Moisture Deficit and Defoliation Effects on White Clover Yield and Demography

J. A. García,* G. Piñeiro, S. Arana, and F. H. Santiñaque

ABSTRACT

Moisture stress and high temperatures during the summer are major factors limiting white clover (Trifolium repens L.) productivity in Uruguay. Our objective was to determine the influence of irrigation and defoliation on the growth of white clover. Two white clover cultivars were sown with tall fescue [Schedonorus arundinaceus (Schreb.) Dumort = Lolium arundinaceum (Schreb.) Darbysh.] and subjected to three defoliation regimes with and without low volume irrigations during the summer. Dry matter (DM) yield, botanical composition, number of stolons, and volunteer seedlings were recorded for 3 yr. Irrigation was the main factor affecting clover growth. Clover yields under irrigation were 5.0, 7.4, and 6.9 Mg DM ha⁻¹ in the first, second, and third year compared with 3.0, 5.1, and 1.6 Mg DM ha⁻¹ for the same years in the nonirrigated swards. Defoliation frequency did not affect pasture yields in the nonirrigated swards but the more frequent defoliation produced higher yields under irrigation after the second year. Deferring defoliation during summer did not improve clover yield or persistence. Stolons increased during autumn and winter and decreased in spring and summer. White clover declined after the second summer in the nonirrigated swards while irrigation maintained good clover stand and yield. Profuse seedlings emergences were observed but only 1% survived, suggesting that they were not a reliable mechanism for persistence. Successful reseeding occurred only at the end of the experiment after a severe drought followed by a long wet season. J.A. García, Instituto Nacional de Investigación Agropecuaria, INIA La Estanzuela, Colonia 70000, Uruguay; G. Piñeiro, IFEVA/Cátedra de Ecología, Facultad de Agronomía, UBA/CONICET, C1417DSE Buenos Aires, Argentina; S. Arana and F.H. Santiñaque, Facultad de Agronomía, EEMAC, Paysandú, Uruguay. Received 21 Oct. 2009. *Corresponding author (jgarcia@inia.org.uy).

Abbreviations: DM, dry matter; HF, high frequency; LF, low frequency; LSD, least significance difference; SC, summer closing.

V THITE CLOVER (Trifolium repens L.) is an important compo-**V** nent of pastures in Uruguay, but it usually fails to persist more than 3 or 4 yr after sowing (García, 1993). Lack of persistence in this environment has been associated with frequent moisture stress and high temperatures during summer (Widdup et al., 2006). Periods of limited soil water content are common during late spring, summer, and early autumn in southern Uruguay because of high temperatures (mean maximum temperature in January is 29°C) and rainfall variability. White clover is also a short-lived perennial in other environments such as the southeastern United States (Brink et al., 1998). Similarly, rapid declines in plant frequency of white clover during summer were observed in Australia when the available soil moisture was below 64% and mean weekly maximum temperatures exceeded 20°C (Archer and Robinson, 1989). Crowder and Craigmiles (1960) concluded that white clover in the southeastern United States was negatively affected by temperatures above 24°C regardless of soil moisture status. Soil moisture deficit, which occurs frequently during high air temperature, can be particularly detrimental to performance of white clover because of its shallow root system and poor transpiration control (Hart, 1987).

Published in Crop Sci. 50 (2010). doi: 10.2135/cropsci2009.10.0614 Published online 16 June 2010.

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Soil water deficit may influence the response of white clover pastures to defoliation management. In the southeastern United States white clover stolon branching was not affected by the grazing method during a wet summer but was reduced under continuous stocking compared with rotational grazing during a dry summer (Brink and Pederson, 1993). With high soil moisture white clover produced greater dry matter (DM) yields when intensively defoliated, while during a dry summer the clover yields were improved by decreasing cutting intensity and frequency (Brink, 1995). The importance of not overgrazing white clover swards during summer has also been reported by Brougham (1970). Furthermore, deferred grazing during summer has been shown to increase the clover content of mixed pastures by reducing soil temperatures and soil moisture deficit (Harris et al., 1997).

White clover pastures usually increase forage yields in response to irrigation. In the southeastern United States irrigation during drought periods increased forage yield of a mixed white clover-tall fescue [Schedonorus arundinaceus (Schreb.) Dumort = Lolium arundinaceum (Schreb.) Darbysh.] sward by 159% and the clover content by 17% (Craigmiles and Crowder, 1960). In Australia, frequently irrigated perennial ryegrass (Lolium perenne L.)-white clover pastures yielded 13.5 Mg DM ha⁻¹, while other treatments with less irrigation produced 8.8 and 5.9 Mg DM ha⁻¹ (Dunbabin et al., 1997). Additionally, other studies have shown that yield increments due to irrigations are generally higher in white clover than in other temperate grasses. Given frequent low volume irrigations, pure white clover swards gave an extra production of 6.1 Mg DM ha⁻¹ yr⁻¹ compared with 1.7 Mg DM ha⁻¹ yr⁻¹ for pure tall fescue swards (Johns and Lazenby, 1973a, 1973b).

