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Plant density in red clover (*Trifolium pratense* L.) pastures as an early predictor of forage production

Rodrigo Zarza[a,*], Mónica Rebuffo[a], Alejandro La Manna[a], Mónica Balzarini[b]

[a] Instituto Nacional de Investigación Agropecuaria, Estación Experimental INIA La Estanzuela, Ruta 50 km 11, Colonia, Uruguay
[b] Universidad Nacional de Córdoba, Facultad de Ciencias Agropecuarias, Córdoba, Argentina

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ABSTRACT

Red clover (*Trifolium pratense* L.) is an alternative of great potential productivity for dairy systems, particularly in heavily compacted soils. Plant density (PD) during pasture establishment can be related to forage production and used as an early indicator of pasture quality. However, biomass predictive models for red clover are not readily available. To predict red clover biomass a few weeks after sowing would help farmers to adopt suitable management practices. The aim of this paper has therefore been to model the relationship between red clover biomass and PD at different time points during pasture establishment to identify the best monitoring moment for estimating future herbage productivity. A multi-environment trial, including several seeding rates simulating different levels of establishment within each of the nine environments, was conducted in Uruguay. Seedlings were counted 3, 7 and 12 weeks after sowing (WS). Biomass of first-cut (C1) harvest was linearly related to PD at 7 WS, whereas a second-order polynomial on PD at 7 WS was a significant predictor of accumulated biomass one (Y1) and two (Y2) years after sowing. PD at 3 WS was a strong predictor of biomass only in high-yielding environments. In such environments, more than 64 plants m^{-2} at 3 WS suggest a high probability of achieving annual yields above 10,000 kg DM ha^{-1}. Therefore, early PD determination (3 or 7 WS) is a good indicator of annual productivity in pure red clover pastures.

1. Introduction

Due to the increase in intensive farming in Uruguay during the 2000s, marginal areas have been used for forage crop rotation. These areas are associated with poor establishment conditions, specifically with excessive soil compaction. Under this scenario, the risks of establishment failure increase, possibly affecting future productivity of cultivated pastures. Although several factors affect pasture survival and production, it is well known that rapid soil cover by forage legume species during establishment is closely associated with annual productivity (Peyraud et al., 2009; Frame et al., 1998; Thomson, 1984). Red clover (*Trifolium pratense* L.) ranks second after alfalfa (*Medicago sativa* L.) in terms of seed production and marketing, and number of forage legume cultivars available worldwide (Boller et al., 2010). Red clover is generally used in sustainable grazing production systems because it has: 1) annual nitrogen fixation capacity (~150 kg N ha^{-1}) (Pirhofer-Walzl et al., 2012); 2) often excessive crude protein above nutritional requirements, which leads to high voluntary intakes and improvements in livestock performance (Lüscher et al., 2014; Frame et al., 1998); and 3) high levels of enzymatic polyphenol that directly improve nitrogen utilization in beef and dairy ruminants (Van Ranst et al., 2011; Lee et al., 2006; Winters and Minchin, 2005). In the Pampas region of Uruguay, red clover is a short-lived perennial forage legume. Early flowering cultivars, without winter dormancy (Annicchiarico et al., 2015), are recommended for short rotation paddocks in dairy systems due to their rapid regrowth, erect growth habit, tolerance to grazing, and high production of autumn-winter forage (Ayala et al., 2010).

Among other factors, animal trampling can have detrimental effects on soil physical conditions and plant growth during pasture establishment. In Uruguay, during the 20th century animal stocking rates increased due to intensification of grazing systems, with the consequent increase in the physical restrictions on the establishment and growth of red clover seedlings in clay soils (Pérez-Gomar Capurro et al., 2014 Response curve relationships between soil physical properties (e.g. macroporosity, air-filled porosity, bulk density) and pasture and crop

Abbreviations: WS, weeks after sowing; C1, first-cut; Y1, first year after sowing; Y2, second year after sowing; PD, plant density; DM, dry matter; AC, achievement coefficient

* Corresponding author.
E-mail address: rzarza@inia.org.uy (R. Zarza).

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Table 1
Soil characteristics at three sites. Cartographic unit (Unit), soil type, soil total porosity (TP), soil apparent density (AD), soil acidity in water (pH), organic Carbon (C), Phosphorus (P Bray I) Potassium (K) and cation exchange capacity (CEC).

