Annual ryegrass managed for reseeding purposes: relationship between heading date and seed production

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Abstract
Annual ryegrass reestablished yearly by allowing natural reseeding is a common practice among Uruguayan farmers. Its success relies on the farmers' ability to balance grazing and seed production during spring. We tested a set of ryegrass varieties covering a wide range of heading dates under defoliation management. Each variety was managed independently according to its phenological stage, resulting in different closing dates. We tested the varieties' ability to produce seeds under such management, the effect of heading date on seed yield components, the resulting amount of straw and its effect on summer soil temperatures. A strong negative association between heading date and most of the seed yield components were found. A negative correlation was also found with the amount of straw that remains after the end of the growing season, and this was associated with higher soils surface temperature peaks during summer. We conclude that early maturing varieties could perform better where reseeding is desired.

Introduction
Annual ryegrass reestablished yearly by allowing natural reseeding is a common practice among Uruguayan farmers, and the success of this practice relies on the farmers' ability to balance grazing and seed production during spring. Cultivar differences in reseeding capacity have been reported (Evers & Nelson, 2000) but information on which plant traits favor the reseeding process and how they interact with management practices is scarce, resulting in no general criteria for selecting among ryegrass cultivars. The plant density resulting from natural reseeding at the beginning of the growing season can be conceptualized as the result of the number of seeds produced at the end of the ryegrass growing season, and the seeds and seedlings persistence through summer. Under grazing, ryegrass seed production, seed yield components, leaf area necessary to fill the grains, and the amount of remnant straw that could protect seeds and seedlings during summer can be negatively impacted depending on grazing intensity and grazing termination date (Young et al., 1996). Heading date is one of the main traits differentiating ryegrass varieties and determines the length of the pasture utilization period, but the interaction with reseeding ability is unknown. Our objectives were to determine whether cultivar differences in heading date interact with seed production and seed yield components under defoliation management, as well as the resulting amount of straw and its effect on summer ground temperatures.

Methods
A set of 18 annual ryegrass cultivars and experimental lines were used, covering a wide range of heading dates from mid-September to early-November (southern hemisphere), and including both diploids and tetraploids (Table 1). The experiment was stablished in April 2021 at Glencoe Research Unit in northern Uruguay (Lat. -32.02°S; Long. -57.16°W). The experimental design was a randomized complete block design with 3 repetitions, and plot sizes of 3 m^{-2} (3m x 1m). Sowing densities were 18 $kg \cdot ha^{-1}$ and 25 $kg \cdot ha^{-1}$ for diploids and tetraploids, respectively. Ryegrass plots were mowed weekly at 10 cm height, simulating a continuous grazing management until closure for seeding was determined. Before mowing, the phenological state of every plot was assessed by determining the percentage of tillers in reproductive stage. A tiller was considered to have entered the reproductive stage after the presence of a node at the base of the tiller was noted (stage 31 according to Gustavsson, 2011). The closure of each plot was defined when 50% of the tillers reached the reproductive stage, resulting in varieties differing in closing date, but at the same phenological stage. In each plot, the total seed production was estimated from the seed yield components. At maturity, the number of spikes was counted in 2 subsamples of 0.5 linear m in the central rows of the plot, obtaining the number of spikes per m^2. A sample of 20 spikes per plot was taken before seed dehiscence, the spike length was measured from the base of the lowermost to the tip of the uppermost spikelet, and the number of spikelets per spike ($spikelet \cdot spike^{-1}$) was counted. The spikes were threshed and cleaned by hand, the average weight of seeds per spike was obtained, and the weight of a thousand seeds was determined. From these parameters, the number of seeds per spike ($Seeds \cdot spike^{-1}$), number of seeds per spikelet ($Seeds \cdot spikelet^{-1}$) and number of seeds per square meter ($TS \cdot m^{-2}$) were estimated. In December, after seeds shattered, the amount of straw in each plot

was determined in a 0.125 m^2 sample cut at ground level in the center of each plot. In summer, on January 12 and January 27, soil temperature was measured in each plot at 7 am and at 2 pm. An analysis of variance and comparison of means was performed using variety as the classification variable. The association between the different variables was analyzed using the Pearson correlation coefficient. A multiple regression analysis was performed with the aim of obtaining a prediction equation to estimate $TS \cdot m^{-2}$ based on $spikes \cdot m^{-2}$ and spike length which are the two easiest components that can be obtained in field observations. The statistical software InfoStat version 2020 was used for the analyses.

