Structural dynamic of the annual ryegrass from defoliation to seeds harvest[1]

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ABSTRACT

The aerial part distribution and arrangement can significantly affect the forage resource's use, both for the leaves and seeds harvesting. This work aimed to verify the structure derived from different times of pasture use and the subsequent seeds production in a long-cycle ryegrass cultivar. Weekly assessments of number and length of living leaves, elongation of the internodes, tillering, plants' height, structural components (leaf, stem, flower, and dead material), seeds yields components, and seeds yield, to determine the pasture structure along its production cycle. The ryegrass cv BRS Ponteio pasture had its structure significantly modified just after 150 days (thermal sum: 1303 degrees-days) from seeding without the defoliation. Defoliation during the vegetative period did not alter the plants' structure until the seeds' production. Defoliation just after the beginning of the internodes' elongation caused a significant tillering growth, which maintained the high seeds production potential, without plants lodging, besides promoting a more significant leaves harvesting. Subsequent pasture use determined predominant stems and flower harvesting, compared to the leaves, and the significant reduction of the production seeds and potential seeds production.

Keywords: life leaves; *Lolium multiflorum*; tillering; forage.

INTRODUCTION

The defoliation performance as living leaves are predominant in the pasture allows the entrance of high-quality radiation at the plant base, includes more tillers to the canopy, and replaces the leaves blades, besides allowing the harvesting of high-quality forage crops. This form of defoliation avoids the excess of senescence at the plant's base and the excessive internodes' elongation (Iqbal *et al.*, 2017). The excessive internodes elongation affects the pastureland structure, most especially to determine the distancing among the leaves, which hampers the animals' grazing (Souza *et al.*, 2012). After defoliation, the residual structure is also hampered by the defoliation delay, as fewer living leaves remain to ease the regrowth (Hinman & Fridley, 2020). Even the same, as ryegrass is an annual plant, independent from the defoliation, internodes' elongation occurs (Soares *et al.*, 2019). Defoliations after the internodes elongations, besides determining lower leaf blades at harvesting, also determines the tillers' death compromising, as a consequence, the seeds' yield. The leaves length per tiller and the tiller length have a strict relationship with the seeds' yield. Larger tillers with larger leaves produce more prominent ears, with more spikelets and heavier seeds

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(Tshikunde *et al.*, 2019). If defoliation causes the death of few tillers, and after that, there are favorable temperatures, radiation, and nutrients, intense tillering will occur. These tillers are smaller than the previous, but their higher number, even the smaller size, increases the seeds' yield. The final structure of these tillers is crucial for the high seed yield. Even the same, the over-usage of the pastureland along with the time (until flowering) may jeopardize the canopy structure, mainly as it refers to the seeds production for the excessive tillers death, lower plant's nutritional reserves, and reduced time to create a new structure, allowing the high production potential of the seeds.

In this sense, this work aimed verify the structure derived from different times of pasture use and the subsequent seeds production in a long-cycle ryegrass cultivar BRS Ponteio, a cultivar obtained from the selection of native plants of the Brazilian South that is highly adapted to this forage environment.

MATERIAL AND METHODS

The experiment was performed in an experimental area in the municipality of Capão do Leão, State of Rio Grande do Sul, Brazil (31°80'S e 52°40'W), at an altitude of 13 m above the sea level), belonging to the Brazilian Enterprise for Agricultural Research, EMBRAPA Clima Temperado/ Estação Terras Baixas. The experiment was implemented using the Cultivar BRS Ponteio of annual ryegrass. The seeding was performed on lines, and the seeding density was 25 kg.ha^{-1} of pure viable seeds, spaced 20 cm one each other. The experimental area soil is classified as haplic eutrophic dystrophic planosol, which was submitted to the conventional preparation (plowing and two rackings). According to the Manuring and Liming Manual for the states of Rio Grande do Sul and Santa Catarina (Tedesco *et al.*, 2004), soil analysis was performed to perform the acidity correction and the basic manuring for cold-season grasses. Urea was used as topdressing nitrogen fertilization (45% Nitrogen), divided into two rounds: the first (50 kg.ha^{-1} nitrogen) was performed on the 6th June, at the tillering beginning; the latter was performed on the 23th September due to the soil temperature and humidity, favorable for the forage crop development and growth. The 1536 m^2 experimental area was divided into 16 parcels, 88 m^2 each. A 2 meters-wide trail was created among the eight parcels. The experiment included four treatments arranged in a randomized-block experimental design with four repetitions.