Site	Longitude W	Latitude S	Soil Unit	Soil type	Total Porosity %	Apparent Density g/cm^{-3}	pH	C %	P Bray I ppm P	K meq/100 g	CEC meq/100 g
Colonia	57°41’	34°20’’	Ecilda Paullier-Las Brujas	Silty clay loam	51.20	1.29	6.2	2.35	24.3	0,63	29
Florida	56°24’	34°20’	Tala Rodriguez	Clay loam	46.86	1.41	5.5	1.54	49.6	0.37	18,9
San José	56°49’	34°15’	Tala Rodriguez	Silty clay loam	49.23	1.34	5.7	1.83	7.2	0.57	27,2

yield were determined by Drewry et al. (2008). These authors indicate that the optimum soil macroporosity for maximum pasture and crop yield ranges from 6 to 17% v/v, but there is a paucity of yield response curves for pastoral systems, particularly related to critical or optimum values of soil physical properties. Furthermore, Martínez et al. (2011) confirmed that the reduction in macropores caused by the increase in soil bulk density has negative effects on the phenological development of annual pastures, since it promotes leaf abscission, reduces height, and increases stem diameter.

Many studies have reported reduced pasture growth when the stubble has been trampled over by livestock (Pérez-Gomar Capurro et al., 2014; Zegwaard et al., 1998). However, the relationship between seeding rate and productivity under this condition has been scarcely studied for pure red clover. Some inconsistent results have been reported on this relationship for interseeded winter dormant red clover cultivars (Singer and Meek, 2012; Queen et al., 2009; Singer et al., 2006; Mutch et al., 2003). Mutch et al. (2003) reported increments in biomass with enhanced plant density (PD) during establishment for the cultivar with intermediate dormancy and only in one of two years, whereas Singer et al. (2006) reported a positive linear relationship between PD and forage biomass. In contrast, Singer and Meek (2012) reported that a simple nonlinear model was better in explaining this relationship. Queen et al. (2009) described a contrasting behavior associated with weather conditions; where the correlation between biomass with either soil gravimetric water content or PD evaluated 3, 5, 7, 14, and 20 weeks after sowing (WS) was statistically significant only at 7 WS in 2005 and 2006 and showed a positive a tendency at WS 5.

Additionally, Hume et al. (1995) evaluated PD at 5 WS in red clover and chicory (*Cichorium intybus* L.) mixtures and obtained 46 and 101 plants m^{-2} from densities of 1 to 6 kg seed ha^{-1}, respectively. Furthermore, Moliterno (2000) reported productivity of three complex mixed pastures, all sown with red clover at 8 kg seed ha^{-1}, where the relationship between PD and seeding rate (achievement coefficient - AC) ranged between 41 and 52% for red clover.

A study conducted in the north of Buenos Aires province by Barletta et al. (2013) analyzed factors that determined stand density of 2-3-year-old white and red clover pasture. The occurrence of root damage and diseases caused by insects, chemical soil properties and the use of pasture establishment technologies were the main factors involved in the reduction of red clover stands. These authors determined a positive correlation between soil texture and red clover density ($p < 0.001$, $r = 0.67$).

Since biomass yield could be used as an indicator of the economic viability of grassland-based dairy systems (Schader et al., 2013), it is crucial to predict it at early crop stages. In commercial plots, production of rotational systems is estimated via forage budgeting, which considers average biomass yields for different environments. The main goal of this paper was to model the relationship between PD at the crop establishment stage and the potential forage production. Different models have been developed to understand the relationships of forage yield with soil and climatic variables (Trnka et al., 2006; Han et al., 2003; Quiroga et al., 1993; Sharratt et al., 1987, 1989; Fick and Onstad, 1984; Selirio and Brown, 1979). However, there are few models that approach forage biomass as a function of PD at early establishment (Shewmaker et al., 2002; Min et al., 2000). This relationship is useful to improve the ensemble of multi-models for perennial grasses, which integrate independent studies that associate climatic and soil conditions, vegetation, and management with pasture productivity (Sándor et al., 2017). In this research, a predictive quantitative model was used to determine the relationship between PD in a pure red clover pasture and forage biomass produced at the first cut (C1) and in the first year (Y1) and second year (Y2) after sowing. The purpose of this study was to identify the best seedling count date in order to use PD as an indicator of forage productivity and pasture quality.