Botanically, white clover is classified as a creeping perennial. The original taproot plant developed from the primary seedling is short-lived (Westbrooks and Tesar, 1955; Jones, 1980), and the subsequent growth depends on stolon development and replacement. In a white clover pasture, stolon growth usually follows a seasonal trend determined mainly by temperature and soil moisture. In New Zealand it has been found that aerial and surface stolon numbers were greatest in late summer and decline to a minimum by the end of winter—early spring (Hay et al., 1987), while in the southeastern United States the maximum stolon number is reached in early summer before declining to a minimum in the autumn (Brink et al., 1998).

Another mechanism for white clover persistence is plant regeneration through seedling recruitment. The role of reseeding in white clover had been incidentally observed in various experiments around the world but very few studies did detailed observations of the fate of the seedlings. In the southeastern United States, Brink et al. (1999) recorded around 225 seedlings emerging for

different white clover ecotypes. In another experiment even greater numbers of seedlings were observed, but the authors suggested that seedlings were not important for the stand persistence (Brink et al., 1998). In the moist hill country of New Zealand, Chapman (1987) found an average of 6.6 seedlings m⁻² yr⁻¹ but only 4% survived to form an established plant, concluding that low recruitment was mainly due to high seedling mortality and not to the lack of seeds in the soil bank. In a cool temperate climate of the United States the rate of seedling recruitments in old closed swards were shown to be limited (Barret and Silander, 1992). In contrast, Fothergill et al. (1997) suggested that seedling recruitment can play a role leading to a more balanced age structure in a clover sward in Great Britain. Overall, vegetative stolon survival is considered the main mechanism for plant persistence in temperate environments (Turkington et al., 1979), while in subtropical environments white clover persistence depends less on stolon survival and seedling regeneration is usually decisive for clover permanence (Jones, 1987).

The objectives of this study were to investigate the forage yield, stolon growth, and seedling recruitment of white clover sown with tall fescue under three defoliation regimes with and without low volume irrigation during summer.

MATERIALS AND METHODS

The experiment was initiated in August 1996 at the Instituto Nacional de Investigación Agropecuaria (INIA), Colonia, Uruguay (34° S) on a typic Argiudol amended with 400 kg ha⁻¹ of superphosphate (46% P). Zapicán and Kanopus white clover cultivars inoculated with Rhizobium were sown with tall fescue cv. Tacuabé in August 1996 and subjected to three cutting regimes with and without irrigation. Seeds were broadcast on a prepared seedbed at 6 kg ha⁻¹ for white clover and 20 kg ha⁻¹ for tall fescue on 2 by 5 m plots. The cultivars used in this study were both erect and large-leaved but differed in flowering pattern and seasonal production. Zapicán is a winter active type with early and profuse flowering (Caradus and Woodfield, 1997) and Kanopus is a ladino cultivar derived from Regal and Osceola with a later flowering period than Zapicán and higher herbage yield in spring-summer (García et al., 1999). Tacuabé is an early flowering tall fescue cultivar flowering at mid-September with no aftermath heading.

From November 1996 through April 1999 an irrigation treatment (with and without) aimed at reducing water deficits in the soil surface and improving clover survival was applied depending on recent precipitation events, from midspring to midautumn each year. Approximately 15 mm of water was applied during 6 h by overhead sprinklers (Naan 501-U, Naan-Dan Jain, Post Naan, Israel). Since most irrigation was done in the summer during daytime, we estimated an irrigation efficiency of 70%. Thus the effective water added per irrigation was approximately 11 mm. The amount of water was measured with a waterflow meter. Daily available soil water was estimated using a water balance by Thornthwaite and Mather with the potential evapotranspiration (ETo) calculated using

the Penman-Monteith method with some adjustments for conditions in Uruguay. The main hypothesis to be tested in this study was that a reduction in the number and length of periods when the soil surface is dry has an important positive effect on the forage yield and persistence of white clover. It has been reported that a dry soil surface inhibits nodal root formation and stolons and this may affect persistence of white clover (Stevenson and Laidlaw, 1985). Consequently, irrigation strategy was not intended to maximize the white clover production but to reduce the frequency and intensity of surface soil water deficit using a minimum amount of water. On average, water was added when the available soil water deficit in the top 20 cm of the soil reached a threshold of 27.4 mm, which represented 44% of the total soil available water for that layer (49 mm). Because soil dries from the surface downward, when soil available water was on average 44% in the top 20 cm of the soil, the soil surface was considered mostly dry. The amount of water at each irrigation (11 mm) was not enough to replenish soil available water to field capacity. Details of irrigation, available water, evapotranspiration, and climate are presented in Fig. 1. Considering the three irrigation seasons there were some months in which precipitation plus irrigation exceeded potential evapotranspiration. However, due to uneven rainfall distribution, monthly averages may have covered short periods of soil surface water stress.

The annual rainfall during the experiment was 1022, 1030, and 994 mm in the first, second, and third year—below normal (1120 mm) in all years. The total amount of water added in the first, second, and third irrigation season (Fig. 1) was 225, 117, and 143 mm and the corresponding number of irrigations was 22, 11, and 13, respectively. Changes in the available soil water during the experiment showed important fluctuations when represented on a mean fortnightly basis (Fig. 1), but daily values (not shown) presented more extreme variations. For example, in the nonirrigated pastures, the number of days when the soil available water was below 20% were 35, 10, and 24 d in the first, second, and third summer, while in the pastures with irrigation there were only 3 d in the second summer. Thus, the irrigation strategy effectively reduced the frequency of the surface soil water deficit.