The following treatments were used to assess the defoliation frequency factor: D0 = without defoliation; D1 = one defoliation performed as the forage mass reached approximatively 1500 kgDM.ha^{-1}. The plants had a mean 14 cm height and a thermal sum of 864 degrees-day, on the 8th August (vegetative period). D2 = two defoliations, performed at a thermal sum of 420 degrees-day after the first defoliation, as the plants reached about 20 cm, on 23th September (pre-flowering period). D3 = three defoliations, performed with a thermal accumulation of 357 degrees-day, after the second, as the plants reached about 35 cm height on 21^{rst} October (flowering period). After the defoliation, the plants were preserved at half the height they reached before the defoliation. After defoliation, the plants' height was 7, 10, and 15 cm for the first, second, and third defoliation, respectively. These conditions were preserved to leave an adequate residual of living leaves after the defoliations and, as a consequence, foster the regrowth. In addition, 0.25 m² samples were collected at each defoliation to separate the sampled material (leaf blades, leaf sheath and stem, flowers, and senescent material) morphologically.

The daily thermal accumulation was calculated by the equation: $[(toMx + toMn) / 2] – 5$, being: t^oMx = maximum temperature, t^oMn = minimum temperature and 5 °C = Basal temperature (Tb) for the annual ryegrass (Confortin *et al.*, 2010). The meteorological data of the experimental period were sampled at the agro-meteorological Station of the Pelotas Federal University, in the municipality of Capão do Leão.

At 45 days after seeding, the structural features were measured according to the "marked tillers" technique (Carrère *et al.,* 1997). Eight tillers were marked with a colored floss in each parcel, representing the pastureland. Each assessment analyzed the mature and growing leaf blades (with visible ligule), and their condition (with or without senescence). The completely extended leaves were measured from the ligule, as long as the growing leaves were measured from the last visible ligule (Davies *et al.,* 1993). Weekly records of the height of the last exposed ligule from the ground height and the tillers numbers per plant were taken. Seventeen observation cycles were performed every 7 or 8 days. After each defoliation, the vegetative units' marking was repeated, including new individuals, to preserve the population representativity.

Days before the seeds' harvesting, plats' population assessments were performed by the plants' number counting (four samples of 50 linear centimeters per parcel). Eight marked plants and two random plants were sampled to determine the yield components, summing ten plants, assessing the tillers' number per plant, fertile tillers, tillers' length, ears' length, spikelets' number per ear, number of seeds on each spikelet, and final yield.

The seeds' harvesting maturity was determined by daily water content monitoring. The ideal moment was defined as the seeds displayed approximately 35% humidity. The daily humidity analyses of the different treatments were performed by the 105 °C oven system (Brasil, 2009). As obtained the desired seeds' humidity, the harvesting was performed by eight samples per parcel (0.25 m²), close to the ground. The first 12 hours' drying was performed on the shadow on a concrete floor, being after that dried in a forced-air circulation stove (30 °C temperature) until the seeds reached 10% and 13% water content, according to Maia (1995). All the drying process was performed with the seeds still into the spikelets. Then, the samples were carried to the forage crop seeds laboratory in the Plant Science department of the Agronomy Faculty Eliseu Maciel (UFPel), where they were manually threshed using sieves and packaged (paper bags) in controlled temperature (15 °C) and humidity (50%) chamber.

The final seeds' humidity was determined by the stove method (105 °C for 24 hours) using 4-5 grams of seeds with two sub-samples (Brasil, 2009).

Statistical analyses were performed with the program SASM-Agri (Canteri *et al.*, 2001) by means comparison using the Tukey test at 5% error probability. The data were subsequently submitted to polynomial regression analysis to describe the variable as a function of time with the same level of significance cited above with the use of the "Statistical Analysis System for Windows - Winstat" Version 1.0 (Machado & Conceição, 2003).

RESULTS AND DISCUSSION

Fifty days after seeding (500 DD - 18th June), the plants had, on average, five leaves, with a mean length of seven centimeters. At this moment began the tillering phase with 470 tillers.m^2 (Figure 1a) when the topdressing nitrogen fertilization was performed. After this, under low temperature and high water availability (Table 1), the tillers density evolved slowly until the first defoliation (100 DAS and 864DD) in a quadratic form, so that, before the cuttings, it displayed about 700 tillers.m^2. The unfavorable water condition between the tillering beginning and the first defoliation can be highlighted by the high amount of roots above the soil level. This roots' arrangement is probably an adaptative strategy of the plant facing water stress. Besides the superficial roots' presence, the reduction of living leaves, from five to three (100 DAE), suggested unfavorable environmental conditions (Figure 1b). On the other side, the new leaves, closer to the medial insertion of the tiller, were wider, which increased the leaf blades' layer thickness (10 cm) at the pre-cutting moment (Figure 1c).