2. Materials and methods

2.1. Data acquisition

A multi-environment trial was set up to evaluate the relationship between PD during red clover establishment, first-cut forage productivity (C1) during spring-summer, and annual accumulated biomass in Y1 and Y2. ‘Estanzuela 116’, the early-flowering diploid cultivar most commonly used in Uruguay, was sown in nine environments consisting of the combination of three sites (Colonia, Florida, San José) repeated over three years (Table 1). Although the three sites under study are located on a Typic Argiudoll soil, Florida proved to be a poorest site than Colonia due to its lowest pH, organic matter content and cation exchange capacity (CEC). Experimental areas were located in plots with more than a 10-year history of no-till and dairy herd grazing. This fact produced an increase in soil compaction which may have a detrimental effect on pasture development.

Pre-sowing cultivation of experimental plots was as similar as possible for all trials, with winter cropping of yellow oat (*Avena byzantina* Koch.) followed by summer cropping of *Sorghum bicolor* (L.) Moench subsp. *drummondii*, managed under grazing. Each trial was established using no tillage, with six seeding rates of pure red clover (3, 6, 9 12, 15, 18 kg seed ha^{-1}) to simulate different crop establishment conditions, with smaller seeding rates corresponding to poorer establishment. The seed sown had 95% germination capacity, 98% purity, and 1.88 g for 1000-seed weight; thus, the seed lot had 495 viable seeds per gram. Each trial was conducted following a completely randomized block design with four replicates.

Seeding was performed after weed control, which consisted of the application of 3 L N-(phosphonomethyl) glycine 480 g/L a.i. three months before seeding, and 2 L of N-(phosphonomethyl) glycine 480 g/L a.i. and 0.5 L of flumetsulam 120 g/L a.i. at the sowing date. During red clover development, weed control was performed using flumetsulam and 2–4 DB isobutyl ester and application rates were adjusted to weed level in each trial. Temperature and rainfall recorded in each environment are shown in Table 2. Cumulative degree-days during the first 12 WS were calculated assuming the base temperature of 5 °C used for similar forage legumes (Sharratt et al., 1989). Fertilization with solid phosphate fertilizer (0–46/47-0) at sowing was defined based on soil analysis to reach the critical phosphorus (Hernández, 2008) levels for red clover in Uruguay (16 ppm of P_2O_5).

Seeding was performed using a self-propelled no-tillage experimental seeder with double disc openers and press wheels. Plots were 6 m long and 1.2 m wide, with six rows and 17-cm row spacing. Red clover seed was treated with commercial inoculant containing

Table 2
Sowing date, cumulative degree days above 5 C° (DD; C°) at 12 weeks after sowing (WS) and total cumulative precipitation (TP; mm) at 12 WAS, year 1 (Y1) and year 2 (Y2) after sowing in nine environments (Colonia, Florida and San José sites repeated over three years).

Locality	Year	Environment	Sowing date	DD	TP		
					12 WS	Y1	Y2
Colonia		COL 2012	1-Jun	510	199	913	1037
Florida	2012	FL 2012	26-Jun	630	336	1016	1464
San José		SJ 2012	22-Jun	709	446	1063	1304
Colonia		COL 2013	1-Jun	569	86	1037	1843
Florida	2013	FL 2013	7-Jun	468	175	1464	1852
San José		SJ 2013	13-May	645	123	1304	1838
Colonia		COL 2014	29-Apr	555	296	1843	914
Florida	2014	FL 2014	24-Apr	493	229	1852	831
San José		SJ 2014	25-Apr	675	256	1838	669

Table 3
Pre-harvest canopy height (cm) of red clover 'Estanzuela 116' for first-cut (C1), and years one and two after sowing (Y1 and Y2, respectively), and number of cuttings per year in different environments (Colonia, Florida and San José sites repeated over three years).

Environment	Height (cm)			Number of cuttings year^{-1}	
	First cut	Year 1	Year 2	Year 1	Year 2
COL 2012	28	35	30	5	6
FL 2012	34	34	23	3	7
SJ 2012	32	40	22	5	6
COL 2013	31	41	33	3	6
FL 2013	18	25	25	4	6
SJ 2013	22	34	28	4	8
COL 2014	38	46	29	4	5
FL 2014	26	35	28	5	4
SJ 2014	27	30	26	6	6
Mean	28	36	27	4	6

Rhizobium leguminosarum bv *trifolii* U-204, following the manufacturer's instructions, and sown at a depth of 10–20 mm. Germinated plants were recorded along 2 m sampling length at the two central rows of each plot (0.68 m^2), leaving 2 m at the end of each row to avoid border effects. Plants were counted at 3, 7 and 12 WS, corresponding to early (cotyledon), intermediate (first trifoliolate leaf) and late (three to four trifoliolate leaves) stages, respectively. The plants counted had no stem branching. The AC was calculated based on the PD at each count time for each environment and viable seed density. First harvest (C1) was made when red clover reached the beginning of flower bud (Table 3), leaving a stubble height of 5 cm. Subsamples for each treatment were dried in a forced-air oven (60 °C) to constant weight and weighed to obtain dry matter production.

