# **Research Paper**

# Effect of sowing rate and date on establishment and growth of *Trichloris crinita*, a native American pasture grass from arid environments, in the Arid Chaco of Argentina

*Efecto de la densidad y fecha de siembra en el establecimiento y crecimiento de Trichloris crinita, una gramínea forrajera nativa de ambientes áridos de las Américas, en el Chaco Árido de Argentina* 

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# Abstract

In arid regions, revegetation with locally adapted native species can improve forage production and help ameliorate soil degradation. We investigated the effects of 3 sowing dates and 3 sowing rates of *Trichloris crinita* cv. Chamical-INTA, a perennial forage grass native to arid and semi-arid regions, on pasture establishment parameters in the Argentinian Arid Chaco phytogeographical region. Sowing date significantly influenced plant density and soil coverage at the end of the growing season, with the latest sowing date increasing mean plant density and soil coverage by 42–66% and 16–38%, respectively, relative to the 1st and 2nd dates. Conversely, the later sowing dates (2nd and 3rd dates) exhibited significantly lower mean values for all plant growth-related traits, i.e. tillers per plant, plant height and percentage of flowering plants. Sowing rate had a strong effect on plant density at the end of the growing season but not on plant growth parameters. Under the conditions of this study, using intermediate sowing densities (7.5 kg seed/ha) and sowing early in the season, when temperatures were still mild, delivered the best results in terms of pasture density and establishment efficacy. Early sowing resulted in a greater percentage of flowering plants and seed set prior to the first winter frosts, which should ensure ongoing establishment of plants in the next wet season. Longer-term studies to examine the survival of plants and possible increase in plant density over time are necessary to determine if this procedure has sustainable benefits for pastures in the area.

Keywords: Degraded areas, forage productivity, pasture management, plant density, vegetation recovery.

# Resumen

En regiones áridas y semiáridas, la siembra de especies nativas adaptadas localmente puede contribuir a mejorar la productividad forrajera y atenuar la degradación del suelo. Este trabajo investigó los efectos de tres densidades y tres fechas de siembra en la implantación y el establecimiento de *Trichloris crinita* cv. Chamical-INTA, una gramínea forrajera nativa de zonas áridas, en la región fitogeográfica del Chaco Árido, Argentina. La fecha de siembra influyó significativamente en la densidad de plantas establecidas y cobertura del suelo al final de la época de crecimiento, con siembras en la tercera fecha mostrando incrementos para estas variables de 42–66% y 16–38%, respectivamente,

Correspondence: .F. Cavagnaro, Instituto Nacional de Tecnología Agropecuaria (INTA) E.E.A La Consulta, Ex Ruta 40 Km 96, San Carlos, 5567 Mendoza, Argentina. E-mail: <u>cavagnaro.pablo@inta.gob.ar</u> comparado con las siembras en la primera y segunda fechas. Contrariamente, las fechas de siembra tardías (segunda y tercera fechas) mostraron valores significativamente menores para todos los parámetros de crecimiento evaluados (p.ej. brotes por planta, altura de planta y porcentaje de floración). La densidad de siembra tuvo un fuerte efecto sobre la densidad final de plantas establecidas, pero no en los parámetros de crecimiento. Las siembras tempranas con densidad intermedia (7.5 kg/ha) tuvieron los mejores resultados en términos de eficacia del establecimiento de la pastura. La siembra temprana resultó en un mayor porcentaje de plantas florecidas al final de la temporada, y las semillas resultantes deberían favorecer el establecimiento de la pastura en la temporada siguiente. A futuro, serán necesarios estudios de largo plazo que monitoreen la supervivencia y densidad de plantas en el tiempo, a fin de determinar si estas prácticas poseen beneficios sostenibles para las pasturas de *T. crinita* en esta región.

**Palabras clave:** Áreas degradadas, densidad de plantas, manejo de pasturas, productividad forrajera, recuperación de vegetación.

# Introduction

Drylands, i.e. arid, semi-arid and dry subhumid regions combined, cover nearly 41% of the Earth's land surface and are home to more than 38% of the total global population (Global Land Project 2005; Millennium Ecosystem Assessment 2005). Severe land degradation is present on 10–20% of these lands, affecting ~250 million people, mainly in developing countries (Millennium Ecosystem Assessment 2005), and current expectations are that these estimates will increase over time due to climate change and population growth.

In arid and semi-arid regions, land desertification, characterized by low fertility and organic matter concentration in the soil, is widespread, and this situation is often aggravated by overgrazing by domesticated animals (Papanastasis 2009). Different studies have estimated that 20–73% (with a mean of 60%, considering estimates from all studies) of the world's grazing areas are moderately to severely degraded (Lund 2007). Moreover, loss of perennial grasses in rangelands, often accompanied by severe soil erosion and salinity, is a frequent component of desertification processes in arid regions (Waters and Shaw 2003).