Table 1: Mean minimum temperature (MmT), mean maximum temperature (MMT), mean temperature (MT), total rainfall (TR), mean radiation (MR), thermal sum (TS), and number of frosts (NF) during the experimental period

Period	**MmT (°C)**	**MMT (°C)**	**MT (°C)**	**TR (mm)**	**MR (cal.cm^2)**	**TS (degrees. day^{-1})**	**NF**
April 29th	14.1	30.4	18.5	6.5	218.0	15.8	0
May	11.1	27.2	15.0	118.3	240.0	342.7	3
June	8.7	17.7	12.4	116.2	182.3	245.6	7
July	7.5	16.2	11.2	71.0	183.1	213.8	3
August	9.2	17.0	12.5	114.2	231.5	250.1	6
September	10.1	30.2	14.4	75.1	331.8	301.2	0
October	13.5	22.1	17.4	75.9	349.1	396.6	0
November 18th	15.1	25.3	19.8	23.7	545.2	456.7	0
Total	11.2	23.3	15.1	600.9	2281.0	2222.5	18

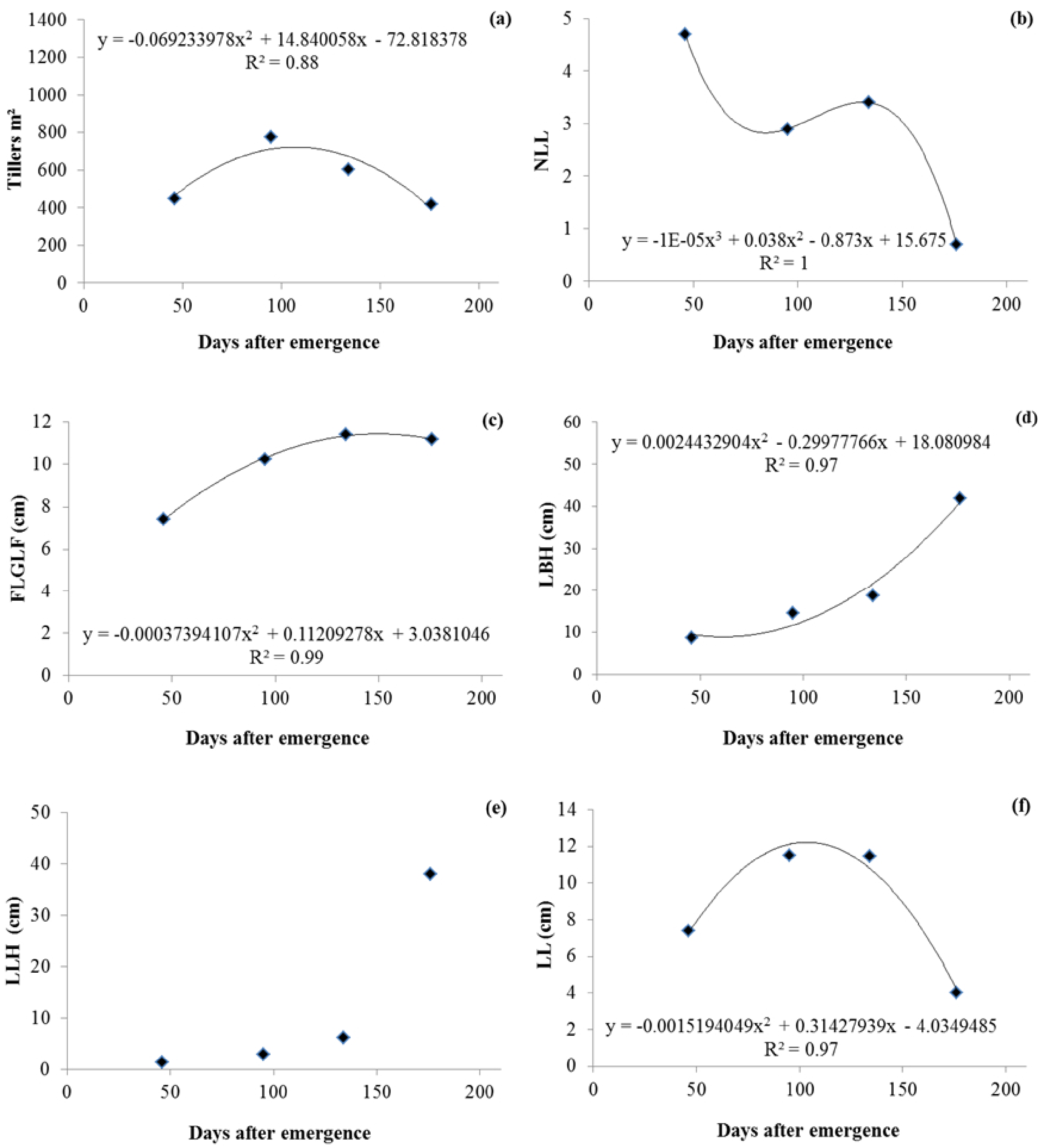

Figure 1: Tiller per square meter, number of living leaves (NLL), final length of the green leaves' fraction (FLGLF), last blade height (LBH), last ligule height (LLH) and leaves' layer (LL) of plants without defoliations.

