

SEEDLING ESTABLISHMENT AND FORAGE ACCUMULATION OF 'CERES TONIC' PLANTAIN IN PURE STANDS AND IN GRASS MIXTURES

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This study evaluates the balance of species 2 mo after sowing, its effects on seasonal and annual dry matter accumulation of *Plantago lanceolata* L. 'Ceres Tonic' sown in pure stands, in binary mixtures with three grasses, and different initial growth rates: annual ryegrass (*Lolium multiflorum* Lam.), tall fescue (*Festuca arundinacea* Schreb.), and orchard grass (*Dactylis glomerata* L.). Plantain monocultures were sown at three densities (4, 8, and 12 kg seed ha⁻¹), while halved densities were used in the mixtures. Each grass was sown at the density that is typical for the region (annual ryegrass = 30, tall fescue = 15, and orchard grass = 6 kg seed ha⁻¹). The trial was carried out in Balcarce (37°45' S, 58°18' W; 130 m a.s.l.), Argentina in 1.4 × 5 m plots sown in April 2005 with a randomized complete block design, a factorial arrangement of pasture type and plantain sowing density, and seasons as a measurement repeated over time. Plant density 2 mo after sowing was not a good estimator of the balance between species for all pasture types at the end of the first year. Total forage accumulation throughout the experimental period was affected by pasture type ($p = 0.08$) and plantain sowing density ($p < 0.05$), but not by their interaction ($p > 0.05$). The plantain and annual ryegrass mixture accumulated 28% more biomass than the mean biomass of all the other pastures (7948 ± 647 vs. 6204 ± 502 kg DM ha⁻¹) mainly because of its higher yield in fall and winter. Total precipitation during the year under study was 15% lower than the historic average so that higher accumulations could be expected in years with higher precipitation.

Key words: *Lolium multiflorum*, *Festuca arundinacea*, *Dactylis glomerata*, plant density, seasonal forage.

Plantain (*Plantago lanceolata* L.) is a short, cosmopolitan perennial herb with origins in Europe and Asia. Given its worldwide distribution, *Plantago* is one of the plant genera that have been widely considered in genetic, chemical, physiological, and ecological studies (Kuiper and Bos, 1992; Australian New Crops Web Site, 2008). Most of these studies have focused on the role of plantain in vegetation diversity, productivity, and stability of diverse environments (Tracy and Sanderson, 2004), and it has been considered as a potential forage only in the last two decades. 'Ceres Tonic' and 'Grassland Lancelot' are two plantain cultivars improved for livestock feeding (Stewart, 1996; Rumball *et al.*, 1997; Stewart and Judson, 2005) because of their erect growth, high nutritive value (especially high Cu, Ca, and Na concentrations), their adaptation to poor soils and other adverse conditions, such as droughts, high temperatures in summer, and low temperatures in winter (Fraser and Rowarth, 1996).

Several studies have been carried out in New Zealand and the United States in the last two decades and have

shown successful seedling establishment in both the mentioned cultivars, not only in pure stands (Sanderson and Elwinger, 2000; Sanderson *et al.*, 2003; Belesky *et al.*, 2007), but also in different forage grass mixtures (Miller and Werner, 1987). However, plantain is a low competitive power species at establishment, especially with light (Kuiper and Bos, 1992). For this reason, when it is sown pure it is rapidly invaded by weeds; in annual ryegrass (*Lolium multiflorum* Lam.) mixtures, seedling establishment as well as contribution to yield are usually poor (Scarlsbrick and Ivins, 1970). In a greenhouse study, the competitive ability of plantain for light and water was higher than for other forage grasses frequently found in old fields, such as *Dactylis glomerata* L., *Festuca elatior* L. (= *Festuca arundinacea* Schreb.), and *Trifolium pratense* L. (Fortner and Weltzin, 2007).

Plant persistence and animal acceptance of 'Ceres Tonic' plantain have not been considered much worldwide (Edwards *et al.*, 2005; Labreveux *et al.*, 2006; Bilbao *et al.*, 2007). Available information suggests that this cultivar resists cattle grazing (Labreveux *et al.*, 2006) and that sheep grazing favors its establishment (Edwards *et al.*, 2005). We also found that cattle select plantain in grass and legume mixtures in the Argentinean Pampas (Bilbao *et al.*, 2007).