The traditional strategy to address these degradation issues has been to reseed the degraded areas with introduced perennial species. However, in recent decades the trend has moved towards the use of native species, with a clear recognition of their intrinsic adaptive and ecological value (Waters and Shaw 2003). Selection of drought-tolerant species, with adequate seed available, and the utilization of appropriate and sustainable management practices, especially with regard to pasture establishment, are critical for a successful revegetation program (Quiroga et al. 2013).

*Trichloris crinita* (Lag.) Parodi [syn. *Leptochloa crinita* (Lag.) P.M. Peterson & N. Snow (<u>Peterson et al.</u> 2012; 2015)] (Chloridoideae, Poaceae) is a perennial

grass native to arid and semi-arid regions of North and South America (Peterson et al. 2007). Under natural conditions, it behaves as a typical aestival species, growing whenever soil water is available and the temperature is above 10 °C (Seligman et al. 1992). In these dry lands, the species is widely recognized for its good forage quality, drought tolerance, resistance to trampling and grazing, rapid growth and aggressive competition with other native species (Kozub et al. 2017). In environments with low water availability, it is used as forage for range grazing and for restoration of degraded rangelands (Passera et al. 1992; Cavagnaro and Trione 2007; Guevara et al. 2009).

These arid environments are typical in the north- and central-west part of Argentina. The 'Arid Chaco' region, located in the 'Chaco' phytogeographical province, is one of these drylands, presenting an east-to-west annual rainfall gradient of 250-550 mm, with 80% of the rainfall occurring in mid-summer (November-January) (Morello et al. 1985). In this region, extensive rearing of beef cattle and, to a lesser extent, goats, is the main productive activity (Rueda et al. 2013), with pasture grasses being the main feed source for livestock. However, in recent decades, forage production has decreased steadily as a consequence of land degradation due to overgrazing, thereby altering the landscape to shrublands with extensive areas of bare soil (Blanco et al. 2005; Karlin 2013). Under this scenario, applying revegetation strategies, for example by sowing seeds of locally-adapted native grasses, such as T. crinita, may be effective in increasing plant numbers and therefore forage production, as reported in previous studies with other grass species (Passera et al. 1992; Blanco et al. 2005; Quiroga et al. 2009; Mora et al. 2013).

Initial seedling growth and establishment are stages of extreme susceptibility to a wide range of stresses, due to the small seedling size, and these stages are critical for achieving productive pastures (<u>Praat 1995</u>; <u>Skinner</u> 2005; Bertram 2008). To date, we are unaware of any studies to evaluate the effects of sowing density and date on seedling survival and pasture establishment using *T. crinita*. We hypothesized that early sowing of *T. crinita* using high sowing densities would improve the implantation and establishment of the pasture in an arid environment, such as the Argentinian 'Arid Chaco' phytogeographical region. The objective of this work was to investigate how different sowing rates and dates affect the establishment and growth of *T. crinita* pasture in the year of implantation.

## **Material and Methods**

A field trial was carried out in the Agricultural Experimental Field of the 'Universidad Nacional de La Rioja Sede Chepes' (31°20' S, 66°38' W). The experiment began with the sowing of the grass in the spring of 2014 and finished with the first frost of autumn of 2015.

Three sowing rates were used:  $0.25 \text{ g/m}^2$ , now referred to as low sowing rate (LR);  $0.75 \text{ g/m}^2$ , intermediate sowing rate (IR); and 1.25  $g/m^2$ , high sowing rate (HR); they are equivalent to 2.5, 7.5 and 12.5 kg seed/ha, respectively, or 300, 900 and 1,500 caryopses/m<sup>2</sup>. An unseeded plot was used as a Control, with the objective to obtain complementary information on the dynamics of the natural vegetation, as determined by the seed bank already on site. Three sowing dates were used: 22 November 2014 (sowing date 1; after the first rains); 22 December 2014 (sowing date 2); and 21 January 2015 (sowing date 3). Spikelets of Trichloris crinita cv. Chamical-INTA with a mean germination rate of 85% were sown into a soil with superficial tillage (5 cm-deep soil movement with a hand rake). After depositing seeds on the soil surface, the soil was raked to incorporate the seeds and to avoid random dispersal by the wind. The soil was classified as typic torriorthent, with the following characteristics: silt loam texture, low organic matter content (1.5% of soil mass), pH of 8.2, conductivity of 2.12 mS/cm, 0.1% total nitrogen and a C:N ratio of 9.7. A complete randomized block design was used. The total trial area was 144 m<sup>2</sup> and consisted of 12 treatments of sowing rate × sowing date combinations, with 3 replicates, totaling 36 experimental units (plots) of 4 m<sup>2</sup>  $(2 \times 2 \text{ m})$  each.

Every 15 days for the following 180 days (until 21 May 2015), the following parameters were measured: density of *T. crinita* plants and other narrow-leaf (grasses) and broad-leaf plants; number of tillers per plant; and plant height of *T. crinita*. The percentage

of flowering plants was estimated on 21 May at the end of the growing season for all treatments, i.e. 180, 150 and 120 days after 1st, 2nd and 3rd sowing dates, respectively. The percentage of the land surface covered with plants was estimated using photo images taken from above on 6 April 2015, i.e. 135, 105 and 75 days after the 1st, 2nd and 3rd sowing dates, respectively. This date was arbitrarily selected as a point in time, when the pasture appeared to have reached its maximum vegetative growth and the leaves of some plants began to senesce. All measurements were performed in selected and standardized areas by using a quadrat of 1 m2 in the center of each experimental unit.