The last ligule height (of the pseudo-stems) was insignificant until the first cutting, which induced a slight evolution of the plant's height (Figure 1e; Figure 1d, respectively), verified by the first-degree (negative), and second degree, very close to zero angular coefficients.

The definition of the first cutting best moment occurred due to the total soil cover by live leaves, which hindered it almost wholly. Under these conditions, it is recommended that the plant is adequately fixed to the ground, with a minimum reduction of the living leaves (Costa *et al.*, 2004). In the pre-cutting moment (101 days and 864 DD after seeding), the ryegrass plants displayed three living leaves with the mean 10 cm length, 700 tillers.m^{-2}, with 14 cm height at the last leaf blade, and with almost no internodes elongation. Even after 100 days after seeding, the reduced elongation of the internodes is a typical feature of long-cycle cultivars (Cunha *et al.*, 2016) such as those of the present study. Thus, the pre-cutting condition was quite favorable for forage crop harvesting. The 10 cm layer composed exclusively of leaf blades (distance between the height of the last ligule and that of the last blade) promoted just the harvesting of this structural component (Figure 1f).

At the first cutting, there was a significant reduction of the leaf blades layer (50% - from 10 to 5 cm) (Figure 2f) and plants' height (14 to 10 cm) (Figure 2d), as displayed, respectively. After the cutting, both the height and leaf blades layer evolution was intense. The leaves length increased by a higher first-degree angular coefficient (0.45) than displayed in the treatment without defoliation (0.11). The last blade height altered the quadratic advance of the control to linear, with a higher first-degree angular coefficient than the defoliated groups. These differences probably occurred due to a compensatory growth after the cutting and for the shorter route trodden by the new leaves until their appearance (Martins *et al.*, 2019). The environmental improvement during August and, most significantly, September (water availability, temperature, and radiation) improved the plants' structure (Abib, 2015) so that the remaining structural variables were almost unaffected by the cutting. In this form, both control plants, and those that received the cuttings, displayed very similar structures until the end of the experiment.

In disagreement with the period until the first cutting (101 days and 864 DD), in the period until the second cutting (147 days and 1284 DD), the living leaves number remained stable, most especially in the control treatment (Figure 1b). Soon after the first defoliation, there was a slight number of living leaves elevation (Figure 2b), but after that, there was a linear decrease of this number until the end of the cycle, reducing one living leaf every 27 days (Figure 3b). During this period, the NLL of the control decreased at a similar rate.

The tillering and final length of the green leaves fraction were similar in the plants not submitted to defoliation and in those submitted to one defoliation that obtained an average of 750 tillers.m^{-2} among avaluations (Figure 2a) and of 12 cm green leaves fraction at 150 DAE (Figure 2c). The defoliation before the elongation of the internodes, preserving a 7 cm residual, and the proper light environment soon after the cutting determined almost all the tillers' survival (Souza *et al.*, 2019).

Living leaves length displayed values proximate to the maximum ones observed along the phenological cycle, both in control and in those plants defoliated once (Figure 3c), approximately 150 days after seeding. Even if both models are quadratic for this variable, after defoliation, the leaves extended their length at a higher rate along the time, highlighted by the angular coefficient (0.1121 for the control and 0.45 for the treatment cut once).

Approximately 150 days after seeding, the last ligule height began displaying higher values, suggesting the beginning of the internode elongation (Figure 2e; Figure 3e). As a consequence of this event, the plants' height was about 20 cm at the second cut performance.

Even the significant leaves loss until the moment of the second cut, compared to the first, defoliation can be performed at 150 days after seeding to obtain a higher forage harvest with only one cutting, without impairment of the remaining structural features. Basically, the long period until the significant internodes' elongation of this cultivar allows this kind of handling.

The second cutting allowed harvesting most of the leaves (leaves blades layer close to 10 cm), but with the first flowers' appearance and significant stems' harvesting. The second defoliation determined the reduction in the residual's number of leaves, leaves size, beheading of a significant amount of main tillers, and, consequently, elimination of the apical dominance (Kebrom, 2017). Nitrogen topdressing fertilization associated with the environmental improvement after the cutting caused a high tillering (Bohn *et al.*, 2020), 75% higher than that verified in the one-cut treatment and control. A quadratic model with a maximum point at 1268 tillers.m^2 represented, at best, the tillering evolution after the second cutting (Figure 3a). This value is higher than the highest tillering observed in free-growing plants and those defoliated just once, which displayed maximum tillering between 700 and 800 tillers.m^2.