Pastures were subjected to three cutting regimes differing in the frequency and intensity of defoliation. In the high frequency (HF) treatment forage was cut to 3.5 cm when it reached 12 cm of height, while in the low frequency (LF) treatment forage was cut to 5 cm when it reached 20 cm. On average, the HF treatment was defoliated every 25 d under irrigation and every 38 d in the nonirrigated swards, while in the LF treatment the corresponding intervals between defoliations were 37 and 48 d, respectively. A third treatment (HF+SC) was performed with the same cutting procedure as HF but not clipped during part of the summer, simulating a summer closing (SC) period or deferred grazing. Closing periods were from November 14 (nonirrigated) and December 17 (irrigated) until February 14 (92 and 59 d) in the summer of 1996-1997, from November 5 until February 17 (104 d) in the summer of 1997-1998, and from October 14 until February 10 (119 d) in the summer of 1998-1999. Seedheads were harvested at the end of each closing period in the HF+SC treatment and the threshed seed was returned to each plot. Forage DM yields were determined by cutting a 5 by 0.5 m area per plot with a rotary mower Honda HRC216 (Honda Motor Co., Tokyo, Japan) with a catch basket. Subsamples for DM content were dried at 80°C. Before each cut, the forage of two plots representing the extremes of the clover content were sampled by cutting with electric hand shears in 0.5 m⁻² quadrats, and the herbage was hand separated and weighed. Based on this calibration, the clover and fescue content of each plot was visually assessed and recorded.

Several demographic measurements were made in the pasture beginning in November 1996. The number of stolon apices (10 mm or longer) were recorded approximately every 40 d using three 125-cm² squares randomly arranged in each plot. Volunteer clover seedlings were recorded monthly in 1997 and 1999 in the HF+SC treatments using three fixed 20-cm diameter rings per plot. Seedlings were also observed during 1998 but were not recorded. Individual seedlings were marked with different colored rings in each sampling date for accurate reidentification, allowing the fate of the seedlings to be followed on different dates. Each month the seedlings previously marked were counted and classified as dead, alive, or established (more than three true leaves). Seedling observations were performed in two periods starting in July 1997 and in April 1999 and recording of new seedlings ceased in October-November of each year. Observations of seedling survival were continued until all seedlings died or were successfully established.

The experiment was arranged in a split-split plot design with four replicates. The main plots were irrigation treatments (with and without), which were divided into the three cutting regimes subplots and the two clover cultivars as sub-subplots. Forage yield data were analyzed separately for each year (Table 1) due to significant (P < 0.01) year × irrigation and year × cultivar interactions. All variables were tested by analysis of variance and treatment means were separated by Fisher's protected LSD (P < 0.05). For analysis and presentation of results individual cuts within each season were added to calculate seasonal yields that may include different numbers of harvest according to cutting frequency. For the yield comparisons of cutting frequencies and their interactions with irrigation and cultivars, analyses of variance including only HF and LF as cutting treatments were performed for each season (Table 2). Number of stolons were analyzed at each sampling date and means separated when significant. Seasonal analyses for number of stolons were performed (Table 3) with the average number for those periods when stolons increased (autumn-winter) and decreased (spring-summer) (Fig. 2). Forage yield was recorded until May 1999 and seedling survival until November 1999. Stolon data are presented until August 1999. Each year goes from March to February and the growing season was divided into autumn (March, April, May), winter (June, July, August), spring (September, October, November), and summer (December, January, February).

RESULTS AND DISCUSSION

Irrigation Effects

Irrigation increased and maintained high white clover yields across years (Tables 1 and 2). Average clover yields in the first, second, and third year of the pasture were 5.0, 7.4, and 6.9 Mg DM ha⁻¹ under irrigation and 3.0, 5.1 and 1.6 Mg DM ha⁻¹ in nonirrigated swards (Table 4). Without irrigation,

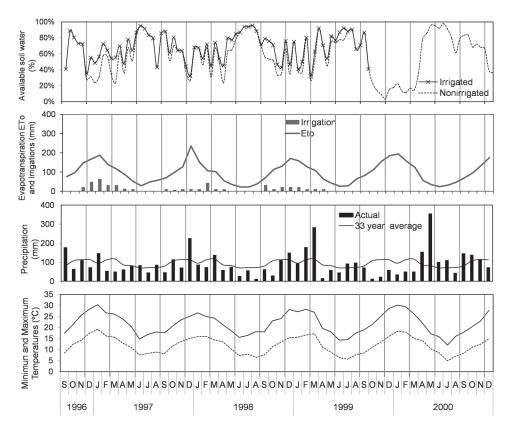


Figure 1. Available soil water, evapotranspiration (ETo) and irrigation, precipitation, and minimum and maximum temperatures.