2.2. Statistical analysis

2.2.1. Stand dynamics

The PD at each count time was analyzed using a generalized linear mixed model (Eq. (1)), with Poisson distribution and log link function:

$$\log(E(PD_{ijkl})) = \mu + E_i + W_j + D_k + B_{l(i)} + E \times W_{ij} + E \times D_{ik} + W \times D_{jk} + E \times W \times D_{ijk} + P_{ijkl} \quad (1)$$

where PD_{ijkl} is the number of emerged plants expressed as plants per m^{-2} for environment i at density k, recorded in week j, in block l.; μ stands for the population mean; E_i is the effect of the *i*-th environment (i = 1,…,9); W_j is the effect of the j-th time of count (j = 1,2,3) and D_k is the effect of seeding rate (k = 1,…,6); $B_{l(i)}$ stands for the effect of block l (l = 1,…,4) within environment i; P_{ikl} is the effect of plot within block l for density k and environment i. The terms $B_{l(i)}$ and P_{ikl} were treated as random. Plot random effect was included in the model in order to induce correlations among observations made in a single plot at different count times. The second- and third-order interactions among the effects of environments, count times and seeding rate were also included in the model. The statistical significance of the effects in the model was evaluated and the adjusted LSD method was used for multiple comparisons of treatment means ($P < 0.05$).

2.2.2. Relationship between plant density and forage productivity

The functional relationship between biomass (kg DM ha^{-1}) and number of established seedlings at each count time was calculated using a linear mixed model with random term for the environmental effect (Eq. (2)).

$$Y_{ij} = \beta_0 + \beta_1 PD_j + \beta_2 PD_j^2 + E_i + \varepsilon_{ij} \quad (2)$$

where Y_{ij} is the biomass (C1, Y1 or Y2); β_0 is the intercept; β_1 is the coefficient that relates the increase in the PD to productivity; β_2 is the regression coefficient included for modeling curvature in the relationship between both variables; PD_j is the number of plants at count time (3, 7 and 12 WS); E_i is the random effect of environment, and ε_{ij} is the random error with normal distribution of zero mean and constant variance. The BLUP (Best Linear Unbiased Predictor) of environmental effects were used for environment assessment. The proxy of good, intermediate and poor environments was obtained by averaging the upper, intermediate and lower thirds of the BLUPs, respectively.

Forage biomass prediction (Eq. (2)) for C1, Y1 and Y2 estimated via plant counts obtained at 3, 7 and 12 WS was evaluated by leave-one-out cross validation (Wong, 2015). This procedure consisted on holding out one environment at the model fitting phase for further use in validation of predictions; this was repeated for all environments. At each validation stage, the observed biomass was compared with predicted values by the fitted model and the calculated prediction errors. Finally, the mean of the absolute prediction errors (MAD) was calculated (Pham-Gia and Hung, 2001). MAD was expressed as percentage of overall mean biomass. The Akaike information criterion (AIC) was used to evaluate the goodness of fit for the biomass values, with low AIC values indicating the best fit (Akaike, 1974). The R software (www.R-org.com) was used.

The fitted model was used to obtain expected biomass values at different count times for the three different environmental qualities. Monte Carlo simulations were run 500 times for each regression model in Table 5. Error parameter values were allowed to vary among Monte Carlo simulations (residual standard deviation was 1000 and 3500 kg DM ha^{-1} for the first cut and annual harvests, respectively). An average value of BLUPs from good environments (Table 5) was added to simulate biomass values from relatively good environments. The same procedure was used to obtain 500 potential biomasses for intermediate and poor environments. The relative frequency along 500 runs of biomass values above a given threshold, under each scenario, was used as estimation of the probability of obtaining high yield. In this way, C1 biomass above 2000 kg DM ha^{-1}, and annual accumulated biomass for Y1 and Y2 greater than 10,000 kg DM ha^{-1} were calculated. These threshold values correspond to yields that are above the average values for the first cut and the cumulative production of year one and two of red clover under the conditions of Uruguay, according to the results of the National Evaluation of Cultivars of the INIA-INASE agreement (INIA, 2017).