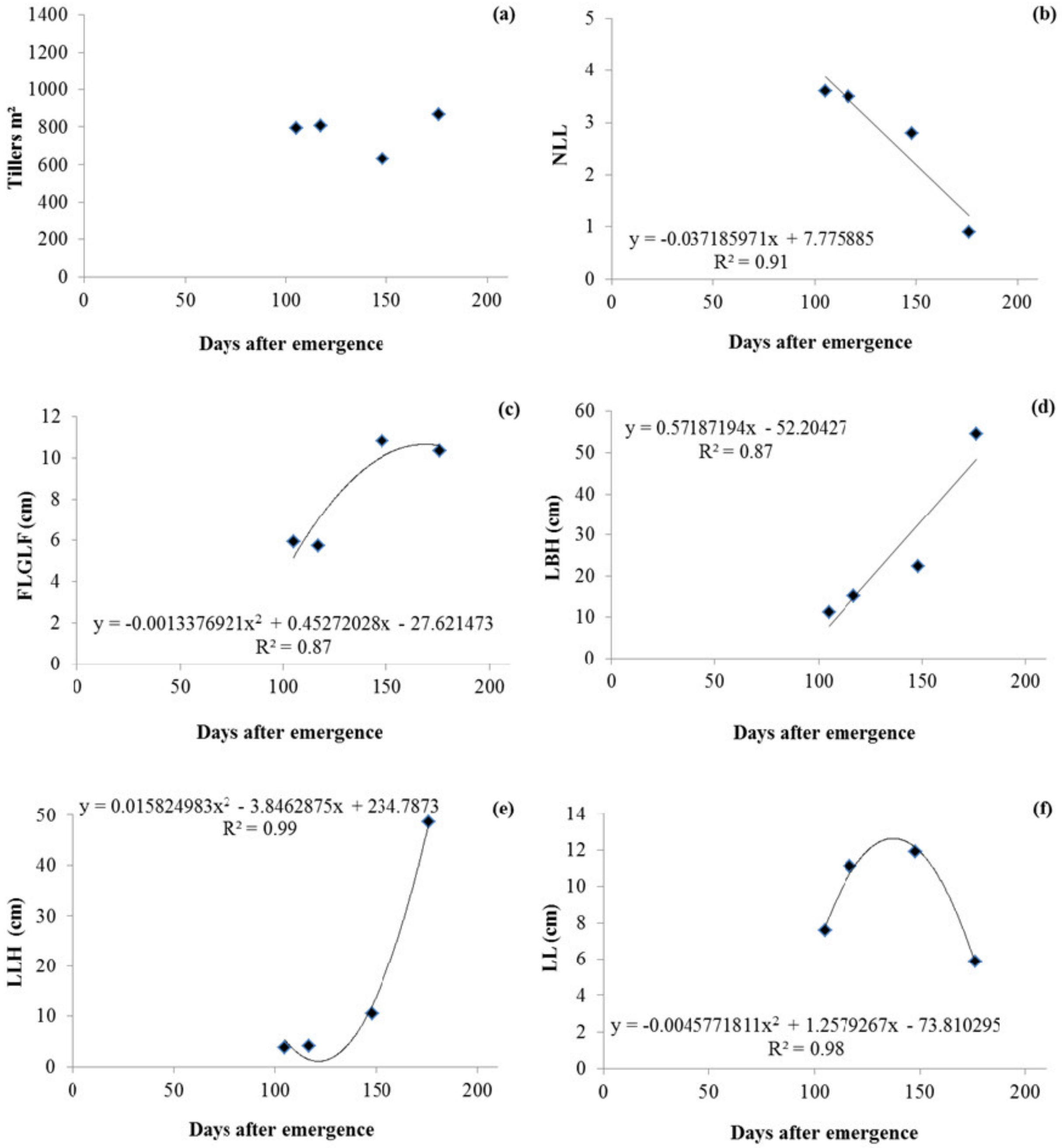

Figure 2: Tiller per square meter, number of living leaves (NLL), final length of the green leaves' fraction (FLGLF), last blade height (LBH), last ligule height (LLH) and leaves' layer (LL) of plants with one defoliations.

The living leaves evolution after the second cut was quadratic (Figure 3b). The number of living leaves decreased approximately one week after the cutting, similar to verified in the remaining treatments and the maximum point of the curve indicates a reduction about 2 cm in the leaf layer by the action of this defoliation (Figure 3f). The decrease occurred due to the tillers' last leaf expansion before the third defoliation, which entailed just the leaves' mortality, not the new leaves' appearance. Another central element for the lower leaves number is the nutrients' drainage from the leaves to elongate the internodes and the flowers' formation before the third defoliation (Barillot *et al.*, 2016). The internode elongation was very intense after the second cutting, highlighted by the first-degree angular coefficient. Even the same, the maximum point was lower (42 cm) and (33,7 cm) than those observed in free-growing plants and plants defoliated only once (Figure 3d; Figure 3e, respectively).