Table 1. Statistical differences for the main effects and their interactions for white clover (WC), tall fescue (TF), and total forage yield (TOT) in each year.

| | | | | | | P | | | | |
|-----------------------|----|-----------|----------------|-----|-----------|-----|-----|-----------|----|-----|
| | | 1996–1997 | | | 1997–1998 | | | 1998–1999 | | |
| Source of variation | df | WC | TF | TOT | WC | TF | TOT | WC | TF | TOT |
| Irrigation (I) | 1 | ** | ** | ** | * | * | * | ** | ** | ** |
| Cutting regime (M) | 2 | ** | NS^{\dagger} | ** | * | NS | * | * | * | NS |
| Cultivars (C) | 1 | NS | ** | NS | *** | *** | ** | NS | * | NS |
| $M \times I$ | 2 | NS | NS | NS | NS | NS | NS | * | NS | NS |
| I × C | 1 | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| $C \times M$ | 2 | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| $M \times I \times C$ | 2 | NS | NS | NS | NS | NS | NS | NS | NS | NS |

^{*}Significant at P < 0.05.

clover yield declined in the third year, producing a strong reduction of the pasture total yield as normally occurs in rainfed pastures in Uruguay and neighboring countries (García, 1993). Clover yields in irrigated plots were higher than in the nonirrigated plots in all seasons in which water was added and from the second summer onward in all seasons including those without water additions (Table 2). Clover contents did not show significant interactions between treatments and were significantly higher in irrigated swards from the second summer onward (Table 5).

Irrigation also increased the yield of tall fescue in the pasture across years but to a lesser extent than white clover (Table 4). However, in the nonirrigated swards tall fescue did

not increase forage yields from the second to the third year, probably due to the lack of nitrogen due to the clover decline. Yield increases due to irrigation were higher on white clover than on tall fescue. Irrigation increased the yield of the white clover fraction by 2.0, 2.3, and 5.3 Mg DM ha⁻¹ in the first, second, and third year when compared to nonirrigated swards, while for the tall fescue component the corresponding increments were 0.4, 0.5, and 2.1 (Table 4). This agrees with findings reported by Craigmiles and Crowder (1960) and Johns and Lazenby (1973b) who observed that white clover was more sensitive than tall fescue to water deficits. In our experiment the average responses to irrigation during the first 2 yr were 13.5 and 2.8 kg DM mm⁻¹ of water added

^{**}Significant at P < 0.01.

^{***}Significant at P < 0.001.

[†]NS, not significant.

Table 2. Statistical differences for the main effects and their interactions for the seasonal clover yield.

| | | | | | | | P | | | | | |
|---------------------------------|----|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | | 19 | 1996 | | 1997 | | | | 19 | 98 | | 1999 |
| Source of variation | df | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn |
| Irrigation (I) | 1 | NS | ** | * | NS | NS | ** | ** | ** | ** | ** | * |
| Cutting regime (M) [‡] | 1 | NS |
| Cultivars (C) | 1 | NS | NS | NS | NS | ** | ** | NS | ** | NS | NS | NS |
| $M \times I$ | 1 | NS | NS | NS | NS | ** | ** | NS | ** | NS | NS | NS |
| I × C | 1 | NS | NS | NS | * | NS | * | NS | * | NS | * | NS |
| $C \times M$ | 1 | NS |
| $I \times C \times M$ | 1 | NS | NS | NS | NS | NS | * | NS | NS | NS | NS | NS |

^{*}Significant at P < 0.05.

Table 3. Statistical differences for the main effects and their interactions for white clover stolon number in different periods.

| | | | | P | | |
|-----------------------|----|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Source of variation | df | Autumn-winter 1997 | Spring-summer 1997 | Autumn-winter 1998 | Spring-summer 1998 | Autumn-winter 1999 |
| Irrigation (I) | 1 | NS [†] | NS | ** | ** | * |
| Cutting regime (M) | 2 | NS | *** | NS | NS | NS |
| Cultivars (C) | 1 | NS | * | NS | NS | ** |
| $M \times I$ | 2 | NS | NS | * | NS | NS |
| I × C | 1 | NS | NS | NS | NS | NS |
| $C \times M$ | 2 | NS | NS | NS | NS | NS |
| $M \times I \times C$ | 2 | NS | NS | NS | NS | NS |

^{*}Significant at P < 0.05.

for white clover and tall fescue, respectively. Similar values of 12.6 and 3.5 kg DM mm⁻¹ of water were obtained in Australia for pure swards of white clover and tall fescue, respectively (Johns and Lazenby, 1973b).

High white clover yields in irrigated plots were sustained by an elevated number of stolons throughout the experiment. A consistent effect of irrigation on stolon production was observed after autumn 1998 and until the end of the experiment (Table 3). In addition to irrigation effects, stolons showed a clear seasonal pattern increasing through autumn and winter and decreasing during spring and summer regardless of any treatment applied (Fig. 2), mainly associated with temperature and water conditions as observed in other subtropical areas of Australia (Jones, 1982), the southeastern United States (Brink et al., 1998), and Argentina (Pagano et al., 1998). Overall, irrigation during summer allowed reaching a similar maximum number of stolons (~1500 stolons m⁻²) each winter that sustained forage production through time (Fig. 2). The higher numbers of stolons in the irrigated plots were responsible for the higher clover yields even when no water was applied as in the winter 1998 (Table 2). It has been suggested that surface soil moisture is a critical factor determining the growth and development of new nodal roots and stolons (Stevenson and Laidlaw, 1985; Thomas, 1987). Therefore, our strategic water additions reduced the frequency and intensity of surface soil moisture deficits during summer and prevented the stolon losses observed in the nonirrigated swards.