Mean daytime temperature (T) and the distribution of rainfallweremonitoredduringtheexperiment. Temperature data were obtained from the agrometeorological station of the National Institute of Agricultural Technology (INTA), located at El Portezuelo (EEA INTA La Rioja AER El Portezuelo), La Rioja, Argentina, located ~50 km from the experimental site. The amount and distribution of precipitation were recorded with a rain gauge located on the site of the experiment.

The data were analyzed using mixed linear models with a factorial structure, treating sowing date, sowing rate and their interactions as fixed effects, while treating the 3 blocks as random effects. Different structures of residual variance were considered, and the best models were selected using the Akaike (AIC) and Schwarz (BIC) information criteria (<u>Di Rienzo et al. 2017</u>). All statistical and graphic analyses were performed with InfoStat version 2018 software (<u>Di Rienzo et al. 2018</u>). The data were expressed as mean  $\pm$  standard error, and P values <0.05 were considered significant, using the DGC test (<u>Di Rienzo et al. 2002</u>).

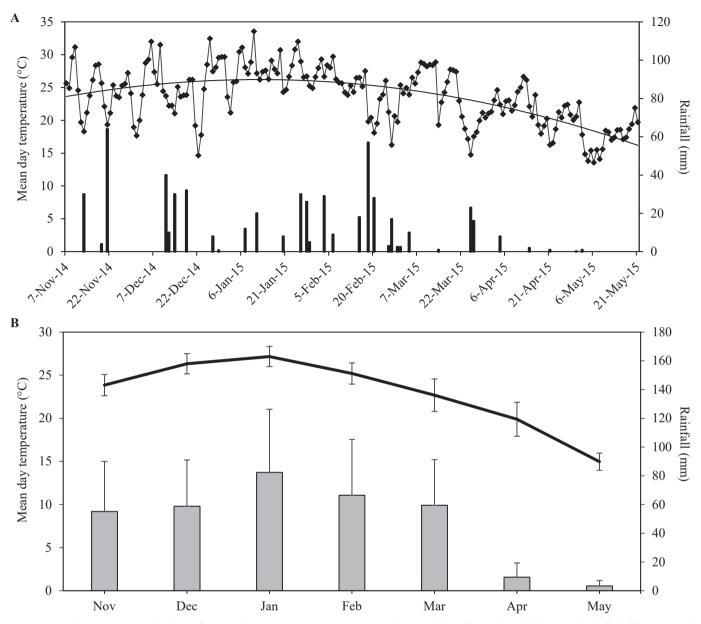
The photographs taken to determine the percentage of the soil surface covered with plants, using canopy area as the criterion, were analyzed with CobCal® 2.0 software (Ferrari et al. 2009), which estimates the area or percentage of vegetation coverage based on colorimetric analysis.

## Results

## Weather conditions during the experiment

Mean day temperature and rainfall throughout the growing season are shown in Figure 1A.

Mean daily temperature for each vegetative period, i.e. from the relevant sowing date to 21 May 2015, was 23.6 °C  $\pm$  4.4; 23.5 °C  $\pm$  4.6; and 22.6 °C  $\pm$  4.3, for the 1st, 2nd and 3rd sowing dates, respectively. In general,



**Figure 1**. A. Time-course variation of mean day temperature (upper graph part with adjusted trend line) and rainfall (histogram in the lower graph part) in the growing season at the site of the experiment. B. Time-course variation of mean day temperature (black line) and mean precipitation (histogram) during the growing season (November–May) at the site of the experiment for the decade 2011–2021. Dispersion bars indicate standard deviations.

mean temperature increased steadily from the 1st sowing date (22 November 2014) until early February (6 February 2015), reaching a maximum of 33.6 °C on 10 January 2015. Additionally, Figure 1B presents weather conditions at the site of the experiment for the last decade.

Total precipitation during the experiment was 548 mm, with amounts for the 3 growing periods being 450 mm for the 1st sowing date, 338 mm for the 2nd sowing date and 289 mm for the 3rd date. In November 2014, 98 mm of rainfall were registered, with 64 mm received before the 1st sowing date (21 November 2014).