Results and Discussion

Varieties differed in closing date and followed a similar pattern than their average heading date as expected (Table 1). There were significant differences in seed production ($TS \cdot m^{-2}$), in seed yield components, and in the amount of remaining straw. With the exception of $spikelets \cdot spike^{-1}$, all other observed differences were negatively associated with closing date but positively associated among them (Table 2). Under the imposed defoliation management, the later a variety was, and hence its closing date, the lower the seed production could be expected. This was associated with a reduction in the production of spikes ($Spikes \cdot m^{-2}$), and a reduction in the spike fertility ($Seeds \cdot spike^{-1}$). The reduction in $seeds \cdot spike^{-1}$ with delayed closing date was not a result of shorter spikes having less spikelets since $spikelets \cdot spike^{-1}$ varied independently from both and from closing date, but was rather due to a reduction in spikelet fertility ($Seeds \cdot spikelet^{-1}$). The number of spikelets and florets in a spike is determined earlier in the season during primordia induction, and although there were varietal differences, they were independent from the closing date. In turn, probabilities of high temperatures and water deficit increases later in the season which are factors known to affect floret fertility ($Seeds \cdot spikelet^{-1}$) (Martiniello and Teixeira da Silva, 2011).

Table 1. Closing date and seed yield components for the 18 ryegrass varieties evaluated under reseeding management. P- value of the ANOVA test and LSD value for mean comparison are provided for each variable.

Variety (ploidy)	Heading date	Closing date	$TS \cdot m^{-2}$ *	$Spikes \cdot m^{-2}$	Seeds· $Spike^{-1}$	Spike length⁺	Spikelets· $Spike^{-1}$	Seeds· $Spikelet^{-1}$	Straw $tt \cdot ha^{-1}$
Lm091 (2x)	Mid-Sep	1-Sep	148	1083	138	28.4	25.0	5.4	5.41
E284 (2x)	Mid-Sep	4-Sep	132	1090	122	27.2	26.3	4.6	5.16
Lm121 (2x)	Late-Sep	10-Sep	119	1141	105	26.3	26.0	4.0	5.39
Lm111 (4x)	Early-Oct	14-Sep	49	549	93	28.1	26.7	3.4	5.53
Lm122 (4x)	Early-Oct	14-Sep	58	572	101	30.5	28.0	3.6	6.16
Cambará (2x)	Mid-Oct	20-Sep	31	679	42	19.8	20.0	2.1	3.88
Winter Star 3 (4x)	Mid-Oct	20-Sep	49	659	73	25.5	28.7	2.5	5.82
Lm192 (2x)	Late-Oct	28-Sep	44	718	62	20.3	27.0	2.3	3.27
Lm194 (2x)	Late-Oct	28-Sep	53	735	72	19.1	29.0	2.4	4.05
Lm195 (2x)	Late-Oct	30-Sep	39	674	56	18.8	23.3	2.4	3.77
INIA Bakarat (2x)	Late-Oct	1-Oct	47	597	76	22.6	25.0	3.0	4.02
INIA Camaro (2x)	Late-Oct	1-Oct	34	608	52	18.8	26.0	2.0	2.98
Lm201 (2x)	Late-Oct	1-Oct	43	657	64	19.3	26.7	2.5	2.58
Lm202 (2x)	Late-Oct	1-Oct	30	418	61	19.8	26.0	2.5	3.08
Lm193 (2x)	Late-Oct	1-Oct	37	587	62	20.1	26.0	2.4	2.93
Lm151 (4x)	Late-Oct	2-Oct	10	276	29	22.1	30.0	1.0	3.18
INIA Titán (4x)	Early-Nov	6-Oct	2	141	17	17.6	29.5	0.6	2.27
INIA Escorpio (4x)	Early-Nov	13-Oct	2	92	18	19.5	30.5	0.6	1.89
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0026	<0.0001	0.0039
	$LSD_{(\alpha=0.05)}$	6	33	308	37	4.0	4.2	1.5	2.19