3. Results

Harvested biomass was highly variable among environments, seeding rate treatments, and crop ages (Tables 4 and 5). Annual biomass in pure red clover pasture of Uruguay ranged from around 2000 kg in a poor environment with poor establishment to more than 15,000 kg DM ha^{-1} in a relatively high-quality environment with high

Table 4
Red clover biomass (kg DM ha^{-1}) harvested at the first cut (C1) and annual accumulated biomass for years one and two (Y1 and Y2, respectively) of the pasture in different environments (Colonia, Florida and San José sites repeated over three years) according to the initial seeding rates (kg seed ha^{-1}).

Harvest period	Seeding rate	Environments								
		COL 2012	COL 2013	COL 2014	FL 2012	FL 2013	FL 2014	SJ 2012	SJ 2013	SJ 2014
C1	3	1861	1733	2067	1029	255	1290	875	819	1467
	6	2834	2237	3620	1195	377	1639	1211	1048	1840
	9	3851	2511	3452	1576	604	2578	1409	1630	2212
	12	3172	3068	3530	1594	569	2483	1469	1549	2572
	15	3283	3344	3197	1575	709	2346	1477	1731	2930
	18	3353	3785	4007	1653	1091	2844	1344	1741	2856
Y1	3	11980	7890	15946	2059	6266	7642	8629	7405	11961
	6	13400	9002	17540	2548	7625	9610	10282	8968	12854
	9	15378	8392	16738	3315	8670	11543	11798	10553	13514
	12	14108	9760	16223	3473	8167	11125	11305	10913	13828
	15	14950	11414	16584	3596	9272	11185	11484	10987	13804
	18	13559	10951	18224	3659	9838	11637	11546	10961	13540
Y2	3	11968	11541	4000	8186	8145	2546	9740	7759	9789
	6	13222	12815	6172	8688	7919	3132	11794	9204	9925
	9	13719	13642	7856	10244	9353	3559	12760	10292	10523
	12	14424	14241	7219	10316	8508	4129	12790	10622	10132
	15	14796	14256	7852	10086	8815	4574	13194	11245	10735
	18	14484	15581	7919	11489	9591	4586	14184	10923	10307

plant density during establishment. The herbage production at the first cut was between 255 kg DM ha^{-1} in the poorest pasture to 4007 kg DM ha^{-1} in the best one. Dynamics of plants during establishment analyzed using Eq. (1) showed that PD responded to the different seeding rate treatments in all environments but in poor ones (FL2013 and FL2014), an increase in plant density was observed only at very high seeding rates (Fig. 1). The PD during establishment depended not only on seeding rate ($P < 0.0001$) and environment ($P < 0.0001$), but also on the count time and their interaction ($P < 0.0001$) (Fig. 1).

The AC was statistically higher ($P = 0.0012$) when plants were counted at 7 than 12 WS (0.44 vs 0.40, respectively). Although significant interactions between environments and both count time and seeding rate ($P < 0.05$) were detected, the main environmental effect was highly significant (Fig. 2). Out of the nine environments, three of the four smallest values were in the same site (Florida).

Biomass production, determined at C1, Y1 and Y2, was related to PD via the mixed linear models reported in Table 5. This relationship was always statistically significant ($P < 0.001$), regardless of the moment when plants were counted (Table 5). Second-order polynomial relationships between Y1 or Y2 and PD at the three count times were found, whereas a linear relationship was identified for C1. Mean BLUPs for environments of relatively good, intermediate, and poor forage yields suggest that the relationships exhibit different intercepts, depending on the environment quality. The intercept of each regression was obtained by adding the BLUP of the environment quality to the β0 coefficient. As a consequence of the reduction of plants over time, forage production per plant (β1), both in Y1 and Y2, increased from 3 to 12 WS.

The timing of plant counts (3, 7 and 12 WS) that was best related to biomass accumulation was determined using the AIC model information criteria and prediction error measurements (Table 6). Prediction errors of accumulated biomass at Y2 were always below 20%. PD was a good biomass predictor for Y1 in environments of intermediate and good quality and for C1 only in good quality environments. Intermediate counts to predict Y1 and Y2 showed the lowest AIC values for most of the environments; however, the prediction errors were similar to those obtained using early plant counts. MAD measurements were similar at the different count times but were consistently smaller in good quality environments than in the other ones.