The maximum leaves length in this period was 10 cm (Figure 3c), but this occurred one week before the third defoliation. At the third defoliation, the leaves length reduced to 8 cm. This reduction occurred because the leaves that precede the flowering have a shorter size than the leaves of the medium tiller insertion (Tshikunde *et al.*, 2019; Barillot *et al.*, 2016). Under the structural aspect, it would be more convenient to defoliate earlier, at the moment of the complete expansion of the last tiller leaf, allowing the harvesting of the maximum number of leaves, with lower requirements of the plant to constitute stem and flowers, which are less relevant structures under the forage point of view (Pedroso *et al.*, 2004). The last leaf expansion occurred 100 DD (one week) after the second cutting. If the defoliation occurred at this moment, the forage harvest would be minimal. For this reason, the defoliation was delayed, mainly due to the pre-determined method of defoliate each 350 DD (Confortin *et al.*, 2010) and to allow a higher forage harvest and enable economically sustainable defoliation.

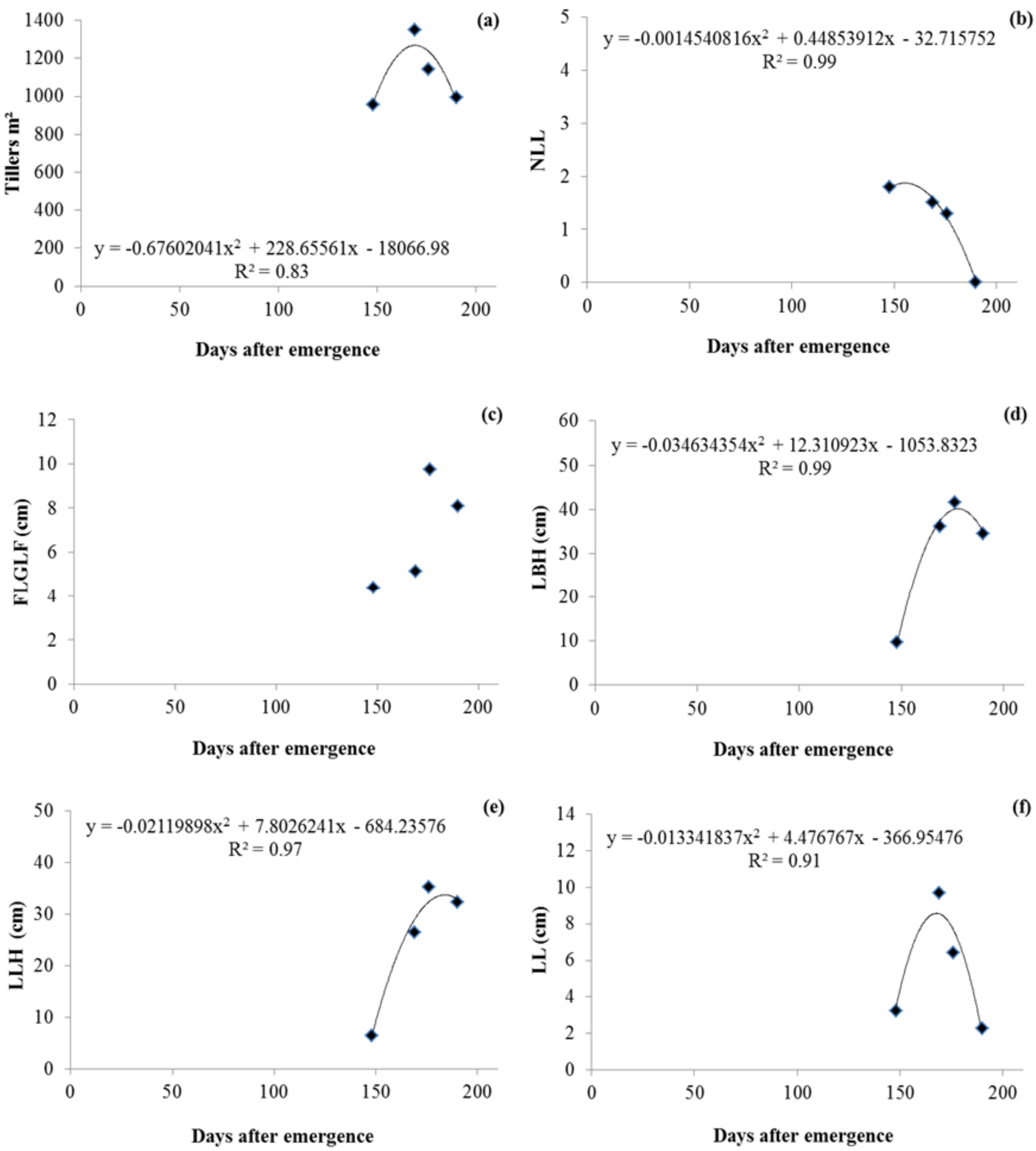

Figure 3: Tiller per square meter, number of living leaves (NLL), final length of the green leaves' fraction (FLGLF), last blade height (LBH), last ligule height (LLH) and leaves' layer (LL) of plants with two defoliations.