#### *Plant density of T. crinita and other species*

Both sowing date (P=0.0051) and sowing rate (P<0.0001) of *T. crinita* seed had significant impacts on final plant density (FPD) at the end of the experiment, i.e. 135, 105 and 75 days after the 1st, 2nd and 3rd sowing dates, respectively, but there was a significant (P=0.0142) interaction between sowing date and sowing rate (Table 1; Figure 2). The highest values for FPD occurred for the intermediate sowing rate (IR; 0.75 g/m<sup>2</sup>) treatment at the 1st and 3rd sowing dates (P<0.05), with means of 135 and

131 plants/m<sup>2</sup>, respectively. The lowest FPD (22 plants/ m<sup>2</sup>) was for the low sowing rate (LR;  $0.25 \text{ g/m}^2$ ) at the 1st sowing date (Figure 2). Among the Control plots, only those for the 2nd sowing date presented *T. crinita* plants, with a mean FPD of 2 plants/m<sup>2</sup>. Presumably, these plants developed from *T. crinita* seeds that were part of the natural seed bank in the soil, as this species is native to arid and semi-arid regions of Argentina, including the Arid Chaco, where the experiment was conducted.

Figure 3 depicts the time-course variation of *T. crinita* plant density throughout the study. At all sowing rates for the 1st sowing date, minimal emergence of *T. crinita* seedlings was observed at 15 days after sowing, but emergence increased significantly in the following 15

days, reaching a maximum plant density (MPD) 45–60 days after sowing (DAS), with mean values of 48, 195 and 199 plants/m<sup>2</sup> for LR, IR and HR, respectively (P<0.05). Plant density then declined steadily until April with final populations of 22, 135 and 81 plants/m<sup>2</sup> for LR, IR and HR, respectively (P<0.05).

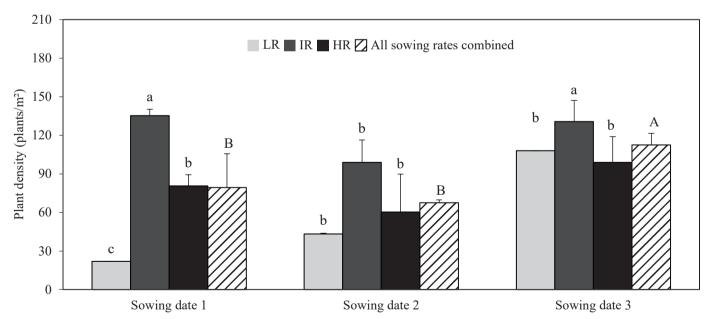
For the 2nd sowing date, there was little emergence at observation dates before 5 February (45 DAS), when peak plant numbers were recorded (58, 145 and 164 plants/m<sup>2</sup> for LR, IR and HR, respectively; P<0.05). A rapid decline in plant numbers occurred in the following 2 weeks, after which plant density remained relatively stable until the end of the experiment, with FPDs of 43, 99 and 60 plants/ $m^2$  for LR, IR and HR, respectively (P<0.05).

**Table 1.** Effects of sowing date and sowing rate, and their interaction, on final plant density of *Trichloris crinita* and other narrowand broad-leaf species, mean number of tillers per plant, plant height and percentage of flowering plants of *T.crinita*, plus percentage of soil covered with vegetation at the end of the study (last measurement).

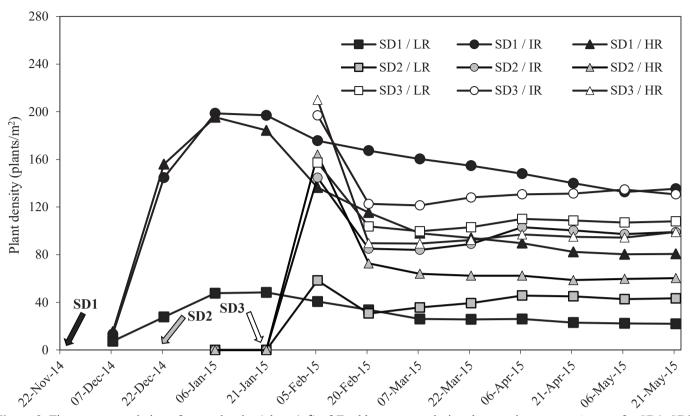
	Plant density at end of study			Tillers	Plant height	% soil	% flowering
	Trichloris crinita	Narrow-leaf species	Broad-leaf species	per plant		coverage	plants
Sowing date	6.8**	ns	ns	6.1**	55.5***	4.6*	16.8***
Sowing rate	37.7***	6.1**	5.1**	ns	ns	19.5***	ns
Date × rate	3.5*	ns	ns	ns	ns	3.8**	ns

Numbers are the F value from ANOVA.

Vegetation cover was determined with the software CobCal 2.0 (Ferrari et al. 2009) using digital photographs taken from above.



**Figure 2.** Plant density at the end of the study (FPD) for *Trichloris crinita* for each sowing date and sowing rate, i.e. at 180, 150 and 120 days after the 1st, 2nd and 3rd sowing dates, respectively. Low (LR), intermediate (IR) and high sowing rates (HR) correspond to sowing densities of 0.25, 0.75 and 1.25 g seed/m2, respectively. Columns represent means and bars are the standard errors. Lower-case letters indicate significant differences among all different combinations of sowing date and rates and upper-case letters indicate comparisons among sowing dates considering all sowing rates for a given sowing date combined. Columns with the same letter are not significantly different at P<0.05.