Profuse seedling emergence was observed each winter and spring that recurrently died before or during the summer. Except in April of 1999, seedling emergence was generally higher in the nonirrigated plots (Table 6) and since no water was added during the winter, those differences should be ascribed to previous effects of irrigation. April of 1999 was very dry and water was added (Fig. 1), and this may have been responsible for the higher number of seedlings that emerged in the irrigated plots at that date. The results agree with the report of Jones (1982) from Australia who found that, compared with a moist site, seedling emergence was normally higher at a dry site although seed reserves were higher on the moist site. He attributed the higher germination on the dry site to the higher soil temperature fluctuations during the previous summer that promoted seed dormancy breaking.

The amounts of seeds produced and returned to the soil during the closing periods (Table 7) suggest that the

^{**}Significant at P < 0.01.

^{***}Significant at P < 0.001.

[†]NS, not significant.

[‡]High frequency and low frequency treatments.

^{**}Significant at P < 0.01.

^{***}Significant at P < 0.001.

[†]NS, not significant.

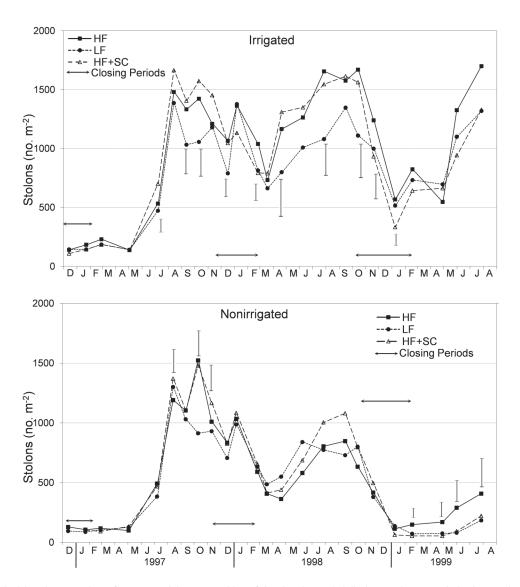


Figure 2. Number of white clover stolons (average of the two cultivars) for the three defoliation regimes and closing periods for the HF+SC treatments, in irrigated and nonirrigated plots. Bars indicate LSD at $P \le 0.05$; otherwise, means are not different.

seed bank was not a limiting factor for seedling recruitment. Although high numbers of seedlings were observed each year, only ~25% of those seedlings reached the stage of established (more than three true leaves) and almost all died before or during the following summer (Table 7, Fig. 3). However, a successful reseeding was observed at the end of the experiment. Irrigation was suspended in the spring of 1999, and after a severe drought during the spring and summer of 1999-2000 (Fig. 1) that killed all white clover plants followed by a long wet season, a new white clover stand regenerated from seedlings. In November 2000, 202 m⁻² new taproot plants were counted in the HF+SC treatments that successfully established and survived after the next summer. The number of new re-established plants was similar to the initial clover plants at the beginning of the experiment in November 1996 (250 plants m⁻²). The reconstituted pasture yielded 1821 kg DM ha⁻¹ after 35 d of regrowth in November 2000, with 49% of white clover (the rest were old tall fescue plants).

In summary, the high white clover forage yields in irrigated plots were sustained by an elevated number of stolons and not by natural reseeding, suggesting that under good climatic conditions white clover favors vegetative reproduction while after severe droughts seedlings may play an important role in clover persistence. Our results agree with those of Archer and Robinson (1989) who found that in New South Wales (Australia) seedling establishment was effective in 1 of 5 yr and was associated with the death of white clover during summer followed by cool and moist weather. Similarly, the low frequency of occurrence (1 out of 5 yr) in our study indicates that reseeding was not a reliable mechanism for clover persistence. The results obtained with our strategy of frequent low volume irrigations support the hypothesis that reductions in the surface soil moisture deficit have important impacts on the stolon production and survival. Furthermore, since the irrigated treatments had water additions only 5% above normal precipitation, probably the rainfall

Table 4. The effect of irrigation, cutting regimes and cultivars on the white clover (WC), tall fescue (TF), and total forage yield (TOT) in each year.[†]