At the third defoliation, the pastureland structure was quite different from that of the moments preceding the defoliation, most especially due to the internodes elongation and living leaves' number reduction (Figure 4b). Consequently, stems and flowers prevailed in this third cutting so that the leaves layer was insignificant, as the last ligule belonged to the last blade (Figures 4d; Figure 4e, respectively). Even with an approximately 15 cm residual persistence after the third defoliation to allow an effective regrowth, a mean negative impact was observed, mainly due to the tillers' high mortality. The third defoliation killed approximately 300 tillers.m^{-2}. After the third cutting, the models describing the variables' behavior against time were quite different among the treatments.

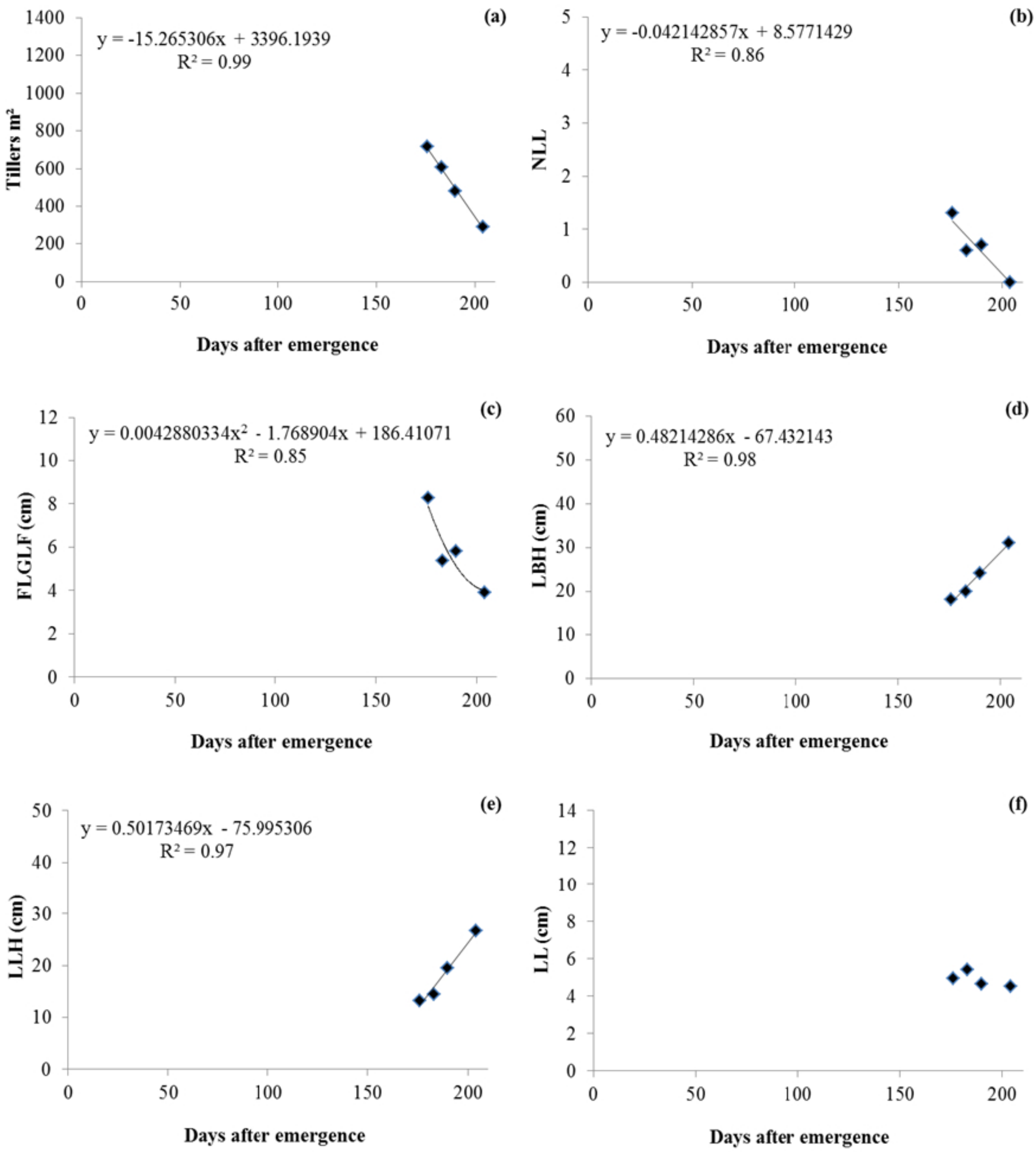

Figure 4: Tiller per square meter, number of living leaves (NLL), final length of the green leaves' fraction (FLGLF), last blade height (LBH), last ligule height (LLH) and leaves' layer (LL) of plants with three defoliations.