Plantago lanceolata 'Ceres Tonic' was introduced in the southeastern province of Buenos Aires (Argentina) at the end of the last decade. Plantain grows year round

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in this region and elongates its floral stalks from late September to late December. Until now, its yield has not been evaluated in pure stands or in forage grass mixtures. Moreover, for the mixtures, it has not been studied whether the established species balance is decisive in their contribution to forage accumulation during the first year. Consequently, the objective of this study was to evaluate seasonal and total forage accumulation of *P. lanceolata* 'Ceres Tonic' in pure stands, in binary mixtures with three forage grasses, and different initial growth rates: annual ryegrass, tall fescue, and orchard grass. Furthermore, the relationship between forage accumulation for each species at the end of the first year and the balance in seedling establishment was analyzed in the mixtures.

MATERIALS AND METHODS

The study was carried out in the experimental field of KWS S.A. located in Balcarce (37°45' S, 58°18' W; 130 m.a.s.l.), Argentina from April 2005 to February 2006. The region has a humid-sub humid climate. Average annual precipitation and temperature are 916 mm, and 13.9 °C, respectively. Average seasonal precipitation and temperature are 295, 210, 149, and 274 mm, and 19.8, 11.7, 8.6, and 15.3 °C, for summer, fall, winter, and spring, respectively (35-yr series, Estación Experimental Agropecuaria, Instituto Nacional de Tecnología Agropecuaria Balcarce).

The evaluated species were plantain 'Ceres Tonic' (PL), annual ryegrass 'Jeanne' (RG), tall fescue (*Festuca arundinacea* Schreb.) 'Enforcer' (TF), and orchard grass (*D. glomerata* L.) 'Oasis' (OG). The trial was carried out in an area with soil apt for agriculture (typical Argiudol) with 4.6% OM and pH 5.5. Sowing was done on 4 April in 1.4 × 5 m plots and 17.5 cm between rows. Plots were allocated in a randomized complete block design (n = 3) in a factorial arrangement of four pastures (PL pure, PL+RG, PL+TF, PL+OG), and three PL sowing densities. Plantain sowing densities were 4, 8, and 12 kg seed ha⁻¹ in pure PL plots, and 50% of these values in the mixtures (low = LD, medium = MD, and high = HD densities). Medium density is empirically recommended for this species in mixtures in the region. Grass mixture sowing densities were 30, 15, and 6 kg seed ha⁻¹ for RG, TF, and OG, respectively. These densities are also usually suggested to achieve adequate plant densities in the first year. By considering the applied densities and a sowing efficiency of 45%, it is possible to obtain approximately 250 pl m². Seed germinability of the four species was 89, 96, 94, and 78% for PL, RG, TF, and OG, respectively. The trial area was fertilized before sowing with a dose equivalent to 70 kg ha⁻¹ of diammonium phosphate. After sowing, but before seedling emergence, a dose of glyphosate, equivalent to 2 L ha⁻¹, was applied for weed control. Plots were irrigated twice with 10 mm water before seedling emergence and 15 d after sowing because

of a moisture deficit.

Successful seedling establishment of each species was estimated 2 mo after sowing by determining plant density (pl m⁻²) on three randomly distributed 50 sectors or the rows by plot. Simultaneously, to illustrate relative plant size of the four species in the mixtures, three plants of each species were randomly collected from the plots where plantain was sown at MD and the intermediate-sized plant was photographed.

Forage accumulation was estimated by harvesting the six central rows of each plot (1.22 × 4.3 m = 5.25 m²) at 8 cm above soil level. Harvest dates were decided based on vegetation height as an indicator (25-30 cm above soil level). Accordingly, the number of fall harvests (and their dates) differed among pastures. The first harvest was 10 d after estimating plant density in the PL, PL+RG and PL+TF plots, and after 30 d for PL+OG. Harvested samples were hand-sorted by species, oven-dried (60 °C, 48 h), weighed to estimate DM forage accumulation in all the pastures and the proportion of grasses in the mixtures. Finally, seasonal and total forage accumulations were calculated.