| | | | | 1996–199 | 97 | | 1997–199 | 98 | | 1998–199 | 19 |
|----------------------|-----------------|---------------------|------|----------|------|------|----------|-----------------|------|----------|-------|
| Irrigation treatment | Cutting regime‡ | Clover cultivar | WC | TF | TOT | WC | TF | TOT | WC | TF | TOT |
| | | | | | | | Mg DM ha | a ⁻¹ | | | |
| With | HF | Average | 5.13 | 0.73 | 5.86 | 7.69 | 2.79 | 10.53 | 8.27 | 3.67 | 12.07 |
| | LF | Average | 5.53 | 0.84 | 6.37 | 7.93 | 2.79 | 10.76 | 6.44 | 4.26 | 11.01 |
| | HF+SC | Average | 4.48 | 0.89 | 5.37 | 6.64 | 3.06 | 9.72 | 6.10 | 4.82 | 11.37 |
| Without | HF | Average | 3.61 | 0.44 | 4.13 | 5.59 | 2.39 | 8.34 | 1.51 | 1.81 | 4.13 |
| | LF | Average | 3.18 | 0.46 | 3.69 | 5.37 | 2.46 | 7.87 | 1.63 | 2.44 | 4.53 |
| | HF+SC | Average | 2.29 | 0.35 | 2.71 | 4.50 | 2.36 | 7.14 | 1.65 | 2.27 | 4.62 |
| | LSE | 0.05 | 0.69 | 0.17 | 0.77 | 1.15 | 0.49 | 1.21 | 0.87 | 0.77 | 1.16 |
| | CV, % | | 14 | 15 | 12 | 12 | 13 | 9 | 27 | 16 | 14 |
| With | Average | Average | 5.05 | 0.82 | 5.87 | 7.42 | 2.88 | 10.34 | 6.94 | 4.25 | 11.48 |
| Without | Average | Average | 3.03 | 0.42 | 3.51 | 5.15 | 2.41 | 7.78 | 1.60 | 2.17 | 4.42 |
| LSD (0,05) | | | 0.60 | 0.13 | 0.58 | 2.05 | 0.10 | 2.49 | 1.86 | 0.82 | 2.19 |
| Average | HF | Average | 4.37 | 0.59 | 4.99 | 6.64 | 2.59 | 9.43 | 4.89 | 2.74 | 8.10 |
| Average | LF | Average | 4.36 | 0.65 | 5.03 | 6.65 | 2.62 | 9.32 | 4.03 | 3.35 | 7.77 |
| Average | HF+SC | Average | 3.39 | 0.62 | 4.04 | 5.57 | 2.71 | 8.43 | 3.88 | 3.55 | 8.00 |
| | LSE | 0.05 | 0.49 | NS§ | 0.54 | 0.81 | NS | 0.85 | 0.61 | 0.55 | NS |
| Average | Average | Kanopus | 4.04 | 0.58 | 4.64 | 7.00 | 2.28 | 9.41 | 4.19 | 3.02 | 7.70 |
| Average | Average | Zapicán | 4.03 | 0.66 | 4.73 | 5.57 | 3.00 | 8.71 | 4.34 | 3.40 | 8.21 |
| | | LSD _{0.05} | NS | 0.05 | NS | 0.45 | 0.20 | 0.49 | NS | 0.30 | NS |

[†]DM, dry matter.

Table 5. White clover percentages averages for each main effect in the different seasons.

| | | Irrig | gation | | Cutting | regime [†] | | Cultivar | | |
|-----------|--------|-------|---------|-----------------|---------|---------------------|----|----------|---------|----|
| Year | Season | With | Without | P | HF | LF | P | Kanopus | Zapicán | P |
| | | | | | | % | | | | |
| 1996–1997 | Spring | 89 | 89 | NS [‡] | 89 | 89 | NS | 89 | 90 | NS |
| | Summer | 86 | 86 | NS | 87 | 85 | NS | 87 | 84 | * |
| 1997–1998 | Autumn | 60 | 55 | NS | 48 | 67 | * | 60 | 56 | NS |
| | Winter | 81 | 78 | NS | 78 | 80 | NS | 80 | 78 | NS |
| | Spring | 76 | 73 | NS | 78 | 71 | * | 81 | 69 | ** |
| | Summer | 66 | 53 | * | 59 | 60 | NS | 70 | 49 | ** |
| 1998–1999 | Autumn | 50 | 27 | * | 40 | 37 | NS | 38 | 39 | NS |
| | Winter | 70 | 54 | * | 67 | 58 | NS | 55 | 69 | ** |
| | Spring | 75 | 52 | * | 67 | 60 | * | 60 | 67 | * |
| | Summer | 62 | 23 | * | 50 | 34 | ** | 44 | 42 | NS |
| 1999 | Autumn | 42 | 17 | * | 36 | 28 | NS | 29 | 31 | NS |

^{*}Significant at P < 0.05.

distribution more than the total annual precipitation could be the main factor involved in the poor white clover persistence in this environment.

The Effects of Defoliation Frequency

When comparing the frequent (HF) vs. the infrequent (LF) defoliation treatments the cutting regime affected white

clover annual and seasonal yields differentially in time. In the first 2 yr, HF and LF treatments produced similar yields (Table 4), but a significant cutting regime × irrigation interaction occurred in the third year (Table 1) because the more frequent defoliation (HF) increased clover yields under irrigation, but there were no differences between defoliation treatments in the rainfed swards. The same occurred on a

[‡]HF, high frequency; LF, low frequency; SC, summer closing.

[§]NS, not significant.

^{**}Significant at P < 0.01.

[†]HF, high frequency; LF, low frequency.

[‡]NS, not significant.

Table 6. Number of seedlings (average of the two cultivars) appearing in different months in 1997 and 1999 in irrigated and nonirrigated plots of the HF+SC treatments.

| Year | Irrigation treatment | July | Aug. | Sept. | Oct. | Nov. | Mean |
|------|----------------------|--------|-------|-------|-----------------|------|------|
| | | | | no. m | 1 ⁻² | | |
| 1997 | With | 137ab† | 102bc | 36cd | 23d | 11d | 62 |
| | Without | 208a | 174ab | 60cd | 51cd | 51cd | 109 |
| | Mean | 172 | 137 | 48 | 38 | 31 | |
| 1999 | | Apr. | June | July | Sept. | Oct. | Mean |
| | With | 237a | 80cd | 146b | 71cd | 5e | 108 |
| | Without | 31de | 114bc | 151b | 145b | 32de | 95 |
| | Mean | 134 | 96 | 149 | 108 | 18 | |

[†]Within each year means with different letters are significant at $P \le 0.05$.