* TS = number of seeds in thousands; ⁺ spike length measured in centimetres.

The amount of straw was also negatively associated with closing date ($r = -0.71$; $p < 0.0001$) with early varieties tending to produce more straw than late varieties. The amount of straw affected the soil surface temperature measured in the afternoon on both dates, which varied between 40 °C and 61.5 °C in January 12 ($r = -0.67$; $p<0.0001$), and between 25 °C and 40 °C in January 27 ($r = -0.30$; $p=0.0413$).

The prediction of the number of seeds produced based on spikes·m^{-2} and spikes length showed a high coefficient of determination with a quadratic term for spikes.m^{-2} (Table 3). This equation requires further adjustments specially under real grazing conditions but can be used as an acceptable approximation to anticipate and make decisions in the field.

Table 2. Pearson correlation coefficients (horizontal) and significance levels (vertical) between closing date, seed production and seed yield components

	Closing date	TS·m^{-2}	Spikes·m^{-2}	Seeds·spike^{-1}	Spikelets·spike^{-1}	Seeds·spikelet^{-1}	Spike length
Closing date	1.00	-0.82	-0.71	-0.78	0.20	-0.76	-0.77
TS·m^{-2}	***	1.00	0.87	0.90	-0.19	0.90	0.62
Spikes·m^{-2}	***	***	1.00	0.71	-0.37	0.74	0.41
Seeds·spike^{-1}	***	***	***	1.00	-0.13	0.98	0.76
Spikelets·spike^{-1}	ns	ns	**	ns	1.00	-0.30	0.06
Seeds·spikelet^{-1}	***	***	***	***	*	1.00	0.70
Spike length	***	***	**	***	ns	***	1.00

ns = not significant; * = significant at 5%; ** = significant at 1%; *** = significant at 0.1%.

Table 3. Regression analysis of TS·m^{-2} based on spikes·m^{-2} and spike length (R^2 Adjusted = 0.88)

	Constant	**Spike length**	**Spikes·m^{-2}**	**(Spikes·m^{-2})$^{\wedge 2}$**
Estimate	-54863.69	2899.59	5.52	0.08
Standard error	12263.64	519.74	24.66	0.02
p-value	0.0001	<0.0001	0.8240	0.0002

$^{\wedge 2}$ = quadratic term for Spikes·m^{-2}

A density of 500 plants per square meter is considered an acceptable stand for maximum forage production in the growing season (Evers and Nelson, 2000) and even the very late varieties were able to produce over 2000 seeds.m^{-2}, but seed viability and seedling survival can be greatly reduced during summer. In a previous work in the same location, less than 1% of the seeds derived in a stablished plant in the following growing season (Do Canto, 2019). In addition, the remaining straw acts as a protection against heat, direct radiation, and desiccation. Later varieties produced less straw, which resulted in higher summer soil temperature peaks, and could reduce even more the chances of a successful re-establishment in the following season.

Conclusions and/or Implications

We conclude that heading date could be used as an indirect selection criterion among cultivars when reseeding ability is considered. Varieties with early heading dates would be preferred for situations were re-establishment of the pasture from the soil seed bank is desired. Late heading varieties in turn would be a better option when ryegrass is part of a crop rotation, and ryegrass becoming a weed is a concern. Intermediate varieties could be used for either purpose but demand caution or additional management practices. Cultivar differences in seed and seedling survival through summer were not assessed in our work but could potentially affect the ryegrass re-establishment.

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