The plants submitted to three cuttings had their structures compromised for the following seeds' production. Tillering decreased in a linear form with an accentuated slope (Figure 4a). The same tendency was observed for the number of living leaves and leaves' length (Figure 4b; Figure 4c). The height of the last ligule (of the pseudo-stem) increased linearly as a time function, with a high slope (50 $cm.day^{-1}$ increase). Even the same, as the plants' height quickly stabilized, at the end of the cycle, the height of the plants' last blade was below 30 cm, low developed tillers to produce high quantities of seeds, as the strong relationship between the tillers' length and the seeds production. The leaf layer was smallest found among all treatments (Figure 4f). This modification observed in the plants submitted to three defoliations might probably associate with the flowering induction soon after the defoliation, the low reserve levels in an annual plant, and the main drainage of nutrients from the leaves at the end of the cycle to extend the internodes, produce flowers, and seeds (Barillot *et al.*, 2016).

During the cycle, the first cutting caused a higher tillering of the plants than the control, mainly due to the lower competition for the light in the temperature and soil water availability most favorable moments. The more extended period without cuttings interference produced a higher leaves number, length, and most significantly, an intense internodes elongation so that the free-growing plants and those defoliated only once lodged completely. The second cut significantly modified the plants' structure at the end of the culture cycle, as the cutting reduced the leaves' number, length, and the pseudo-stem length (with the tillers' cutting higher than that observed with one single cut). On the other hand, the second cutting increased the tillers' number, which fostered a denser structure. As a consequence, the plants of this treatment did not lodge.

The aerial tillering was not affected by the number of defoliations, which remained between 30 and 50% of the plant's tillers (Cunha *et al.*, 2022). This structural feature has rarely been discussed earlier in literature for this plant. This characteristic is more visible in annual than in perennial grasses due to some limitation of the base tillers to flower and produce seeds (Durães *et al.*, 2003). It would be a plant's compensation to preserve the high seeds' yields, even if the seeds produced by these tillers display less vigor (Cardoso *et al.*, 2021).

The yields' components remained similar, except for the fertile tillers amount and seeds' number per spikelet. As considering the same seeds' weight in all treatments of 2g to determine the potential yield (Brasil, 2009), the treatment with just one cutting determined the higher seeds' productive potential for (12.360 $kg.ha^{-1}$) due to the high number of fertile tillers and the higher spikelets' amount per ear. On the other side, the higher fertile tillering of plants submitted to two cuts allowed a similar potential yield (10.848 $kg.ha^{-1}$) with a more appropriate structure for the direct harvesting due to the missing plants' lodging. The lower number of fertile tillers observed in control and the plants cut three times determined the lower seeds' production potential. Even the same, as the higher number of spikelet/ear in the treatment that received no cuttings, this displayed a higher productive potential (2.753 $kg.ha^{-1}$) than the treatment submitted to three cuttings (1.668 $kg.ha^{-1}$). These trends reflect in a similar form on the real seeds production (Table 2), most especially for the plants submitted to one or two defoliations.

Table 2: Number of plants $hectare^{-1}$ (Pl/ha), tiller $plant^{-1}$ (T/P), fertile tillers $plant^{-1}$ (FT/Pl), tiller length (TL), spikelets ear^{-1} (S/E), seeds $spikelet^{-1}$ (S/S) and seeds production (SP) of annual ryegrass plants subjected to different defoliation frequencies

Defoliation	Pl/ha (milh.)	T/P	FT/Pl	TL (cm)	S/E	S/S	SP ($kg.ha^{-1}$)
Zero	1.4 a	3.4 b	3.4 a	85 a	16.7 ab	4.7 a	777.7 a
One	1.2 a	4.4 ab	3.7 a	92 a	18.8 a	4.2 a	736.6 a
Two	1.3 a	5.3 ab	4.3 a	71 b	15.4 b	4.1 a	624.5 a
Three	1.3 a	6.2 a	3.3 a	56 c	15.3 b	4.1 a	234.4 b
CV%	11.2	19.4	9.5	7.7	7.9	17.2	18.3

Means followed by the same same letter in the same column do not differ according to Tukey's test at 5% significance.

CONCLUSIONS

The defoliation during the vegetative time increases the seeds production potential of ryegrass cultivar BRS Ponteio. Due to the long cycle of this cultivar, main modifications to the structure occur just 150 days after seeding,

The defoliation performed soon after the internodes' elongation of the main tillers reduces the leaves' length but increases the tillering, preserving the high seeds production potential. According to the present research results, the first flowers' appearance should represent the ideal moment for the last cutting execution, maintaining the half of the pasture height as a residual (lowering from 20 to 10 cm of height of the last blade) to ease both forage and seeds harvest.

The defoliation during the full flowering radically affects the leaf blades harvesting and the plant's structure for the seeds' production. Independently from the defoliation, the cv BRS Ponteio displays a significant proportion of aerial tillers at the moment of the seeds' harvesting.

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We also declare that there are no conflicts of interest.

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