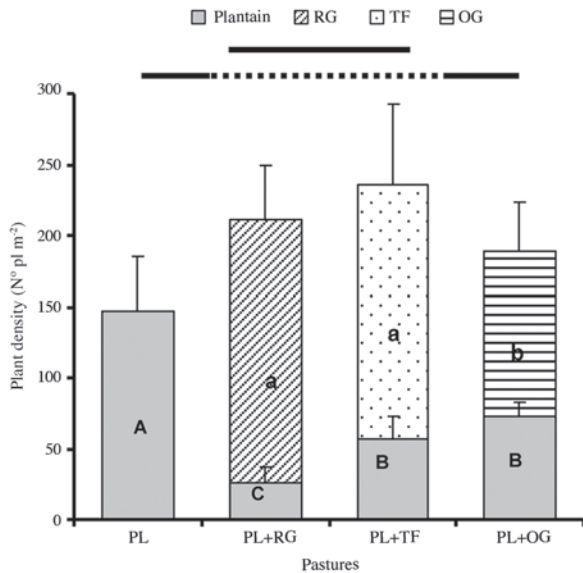
Data were analyzed with a model that considered blocks, a factorial arrangement of pasture type and plantain sowing density as the sources of variation. In the seasonal forage accumulation data analysis, seasons were considered as measurements repeated over time. Data normality was explored through residual graphs, and the Levene test was applied for data homogeneity by (Steel and Torrie, 1980). Seasonal forage accumulation data had to be transformed by \sqrt{x} . Differences among means were evaluated by the Tukey test. All data are shown without transformation, and standard error (SE) is shown with the mean value in every case. Unless otherwise stated, all the differences are $p < 0.05$.

RESULTS

Total precipitation during the trial period was 779 mm, 15% less than the 35-yr series average. Although the year was generally dry, spring and summer were wet. In fall (100.8 mm) and winter (155.7 mm), precipitation was 52 and 5% lower than the historic average, respectively. However, in spring (250 mm) and summer (272.2 mm), it was 9.4 and 8.3% higher, respectively. Average seasonal temperatures did not differ from the long-term average (12.7, 8.6, 14.7, and 19.4 °C in fall, winter, spring and summer, respectively).

Plant density

Plantain sowing density did not interact with pasture type for plant density 2 mo after sowing, but both factors affected it (Figure 1). Total plant density was 35% higher in PL+RG and PL+TF than in PL+OG and PL plots (216 ± 58 vs. 161 ± 37 pl m⁻²). Grass plant density differed among mixtures and, on the average, OG was 65% lower



PL = plantain, RG = annual ryegrass, TF = tall fescue, and OG = orchard grass. PL: 4, 8, and 12 kg seed ha⁻¹ in pure stands and halved densities in mixtures; RG: 30, TF: 15, and OG: 6 kg seed ha⁻¹. Sowing date = 4 April 2005. Means of pastures below the same horizontal lines, and those with similar upper (plantain) or lower (grasses) case letters do not differ ($p > 0.05$).

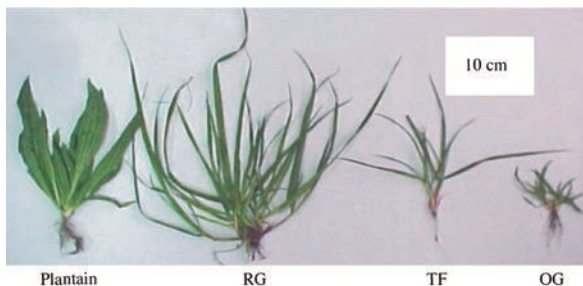
Figure 1. Plant density (mean \pm SE; 2 mo after sowing) in ‘Ceres Tonic’ plantain plots in pure stands and in grass mixtures.

than RG and TF (RG = 183 ± 19 , TF = 171 ± 18 , and OG = 107 ± 19 pl m⁻²). This affected plantain seedling establishment ($p < 0.001$), for which plant density in the PL+RG plots was only 26% of the pure plantain plot density (27 ± 3 vs. 149 ± 12 pl m⁻²). In the PL+TF and PL+OG plots, density was 35% (54 ± 4 vs. 149 ± 12 pl m⁻²) of pure plantain stand density. The relative plant size of the four species in the MD plots is shown in Figure 2.

Plantain sowing density only affected plant density of this species. The mean of the four pasture types showed plantain plant density was 28% higher when it was sown at HD than at the other two densities (HD = 83 ± 4 , MD = 67 ± 3.5 , and LD = 61 ± 4.3 pl m⁻²).

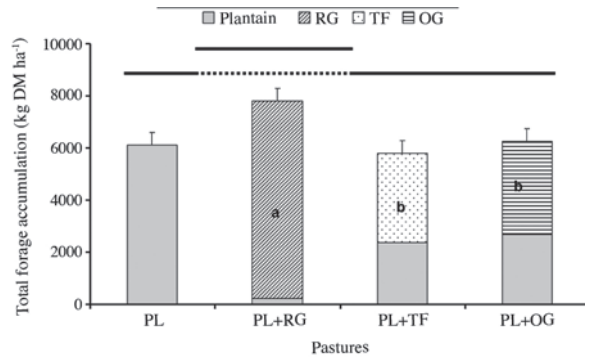
Seasonal and total forage accumulation

Fall harvest dates were the following: PL and PL+RG on



RG = annual ryegrass, TF = tall fescue, and OG = orchard grass. Sowing density: 4, 30, 15, and 6 kg seed ha⁻¹ for plantain, RG, TF, and OG, respectively. Sowing date = 4 April 2005.