Table 7. Seed yield of white clover (average of the two cultivars) harvested and returned to the plots in the high frequency plus summer closing (HF+SC) treatments and number of seedlings (average of the two cultivars) appearing and surviving in the subsequent months.[†]

| | Seed | yield | Seedlings appearing | | | llings iving |
|------|------|---------------------|---------------------|------|-----------------|-----------------|
| Year | NI | I | NI | I | NI | I |
| | kg l | na ⁻¹ —— | | no. | m ⁻² | |
| 1997 | 27a | 36a | 544a‡ | 308b | 4a | За |
| 1998 | 45a | 62b | NR | NR | NR | NR |
| 1999 | nil | 75 | 473a | 539a | 8a | 4a |

[†]NI, nonirrigated; I, irrigated; NR, not recorded.

seasonal basis, when cutting frequencies had no significant effects on white clover yield on any season, but presented a significant irrigation × defoliation interaction during the second half of the second year and in the winter of the third year (Table 2). During the spring of 1997 LF outyielded HF under irrigation while HF produced more than LF in nonirrigated pastures (Table 8). In the summer of the second year the HF produced more than the LF treatment irrespective of clover cultivar in the irrigated pastures, while in the nonirrigated swards Kanopus yielded more with the LF treatment and Zapicán was not affected by cutting frequency. In the winter 1998 HF outyielded LF treatment in the irrigated plots, but there were no differences in the nonirrigated swards (Table 8). On the other hand, tall fescue yields were not affected by the cutting regimes during the first 2 yr but in the third year and in contrast with white clover, tall fescue yield with HF treatment was lower than with the LF treatment (Table 4).

The number of white clover stolons was not significantly affected by the cutting treatments (Table 3) except in the spring–summer 1997 when the HF treatment (1127 stolons m⁻²) had higher stolons compared with the LF treatment (954 stolons m⁻²). An interaction between cutting regime and irrigation occurred in the autumn–winter 1998 when the more frequently defoliated treatments showed higher numbers of stolons (1204 for HF and 1249 for HF+SC stolons m⁻²) than the LF treatment (888 stolons m⁻²) under irrigation, while in the nonirrigated swards

there were no differences between cutting regimes (Fig. 2). This explains the observed cutting frequency × irrigation interaction for clover yield in the winter 1998 (Table 2), when the highest clover yields in the irrigated plots were produced by the frequent defoliation. The cumulative effects of irrigation and cutting regimes on clover yield were evident in the third year when white clover declined in nonirrigated swards regardless of defoliation management, while in the irrigated plots clover yields were higher with the more frequent defoliation (Tables 4 and 8).

The Effects of Summer Closing

Summer closing (HF+SC treatment) decreased white clover forage production collected at the end of the closing period when compared to HF defoliations, except for the first year under irrigation when both defoliation treatments produced similar forage yields (Table 9). The senescence of clover leaves during the closing period was probably the main reason behind clover yield declines. Herbage losses of white clover due to desiccation and senescence during summer under LF defoliations were observed in the United States by Brink (1995). In Australia it was found that during the hottest months up to 70% of the white clover herbage died in nonirrigated swards and 20% under irrigation (Johns and Lazenby, 1973a). The similar forage yields of HF and HF+SC observed in the first year in irrigated swards (Table 9) could be due to the relatively short closing period that year (59 d) that probably reduced losses by senescence.

Summer closing did not increase white clover growth rates in the following autumn after closing as expected initially. White clover yields after the SC periods in the HF and HF+SC treatments were similar except in the first year in the irrigated plots where HF+SC outyielded the HF treatment (Table 9). In that first summer the closing period occurred 140 d after sowing, when the pasture was dominated by white clover (Table 5) based mainly on taproot plants and with a low stolon density of about 200 stolons m⁻² (Fig. 2). During the deferred period in the irrigated plots that year (59 d) the HF treatment was defoliated four times and this may have depleted the energy reserves of that predominantly taproot plant population reducing autumn yield compared to the HF+SC treatment. A different

 $^{^{\}ddagger}$ For each year means with different letters are significant at $P \le 0.05$.

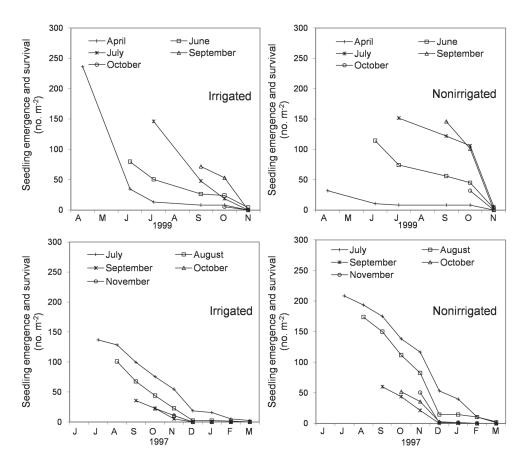


Figure 3. White clover seedlings emergence and survival (average of the two cultivars) in different months in 1997 and 1999, in irrigated and nonirrigated plots.