**Figure 3.** Time-course variation of mean density (plants/m<sup>2</sup>) of *Trichloris crinita* during the growing season. Arrows for SD1, SD2 and SD3 indicate the 1st (22/11/2014), 2nd (22/12/2014) and 3rd (21/01/2015) sowing dates, respectively. For each sowing date, 3 sowing rates were used: low (LR; 0.25 g seed/m<sup>2</sup>), intermediate (IR; 0.75 g seed/m<sup>2</sup>) and high (HR; 1.25 g seed/m<sup>2</sup>).

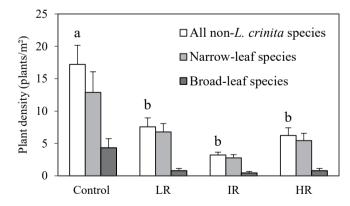
For the 3rd sowing date, peak plant emergence was detected 15 DAS, with plant densities of 157, 197 and 210 plants/m<sup>2</sup> for LR, IR and HR, respectively (P<0.05). During the next 15 days, plant density decreased rapidly and then remained relatively stable until the end of the experiment, when plant numbers were 108, 131 and 99 plants/m<sup>2</sup> for LR, IR and HR, respectively.

While all plots contained some non-*T. crinita* plants, with monocots more abundant than dicots (Figure 4), Control plots had higher frequency of these plants (P<0.05) than plots where *T. crinita* was sown (Table 1; Figure 4). The most frequent grasses were: *Aristida adscensionis, Digitaria californica, Cenchrus ciliaris, Pappophorum caespitosum, Chloris virgata, Sporobolus pyramidatus and Setaria leucopila*, whereas the main broad-leaf species were: *Flaveria bidentis, Gomphrena tomentosa, Allionia incarnata* and *Solanum elaeagnifolium*.

## Production of tillers

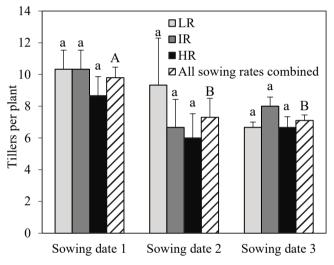
For *T. crinita*, while tiller density at the end of the study was not significantly affected by sowing rate on any sowing date (P>0.05), when data for different

sowing rates were combined within sowing dates, tiller numbers were greater for the first sowing date than for the subsequent sowing dates (9.8 vs. 7.3 and 7.1 tillers/ plant for progressive sowings) (Table 1; Figure 5; P=0.105). Production of tillers throughout the growing



**Figure 4.** Plant density (plants/m<sup>2</sup>) for non-*Trichloris crinita* species at the end of the growing season for Control (0 g seed/m<sup>2</sup>) and the sowing rates low (LR; 0.25 g seed/m<sup>2</sup>), intermediate (IR; 0.75 g seed/m<sup>2</sup>) and high (HR; 1.25 g seed/m<sup>2</sup>). Columns represent mean value  $\pm$  standard error. Columns for all non-*T. crinita* species with the same letter are not significantly different at P<0.05.

season revealed that, despite the lack of significant variation among sowing rate treatments at the end of the experiment, there was a tendency for higher tillers per plant (TPP) in the IR treatment than in LR and HR treatments for the 3 sowing dates (Supplementary Figure S1). For the 1st sowing date, mean TPP remained at 1 during the first 60 DAS, then increased steadily to reach an overall mean of 9.8 TPP at the end of the experiment. TPP for the IR treatment was higher than for LR and HR treatments during most of the growing season (Supplementary Figure S1 A). In general, similar TPP variation patterns were observed for all sowing dates, with different sowing rate treatments not varying much from each other, except for a tendency in favor of IR for the early part of the growing season (Supplementary Figures S1 B and C).

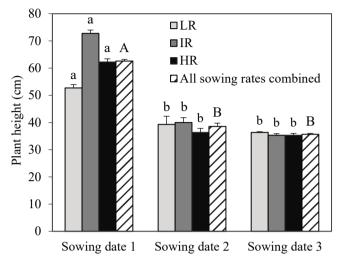


**Figure 5.** Tillers per plant in *T. crinita* at the end of the growing season, as influenced by sowing date and sowing rate. Low (LR;  $0.25 \text{ g seed/m}^2$ ), intermediate (IR;  $0.75 \text{ g seed/m}^2$ ) and high sowing rates (HR;  $1.25 \text{ g seed/m}^2$ ) were used on the 1st (22/11/2014), 2nd (22/12/2014) and 3rd (21/01/2015) sowing dates. Columns represent mean value ± standard error. Lower-case letters indicate differences among the different combinations of sowing dates and rates, while upper-case letters indicate differences among sowing dates combining all sowing rates. Columns with the same letter are not significantly different (P>0.05).