Figure 2. Relative plant size (2 mo after sowing) of ‘Ceres Tonic’ plantain and three grasses in binary mixtures.

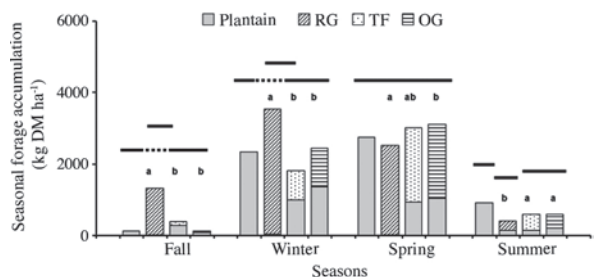


PL = plantain, RG = annual ryegrass, TF = tall fescue, and OG = orchard grass. PL: 4, 8 and 12 kg seed ha⁻¹ in pure stands and halved densities in mixtures; RG: 30, TF: 15, and OG: 6 kg seed ha⁻¹. Sowing date = 4 April 2005. Means of pastures below horizontal lines (total plot forage accumulation), and those with the same letter (grass accumulation) do not differ ($p > 0.05$).

Figure 3. Total forage accumulation (mean \pm SE; 11 mo after sowing) in ‘Ceres Tonic’ plantain plots in pure stands and in grass mixtures in the fall.

21 June and 11 July; PL+TF on 21 June, and PL+OG on 11 July. In the other seasons, the four pastures were simultaneously harvested on the following dates: winter (12 August, 6 and 27 September); spring (19 October, 16 November, and 14 December); and summer (15 February 2006). Total forage accumulation represents forage harvested periodically during 11 mo.

Total forage accumulation differed among pasture types ($p = 0.08$; approximate significant), and was affected by plantain sowing density, but not by the interaction of these two effects (Figure 3). PL+RG plots accumulated 28% more biomass than the other three pasture types (7948 ± 647 vs. 6204 ± 502 kg DM ha⁻¹). Furthermore, total accumulated biomass by PL+RG mixtures was almost exclusively made up of grass (RG = 97%), while the percentage of grass was only slightly higher than plantain in the other two mixtures (TF = 59%; OG = 57%). The mean of the four pastures showed a total forage accumulation that was 22% higher when plantain was sown at HD than LD (6966 ± 487 and 5689 ± 431 kg DM ha⁻¹).



PL = plantain, RG = annual ryegrass, TF = tall fescue, and OG = orchard grass. PL: 4, 8 and 12 kg seed ha⁻¹ in pure stands and halved densities in mixtures; RG: 30, TF: 15, and OG: 6 kg seed ha⁻¹. Sowing date = 4 April 2005. Means of pastures below horizontal lines (total plot forage accumulation), and those with the same letter (grass accumulation) do not differ ($p > 0.05$).

Figure 4. Seasonal forage accumulation (mean \pm SE) in ‘Ceres Tonic’ plantain plots in pure stands and in grass mixtures.

The seasonal forage accumulation pattern differed among pastures (season \times pasture type interaction <0.001), and increased in all of them when plantain sowing density increased ($p < 0.001$) (Figure 4). During fall, the PL+RG pasture accumulated four times more biomass than the others (1360 ± 131 vs. 242 ± 77 kg DM ha⁻¹), almost twice the amount during winter (3585 ± 261 vs. 2272 ± 292 kg DM ha⁻¹), but the amount of forage accumulated by the four pastures was similar during spring (2883 ± 278 kg DM ha⁻¹). The highest forage accumulation in summer was in the pure plantain plots (937 ± 80 kg DM ha⁻¹), the PL+RG mixture was the lowest (412 ± 36 kg DM ha⁻¹), and PL+TF and PL+OG mixtures were intermediate (597 ± 7 kg DM ha⁻¹). Among the grasses, RG accumulated the highest percentage of the mixture biomass during fall (RG = 98 ± 0.5 ; TF and OG = $27 \pm 9.0\%$) and winter (RG = 99 ± 0.4 ; TF and OG = $45 \pm 4.9\%$). In spring, the proportion of RG in the mix was also high, but similar to that of FA, and both were higher than OG (RG 99 ± 0.2 ; TF 71 ± 4.6 and OG $68 \pm 4.6\%$). However, RG was the grass with the lowest contribution to the mixture biomass in summer (RG = 62 ± 6.3 ; TF and OG = $70 \pm 5.6\%$). Total accumulated forage (mean of all pastures) was higher at the highest plantain sowing density (17% higher when it increased from LD to MD, and 35% when it increased from LD to HD).

