.br/pdf/cr/v47n1/1678-4596-cr-47-01-20151582.pdf>. Accessed: Jan. 19, 2020. doi: 10.1590/0103-8478cr20151582.

SANTOS, H.G. et al. Sistema Brasileiro de Classificação de Solos. Brasília: Embrapa Solos, 2018. 356p.

SILVA. A. E. L. et al. Identification and quantification of fungi associated with seeds of ryegrass (*Lolium multiflorum* Lam.). Summa Phytopathologica, v.40, n.2, p.156-162, jun. 2014. Available from: https://www.scielo.br/pdf/sp/v40n2/v40n2a08. pdf>. Accessed: MaY, 15, 2019. doi: 10.1590/0100-5405/1935.