## Plant height

Plant height was significantly influenced by sowing date (P<0.0001) but not by sowing rate (Table 1). Overall, *T. crinita* plants sown earliest (1st date) were 62-75% taller than those sown on the 2nd and 3rd dates (Figure 6). The time-course variation for this trait, as influenced by sowing date and rate, is presented in Supplementary Figure S2. Variation among the sowing rate treatments

was evident for the 1st sowing date, with IR presenting the tallest plants, followed by HR and LR (Supplementary Figure S2 A), whereas no clear differences among the sowing rate treatments were observed for the 2nd and 3rd sowing dates (Supplementary Figures S2 B and C).



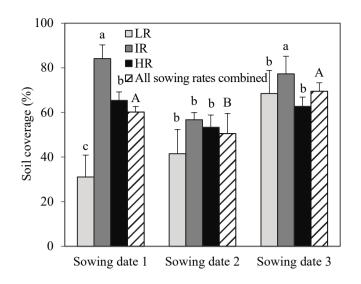
**Figure 6.** Height of *Trichloris crinita* plants at the end of the growing season for each sowing date and rate. Low (LR; 0.25 g seed/m<sup>2</sup>), intermediate (IR; 0.75 g seed/m<sup>2</sup>) and high sowing rates (HR; 1.25 g seed/m<sup>2</sup>) were used on the1st (22/11/2014), 2nd (22/12/2014) and 3rd (21/01/2015) sowing dates. Columns represent mean value  $\pm$  standard error. Lower-case letters indicate differences among the different combinations of sowing dates and rates, while upper-case letters indicate overall differences between sowing dates. Columns with the same letter are not significantly different (P>0.05).

#### Vegetation cover

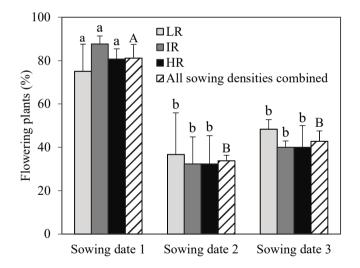
The percentage of soil coverage was significantly affected by sowing rate (P<0.0001), sowing date (P=0.0214) and their interaction (P=0.0094) (Table 1; Figure 7). Soil coverage was highest in the IR treatment at all sowing dates but differences were significant for only the 1st (84.6% coverage) and 3rd (77.3% coverage) sowing dates. The lowest soil coverage was observed in the LR plots sown early in the season.

#### Percent of flowering plants

The percentage of flowering plants of *T. crinita* at the end of the growing season was affected by sowing date (P=0.0001) but not sowing rate (Table 1). Early-sown plots (1st date) had significantly higher percentage of flowering plants (75–88%) than plots sown on the 2nd (32–37%) and 3rd (40–48%) dates, regardless of the sowing rates used (Figure 8).



**Figure 7.** Soil coverage (%) of established *Trichloris crinita* plants at the end of the growing season for each sowing date and rate. Low (LR; 0.25 g seed/m<sup>2</sup>), intermediate (IR; 0.75 g seed/m<sup>2</sup>) and high sowing rates (HR; 1.25 g seed/m<sup>2</sup>) were used on the 1st (22/11/2014), 2nd (22/12/2014) and 3rd (21/01/2015) sowing dates. Columns represent mean value  $\pm$  standard error. Lower-case letters indicate differences among the different combinations of sowing dates and rates, and upper-case letters indicate overall differences between sowing dates. Columns with the same letter are not significantly different (P>0.05).



**Figure 8.** Percentage of *Trichloris crinita* plants flowering at the end of the growing season for each sowing date and rate. Low (LR; 0.25 g seed/m<sup>2</sup>), intermediate (IR; 0.75 g seed/ m<sup>2</sup>) and high sowing rates (HR; 1.25 g seed/m<sup>2</sup>) were used on 1st (22/11/2014), 2nd (22/12/2014) and 3rd (21/01/2015) sowing dates. Columns represent mean value  $\pm$  standard error. Lower-case letters indicate differences among the different combinations of sowing dates and rates, and upper-case letters indicate overall differences between sowing dates. Columns with the same letter are not significantly different (P>0.05).

## Discussion

To the best of our knowledge the present study examined, for the first time, the effects of sowing rate and date on establishment of *T. crinita* pastures. Although *T. crinita* has been used for decades, as forage and for revegetation purposes, it was not until recently that cultivars of this species were developed and seed was made available in sufficient quantities for widespread use. To date, 6 *T. crinita* cultivars can be found at the Argentinian Cultivar Registry of the National Institute of Seeds (INASE). Thus, the use of a reliable and genetically-certified seed source of *T. crinita* cv. Chamical-INTA, the cultivar used in this study, provides confidence in the genetic homogeneity of the plant material used in this study.