Based on the biomass models that used PD at 3 WS and BLUPs that measured the environment quality (Table 5), the expected probabilities of exceeding certain mean yields were calculated (Fig. 3). The probability of achieving yields above 2000 kg DM ha^{-1} in C1 was greater than 75% in good quality environments, regardless of PD (Fig. 3 a). It was necessary to achieve at least 400 plants m^{-2} to exceed 75% probability of good yields in intermediate environments, whereas in poor

Table 5
Regression coefficients and environment quality (mean of BLUPs) for red clover biomass models for first cut (C1) and accumulated biomass in the first (Y1) and second year (Y2), based on plant density during establishment at 3, 7 and 12 weeks after sowing (WS).

Harvest period	Count time	β_0 [a]	B_1 [b]	B_2 [c]	Means of BLUPs		
	WS				Good kg DM ha^{-1}	Intermediate	Poor
C1	3	1325.84	4.22		1014.5	−88.4	−926.1
	7	1259.68	4.35		925.9	−9.8	−916.2
	12	1194.98	5.00		910.4	−63.9	−846.4
Y1	3	9195.00	9.15	−0.01	3863.2	−314.1	−3549.1
	7	8554.76	13.45	−0.01	3805.8	−254.3	−3551.5
	12	8566.13	15.26	−0.02	3677.6	−105.5	−3572.2
Y2	3	8221.10	10.32	−0.01	3504.7	−110.9	−3393.8
	7	7834.40	12.68	−0.01	3172.2	84.9	−3257.1
	12	7638.30	15.48	−0.02	3294.4	−20.9	−3273.5

[a] β0: intercept.
[b] β1 linear coefficient of regression that relating biomass to plant density.
[c] β2 quadratic coefficient of regression.