DISCUSSION

Few studies have related 'Ceres Tonic' plantain forage production, either pure or in grass mixtures, to successful establishment in the first year (Sanderson and Elwinger, 2000; Sanderson *et al.*, 2003; Labreveux *et al.*, 2004). These studies were done in Pennsylvania (USA) where the average annual precipitation (1000 mm) is similar to that of the southeastern province of Buenos Aires and more rigorous winter temperatures depress growth. In southeastern Buenos Aires, it has been empirically observed that plantain is successfully established in summer and spring sowings in complex grass and legume mixtures. In addition, it persists until the end of the first year although its abundance decreases during the second year (A. Farengo, 2005, personal communication). In Pennsylvania, the high seed germination rate and seedling initial growth rate determined that 58% of plantain seeds in pure stands had germinated and produced relatively large plants (10 mg DM pl⁻¹) 15 d after summer sowing (Sanderson and Elwinger, 2000). In addition, 5 to 6 mo after spring sowing with 11 kg seed ha⁻¹, plantain plant density was 300 pl m² under 3- to 5-wk cutting regimes (Sanderson *et al.*, 2003), or grazed with different intensities (5 and 15 cm stubble, respectively) from spring to summer (Labreveux *et al.*, 2004). This plant density was almost constant during the first year in both trials, but strongly decreased in the grazing trial in the second year. Plantain density obtained in these two studies is 68% higher than

the one registered in our study 2 mo after sowing (149 pl m²). However, fall precipitation was low in the year of our study and suggests that plantain establishment could be more successful in more favorable years. Results of our study and those obtained by Sanderson *et al.* (2003) and Labreveux *et al.* (2004) are based on sowings done in different seasons, and harvests at times different from sowing. However, by considering both and independently of the abovementioned factors, we can infer that, 11 to 12 kg seed ha⁻¹ of 'Ceres Tonic' plantain ensures a good stand of plants during seedling establishment in cold and temperate sub-humid regions.

Seasonal and total forage accumulation increased as plantain sowing density increased and reached 7300 ± 485 kg DM ha⁻¹ (mean of all pasture types). This indicates that sowing 12 and 6 kg seed ha⁻¹ of plantain in pure stands and in mixtures, respectively, generates high yields. Plantain forage accumulation obtained in our trial with 12 kg seed ha⁻¹ is similar to values registered by Sanderson *et al.* (2003) and Labreveux *et al.* (2004), with 7000 and 8870 kg DM ha⁻¹ yr⁻¹, respectively. Forage yield from sowing in the second year was recorded by N. Bertolotti (2007, personal communication) when forage accumulation of the three pure plots sown with HD obtained a mean value of $10\ 000$ kg DM ha⁻¹. Consequently, it is possible to assume that in the southeastern province of Buenos Aires, potential 'Ceres Tonic' plantain forage yield ranges from 7 to 10 t DM ha⁻¹ yr⁻¹.

It is usually accepted that the initial growth rate of each species plays an important role in seedling establishment when several species are mixed. Even though the relative abundance of the species can be later modified by climatic conditions and defoliation (cutting or grazing), this initial species balance is decisive in the evolution of each species, and consequently, in its contribution to the forage offer (Harris, 1990). However, the results of our study show that establishment plant density is not necessarily a good indicator of biomass accumulation in binary plantain and forage grass mixtures in the first year.

Total plant density at seedling establishment was higher in PL+RG and PL+TF plots than in PL+OG and plots with pure plantain, representing 86 and 64%, respectively of the 250 pl m², which was defined as the objective for the grass sowing densities. Grass proportion in the mixtures reflected its specific competitive ability. Two months after sowing, RG and TF plant density in the mixtures was similar (177 ± 19 pl m²). However, RG plants were larger than TF, and consequently, plantain density was lower in the mixtures with the former (27 ± 4 vs. 46 ± 5 pl m²). The RG plant density interfered with plantain growth, affecting its contribution to the total biomass as early as in the fall harvests. On the contrary, the same TF plant density allowed the expression of a more balanced biomass proportion between grass and plantain. On the other hand, although OG plant density was only 107 ± 11 pl m², proportions of this species in

seasonal growths were similar to TF. The size of TF and OG plants registered before fall harvests was similar. It is therefore suggested that OG plants, in spite of their low density, were heavier because they contributed to the mixture biomass in the same proportion as TF. Late germination and growth of OG could have caused its high proportions in the winter to summer harvests.