# Effects of sowing rate

The plots in which intermediate sowing rates (IR) were used had higher plant density and soil coverage at the end of the growing season than plots of low (LR) and high sowing rates (HR) and this was consistent for all sowing dates (Figures 2 and 7). Competition among individuals may explain the effects of sowing rate on final plant density in the experimental plots. According to Harper (1977), Burton et al. (2006) and Brooker et al. (2008), the density of established plants increases with sowing rate up to a maximum, which depends on the characteristics of the crop and its interaction with the environment, and then decreases due to excessive competition for resources among the plants. This behavior was clearly observed throughout the growing season for all sowing rates and dates analyzed (Figure 3). In other words, all treatments revealed an initial peak in plant density followed by a decrease, presumably due to the high competition among the plantlets, but the IR treatment showed a much higher initial peak than the LR treatment, and a more gradual decline after the peak than the HR treatment, resulting in the IR treatment having the highest density of established plants and highest soil coverage at the end of the experiment. Thus, in terms of density of a T. crinita pasture under agroecological conditions similar to those used in the present study, our results suggest an optimum sowing rate of 0.75 g seed/  $m^2$ , which translates to 7.5 kg seed/ha.

The present study was conducted during the growing season (from spring to autumn), in which implantation and establishment of the pasture takes place. After the non-vegetative winter period, and re-sprouting of the plants in spring, we evaluated plant density, considering only those plants that were visually sprouted and had resumed their vegetative growth; these densities were similar to those recorded at the end of the previous season (data not presented). This suggests that at the end of the first growing season the pasture was fully established.

A significant negative relationship was found between the density of established T. crinita plants and the density of all other (non-T. crinita) plants [the Pearson's correlation coefficient (r) value was -0.66; P<0.0001], which probably resulted from the natural seed bank in the soil and included other grasses and broad-leaf species. IR and Control plots had the lowest and highest density of non-T. crinita plants, respectively (Figure 4). The number of non-T. crinita plants in Control plots increased slowly and gradually during the growing season, whereas in plots sown with T. crinita the number of plants of other species did not vary throughout the experiment (data not presented). Altogether, these data clearly reflect the competitive pressure of T. crinita plants on other native grasses and dicots in determining the final pasture composition of plant species. This is in agreement with Quiroga et al. (2009) and Blanco et al. (2013), who proposed that the sowing of seeds in semi-arid environments can effectively increase the plant density of desirable species in the short term. In the particular case of T. crinita, sowing of this species in dry regions of South America, where much of the land is extensively degraded, may contribute to the recovery of the ground cover and increase forage grass availability.

## Effects of sowing date

Sowing date had significant effects, although smaller than those of sowing rate (Table 1), on plant density of *T. crinita* at the end of the growing season, with the latest sowing date presenting higher overall mean plant numbers (for all densities combined) than the earlier sowing dates (Figure 2). These results are likely due to the more favorable (lower) temperatures and higher rainfall received during the establishment phase of the plantlets after the 3rd sowing date, as compared with the warmer and drier conditions affecting plantlets from the 1st and 2nd dates (Figure 1).

As was to be expected, vegetative growth parameters of individual plants, such as plant height and number of tillers per plant (TPP), as well as the percentage of flowering plants at the end of the growing season, were strongly influenced by sowing date. The greater plant heights in plots from the earliest sowing date, regardless of sowing rate, were likely due to the longer vegetative growth period of these early-sown plants, as compared with those sown later in the season, as evidenced from comparisons of the time-course variation for this trait among different sowing dates (Supplementary Figure S2). Similarly, overall production of tillers per plant was significantly higher for plots sown on the 1st date than for plots sown on the 2nd and 3rd dates (Figure 5), and monitoring of this trait revealed similar variation in tillering as for plant height (Supplementary Figure S1). Thus, a significant positive correlation (r=0.66; P=0.0002) was observed between *T. crinita* plant height and TPP. This positive association is not surprising, as both traits are considered sub-components of the same overall vegetative growth.

The strong effect of sowing date on percentage of plants of *T. crinita* flowering at the end of the growing season, with the early planting presenting, on average, a 0.9–1.4-fold increase compared with later sowings, was likely a function of plants from the earlier sowing being more mature following a longer growth period, thereby allowing a larger number of plants to reach the reproductive stage, as compared with plants sown later. Flowering is affected by a combination of physiological age and day length, and older plants experienced a longer growth period and more hours of day-length than younger plants.

## Influence of climatic factors

Mean annual rainfall in the Arid Chaco region varies from 450 mm in the east to 200 mm in the west, with 80% occurring between November and March (<u>Morello et al. 1985</u>). Thus, the climatic conditions in the present study were rather atypical for this region, given that 548 mm of rainfall occurred during the experiment (Figure 1). For this reason, the results of the present study are suitable for seasons and/or regions with this amount of rainfall. Therefore, there is a need to verify the results of this experiment in other seasons with different water conditions.

From the beginning of the experiment until 6 January 2015, when the highest plant density for the 1st sowing date was reached, a total of 219 mm of rainfall was received, representing 40% of the total rainfall received. This provided plants from the 1st sowing date with adequate soil moisture in the early stages and throughout the growing season.