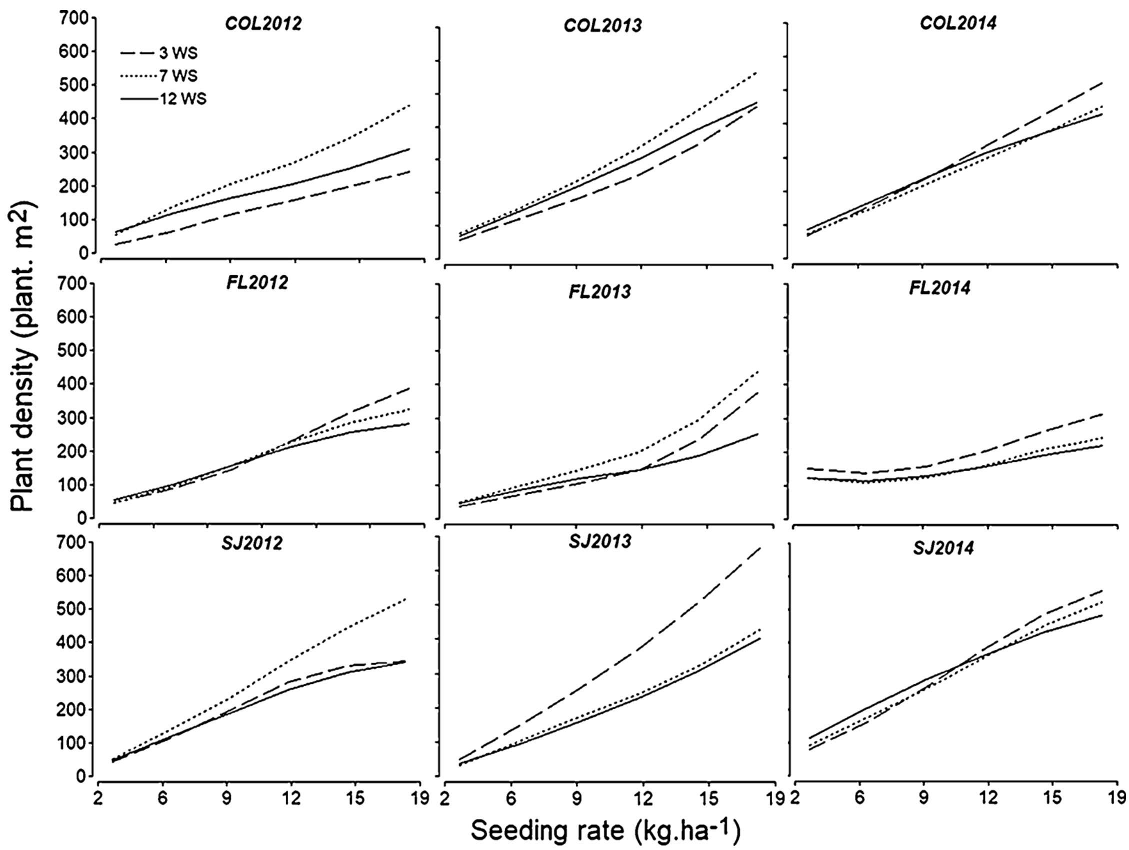

Fig. 1. Number of plants per m^2 as function of seeding rate, at three count times: early, intermediate and late (3, 7 and 12 weeks after sowing - WS, respectively) in three sites (Colonia, Florida, and San José) and three sowing years (2012–2014). Different letters indicate a significant difference between means of number of plants per m^2 ($P < 0.05$).

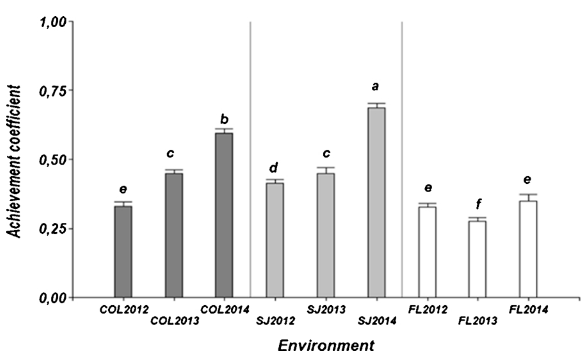

Fig. 2. Red clover achievement coefficient (established plants expressed as proportion of viable seeds sown) for nine environments (Colonia, Florida and San José sites repeated over three years). Different letters indicate a statistical difference between environments ($P < 0.05$).

ones, the same probability value required 466 plants m^{-2} at 3 WS monitoring. Environment quality had a greater impact than PD on the probability of reaching yields above 10,000 kg DM ha^{-1} for both Y1 and Y2 (Fig. 3b and c). In good-quality environments, probabilities were above 80% with PD as low as 64 plants m^{-2}; nevertheless, in more restrictive environments, with the same PD the probabilities were below 50% and 20% in intermediate and poor ones, respectively.

4. Discussion

It is critical to examine the dynamics of grassland biomass production in dairy systems, where management decisions play a role by influencing the temporal forage availability and the interactions between herd and grassland. This research paper was intended to model the relationship between plant stand during the establishment of a pure red clover pasture and its forage productivity to generate a tool for predicting biomass production from early establishment. Experimental studies carried out to this purpose showed that the PD achieved during pasture establishment was proportional to the seeding rate used to simulate different establishment successes. Blaser et al. (2006) observed a

Table 6

Fitting criteria (AIC and Prediction Error) for regression models estimating red clover first-cut biomass (C1) and accumulated biomass in the first (Y1) and second year (Y2) as a function of plant number per m^2 at different count times: 3, 7 and 12 weeks after sowing (WS) in different environments.

Environment Quality [a]	Count time (WS)	Biomass harvest period					
		C1		Y1		Y2	
		AIC [b]	Error [c] (%)	AIC	Error (%)	AIC	Error (%)
Good	3	1148	18	1294	7	1258	8
	7	1145	18	1293	7	1245	8
	12	1142	18	1294	6	1247	8
Intermediate	3	1137	22	1244	10	1227	11
	7	1136	20	1234	10	1216	10
	12	1134	25	1230	9	1217	9
Poor	3	1054	21	1350	28	1326	17
	7	1080	24	1336	26	1315	17
	12	1062	22	1341	26	1323	17

[a] Defined as the environment effect (Best Linear Unbiased Predictors of environmental quality in Table 5).
[b] Akaike Information Criterion; a lower value indicates a better model.
[c] Expressed as percentage of the overall biomass mean.