The total amount of accumulated forage in pure plantain plots as well as PL+TF and PL+OG mixtures were similar, but PL+RG was 28% higher. In the latter mixture, plantain establishment was poor and its contribution to the total biomass, mean of all seasons, was only 3%. On the contrary, in the PL+TF and PL+OG mixtures, plantain was an important component of the biomass (59 and 57%, respectively), reflecting the lower aggressiveness of these grasses. Furthermore, the seasonal pattern of mixture forage accumulation was different. The mixture with RG produced the most forage in fall and winter because of the higher initial aggressiveness of this annual grass, which can reach yields of 45 and 25 kg DM ha⁻¹ d⁻¹ in fall and winter, respectively, in the southeastern province of Buenos Aires (Di Rocco, 2000). The higher forage accumulation of PL+TF and PL+OG mixtures in spring was related to the change from the vegetative to reproductive stage of these perennial grasses. The seasonal forage accumulation of the four pastures declined in summer. In the mixtures, grass growth deceleration caused by summer stressors (high temperatures and water deficits) are characteristic of the region and can explain this decline (Suero *et al.*, 2001). For the PL+RG mixture, the fact that the grass growing cycle ends in summer must also be considered. The present study is the first one related to 'Ceres Tonic' plantain forage accumulation, in pure stands and in binary grass mixtures in southeastern Buenos Aires, Argentina. Data correspond to the establishment year and provide basic information on the adaptation and forage accumulation of this cultivar in the region.

CONCLUSIONS

The mean of all pasture types for seasonal and total forage accumulation increased as plantain sowing density increased reaching 7300 ± 485 kg DM ha⁻¹. Mixtures with annual ryegrass produced higher total forage accumulation than those with either tall fescue or orchard grass. A density of 6 kg seed ha⁻¹ of plantain was adequate for mixtures with tall fescue and orchard grass while this density should be higher in those with ryegrass because of the aggressiveness of this species.

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Argentina for providing the facilities and equipment to develop the study.

Implantación y acumulación de forraje de plantago 'Ceres Tonic' puro y en mezcla con gramíneas. Se evaluó si el balance de especies logrado a los 2 meses de la siembra incide en la acumulación estacional y anual de *Plantago lanceolata* L. 'Ceres Tonic' en cultivos puros y en mezclas binarias con tres gramíneas de diferente velocidad de crecimiento inicial: raigrás anual (*Lolium multiflorum* Lam.), festuca alta (*Festuca arundinaceas* Schreb.), y pasto ovillo (*Dactylis glomerata* L.). Plantago fue sembrado puro a tres densidades (4, 8 y 12 kg semilla ha⁻¹) y con la mitad de ellas en las mezclas. Cada gramínea se sembró con la densidad que usualmente se utiliza en la región (raigrás anual = 30, festuca alta = 15, pasto ovillo = 6 kg semillas ha⁻¹). El ensayo se realizó en Balcarce (37°45' S, 58°18' W; 130 m.s.n.m.), Argentina, en parcelas de 1,4 × 5 m sembradas en abril de 2005. Se utilizó un diseño en bloques completos aleatorizados, con arreglo factorial de tipo de pastura y densidad de siembra de plantago y estación como medida repetida en el tiempo. La densidad de plantas a los 2 meses de la siembra no fue un buen estimador del balance entre especies logrado durante el primer año en todas las mezclas. La acumulación total de forraje fue afectada por el tipo de pastura (p = 0,08) y por la densidad de siembra de plantago (p < 0,05), pero no por su interacción (p > 0,05). La pastura de plantago y raigrás anual acumuló 28% más biomasa que las otras en promedio (7948 ± 647 vs. 6204 ± 502 kg MS ha⁻¹), dado su mayor rendimiento otoño-invernal. En el año del estudio la precipitación fue 15% menor que el promedio histórico, por lo que en años con mayores precipitaciones podrían esperarse mayores rendimientos.

Palabras clave: *Lolium multiflorum*, *Festuca arundinacea*, *Dactylis glomerata*, densidad de plantas, forraje estacional.

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