January was the warmest month, presenting the highest monthly mean temperature (27.4 °C) (Figure 1). This may explain why seedlings from the 2nd sowing

date (22 December 2014) did not emerge until the first week of February, i.e. 45 DAS, which coincided with the emergence of seedlings from the 3rd sowing date (Figure 3). January also coincided with the beginning of the decline in plant density for the 1st sowing date. This suggests that the abundant rainfall received before January favored the initial establishment of seedlings from the first sowing, whereas the subsequent high temperatures in January were less favorable for seedling establishment and general plant growth, i.e. plant height and TPP, in plots from the 2nd and 3rd sowing dates. Nonetheless, comparably higher plant density and soil coverage were observed in plots from the 3rd sowing date (Figures 2 and 7), presumably due to the lower temperatures and rainfall received in this period (Figure 1).

# Conclusions

To the best of our knowledge, this study is the first to document the effects of sowing date and sowing rate on the establishment and plant growth parameters of Trichloris crinita, a good-quality pasture grass native to arid regions. Results showed that, under the environmental and management conditions of the present work, sowing date significantly influenced plant density and soil coverage at the end of the growing season, with the latest (3rd) sowing date increasing both parameters. On the other hand, sowing date had the opposite effect on individual plant growth-related parameters evaluated, with the earliest (1st) sowing resulting in greater production of tillers, plant height and percentage of flowering plants at the end of the season. Since the study terminated with the first frost, earlier flowering from the first sowing should ensure more seed was produced to support plant populations over the subsequent year. Sowing rate had a strong significant effect on the final plant density of the pasture (and therefore on soil coverage) but not on individual plant growth parameters, with intermediate sowing densities yielding the densest plant stands at the end of the growing season. Thus, using ~0.75 g seed per m<sup>2</sup> (7.5 kg/ha) and sowing early in the season, when temperatures were still mild, delivered the best results in terms of pasture density and establishment efficacy, which is likely due to the more favorable initial growing conditions in which the plants were able to take advantage of available water, nutrients and light resources and develop sufficiently to combat later periods of high temperatures. We used aerial photos to assess ground cover, i.e. area of canopy was the criterion. A more effective assessment of amount of soil coverage

is the measurement of basal area of the stand and this method should be utilized as well in future studies. In addition, longer-term studies are warranted to determine the survival of the species over time and its contribution to the pasture in terms of plant numbers and dry matter yields. Since the rainfall received during the study was at the top end of the range of annual precipitation expected in the region, further studies are needed to determine the outcome of sowings where more typical rainfall patterns and amounts were received.

# Acknowledgments

The authors acknowledge the National University of La Rioja (UNLaR) 'Sede Chepes' for providing the necessary infrastructure for the development of the experimental work, and the National Institute of Agricultural Technology (INTA) E.E.A. La Rioja, for providing seed of *Trichloris crinita* cv. Chamical-INTA.

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(Note of the editors: All hyperlinks were verified 14 July 2021).

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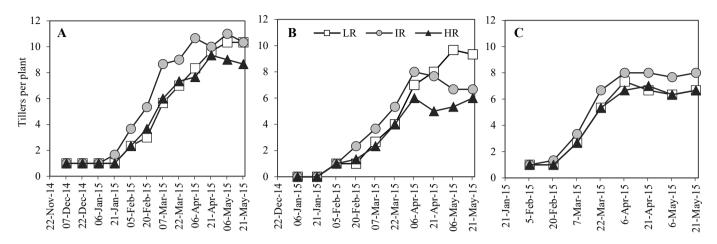
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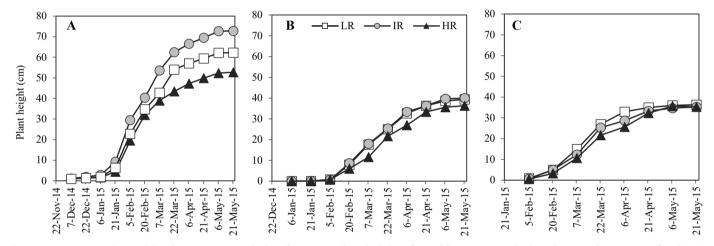
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#### **Supplementary Data**



**Supplementary Figure S1.** Time-course variation of the mean number of tillers per plant produced by Trichloris crinita plants during the growing season, for the 1st (22 November 2014) (A), 2nd (22 December 2014) (B), and 3rd (21 January 2015) (C) sowing dates. Data points for low sowing rate (LR, 0. 25 g seed/m<sup>2</sup>) are indicated by clear squares, intermediate sowing rate (IR, 0.75 g seed/m<sup>2</sup>) by gray circles and high sowing rate (HR, 1.25 g seed/m<sup>2</sup>) by black triangles.



**Supplementary Figure S2.** Time-course variation for mean plant height of *Trichloris crinita* during the growing season for the 1st (22 November 2014) (A), 2nd (22 December 2014) (B), and 3rd (21 January 2015) (C) sowing dates. Data points for low sowing rate (LR, 0. 25 g seed/m<sup>2</sup>) are indicated by clear squares, intermediate sowing rate (IR, 0.75 g seed/m<sup>2</sup>) by gray circles and high sowing rate (HR, 1.25 g seed/m<sup>2</sup>) by black triangles.

(Received for publication 19 October 2020; accepted 6 July 2021; published 30 September 2021)

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