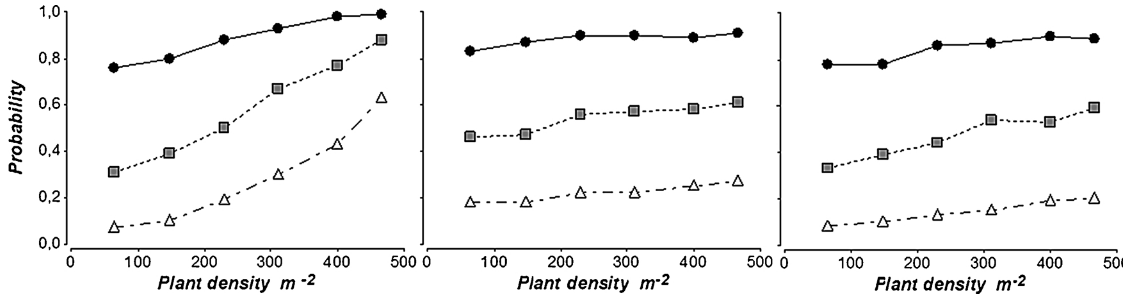

Fig. 3. Probability of reaching more than 2000 kg DM ha^{-1} of a pure red clover pasture at the first cut (a), and more than 10,000 kg DM ha^{-1} in the first (b) and second (b) year according to the plant density counted of TP at 3 weeks after sowing for three different environment qualities: good: circle; intermediate: square; poor: triangle).

similar behavior for undersown red clover. PD reduction between 7 and 12 WS was higher at the highest seeding rate for all environments. The drop in PD after 7 WS can be related to seedling mortality due to competition, as reported by Black (1960). Teixeira et al. (2007) indicated that the reduction in PD at this stage may be partially or totally offset by the increase in some yield components; in particular, stem density below 200 stems m^{-2} is one of the components that significantly reduce forage production (Cummings et al., 1999).

Establishment success measured as AC was also influenced by the seeding rate but mainly by the environment quality. The AC ranged between 0.68 and 0.28 plants per viable seed from the good through the poor environments. A relative smaller AC establishment efficiency was consistently found in Florida site (0.35 to 0.28 plants per viable seed). This experimental site exhibited the soil with the highest apparent density and the lowest total porosity (Table 1). These soil characteristics were associated with physical structure and compaction restrictions on plant growth, as well as biological activity, by Da Silva et al. (2014), Glab (2014) and Pérez-Gomar Capurro et al. (2014).

The PD and biomass production, either for C1 or annual yields, were statistically related through polynomial models, regardless of the moment when establishment was evaluated (Table 5). The linear relationship of C1 biomass with PD might mean that there is no compensation for crown branching of stems at this stage. The negative slope for the quadratic terms of the fitted models to explain Y1 and Y2 biomass (Table 5) indicates that forage production did not increase in the same proportion as the increment of PD during establishment. Such inverse relationship has been reported for other legume-based pastures (White and Harper, 1970; Black, 1960).

Our results suggest interactions among environmental conditions, establishment success, and subsequent forage productivity. Because of the great heterogeneity among environmental effects, the use of environmental quality predictor (BLUPs) allowed us to improve biomass prediction as a function of PD. The data presented in Table 6 indicate that 7 WS and 12 WS plant counts can predict biomass with prediction errors smaller than 20% in good quality environments for all harvest periods, as well as for Y1 and Y2 in intermediate ones, and for Y2 in poor environment quality. However, by monitoring the pasture establishment at an stage as early as 3 WS, PD can also be used as a predictor of biomass production in high-yielding red clover pure stands. The estimations made from the PD measured at 3 WS during pasture establishment always showed prediction errors smaller than 20% in good quality environments for all harvest periods, as well as for Y1 and Y2 in intermediate ones. Several management practices could be implemented in response to predicted low herbage production from monitoring plant stand during establishment. A common practice is replanting, but usually farmers make this decision at 12 or more WS, which could be too late for a new sowing. Many studies associate this delay in the sowing date with a reduced biomass production (Kirby, 1969; Mueller and Chamblee, 1984; Spink et al., 2000; Nevzat et al., 2010). Therefore, by highlighting the need of replanting at 3 WS instead of at 12 WS, management effectiveness will increase. The fitted predictive model using PD at 3 WS is a tool that can be used very early during the establishment stage at least in non-limiting environments.

5. Conclusions

The PD reached a few weeks after sowing a pure red clover pasture is a good indicator of cumulative annual productivity, at least for the first and second year of the pasture. PD evaluated at 3 WS is enough to obtain good biomass predictions; consequently, it is a valuable tool for early forage budgeting. However, forecast precision depended on environment; hence, having information about the expected environment quality would allow farmers to improve herbage productivity predictions. First-cut biomass prediction based on PD might be successful only in environments that do not exhibit restrictions on this legume.

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