Table 8. The effect of irrigation, cutting regime, and cultivar on the seasonal clover yield.†

| | | | 19 | 996 | | 19 | 97 | | | 19 | 98 | | 1999 |
|------------|----------------|----------|--------|--------|--------|--------|---------|------------------------|--------|--------|--------|--------|--------|
| Irrigation | Cutting regime | Cultivar | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn | Winter | Spring | Summer | Autumn |
| | | | | | | | | kg DM ha ⁻¹ | | | | | |
| With | HF | Zapicán | 1880a‡ | 2884b | 1024ab | 1955a | 2310cd | 1726bc | 1759a | 2477a | 2366a | 1915b | 805a |
| With | HF | Kanopus | 1855a | 3644ab | 850ab | 1632ab | 3056ab | 2837a | 1454a | 1361bc | 2443a | 2760a | 932a |
| With | LF | Zapicán | 1882a | 3813a | 1545a | 1708ab | 2854bc | 1165de | 1784a | 1740b | 2168ab | 1390b | 778a |
| With | LF | Kanopus | 1672a | 3698a | 1402a | 1576ab | 3489a | 2122b | 1404a | 1085cd | 1766b | 1872b | 682a |
| Without | HF | Zapicán | 1951a | 1637c | 440b | 1443b | 2186d | 954ef | 492b | 907cde | 355c | 172c | 85b |
| Without | HF | Kanopus | 1888a | 1756c | 425b | 1584ab | 2913abc | 1234cde | 331b | 444e | 216c | 111c | 33b |
| Without | LF | Zapicán | 1839a | 1409c | 541b | 1314b | 1740d | 632f | 389b | 948cde | 289c | 76c | 42b |
| Without | LF | Kanopus | 1748a | 1373c | 745b | 1430b | 2747bc | 1594cd | 572b | 727de | 191c | 63c | 37b |

[†]DM, dry matter; HF, high frequency; LF, low frequency.

situation occurred in the second and third summer in the irrigated plots when the clover population was in the clonal phase (Brock and Hay, 2001) with more than 1200 stolons m⁻² (Fig. 2). In both years, the accumulated clover yields during the deferred periods were increased as cutting intervals decreased and the subsequent autumn yields were not affected by the summer defoliation management (Table 9).

Our results suggest that summer closing was not a good alternative for maintaining high white clover contents in the pasture or increasing its forage yields as was reported to occur in other environments (Watson et al., 1996; Harris et al., 1997), while tall fescue seems to have capitalized on the beneficial effects of summer resting periods, especially in irrigated treatments. In contrast to white clover, summer closing typically increased tall fescue forage production in irrigated plots both during and after the closing period but did not produce important differences in nonirrigated treatments (Table 9). In general, the results indicate that white clover was more sensitive than tall fescue to variations in defoliation frequency and moisture conditions during summer. Without irrigation, SC did not increase stolon survival (Table 3 and Fig. 2)

[‡]Within each season, means with different letters are significant at $P \le 0.05$.

Table 9. The effect of deferring pasture over summer on the white clover and tall fescue yield (means of two clover cultivars), with and without irrigation in each year.

| | | | Nonirrigated | | | Irrigated | |
|--------------|----------------|---------------------|--------------|----------------------|---------------------|-----------|----------------------|
| | | Deferred | period | | Deferred | period | |
| Year | Cutting regime | No. of defoliations | Yield | Next autumn yield | No. of defoliations | Yield | Next autumn yield |
| | | | kg l | OM ha ⁻¹ | | kg D | M ha ⁻¹ |
| White clover | | | | | | | |
| 1996-1997 | HF | 3 | 1695a‡ | 431 | 4 | 1477a | 936a |
| | LF | 2 | 1390a | 643 | 3 | 2584b | 1473ab |
| | HF+SC | 1 | 561b | 477 | 1 | 1448a | 1554b |
| 1997–1998 | HF | 3 | 1725a | 411 | 4 | 3134a | 1605 |
| | LF | 3 | 1712a | 479 | 3 | 2679b | 1594 |
| | HF+SC | 1 | 608b | 579 | 1 | 1218c | 1814 |
| 1998-1999 | HF | 1 | 141 | 58 | 5 | 3177a | 868 |
| | LF | 1 | 70 | 39 | 3 | 1925b | 730 |
| | HF+SC | 1 | 115 | 25 | 1 | 1247c | 655 |
| Tall fescue | | | | | | | |
| 1996-1997 | HF | 3 | 226 | 331 | 4 | 292a | 539a |
| | LF | 2 | 254 | 335 | 3 | 501b | 546a |
| | HF+SC | 1 | 142 | 303 | 1 | 457b | 703b |
| 1997-1998 | HF | 3 | 1179 | 1021 | 4 | 1314ab | 1380a |
| | LF | 3 | 1131 | 1116 | 3 | 1223b | 1773b |
| | HF+SC | 1 | 1274 | 1111 | 1 | 1574a | 1696b |
| 1998–1999 | HF | 1 | 276a | 201 | 5 | 1095a | 848 |
| | LF | 1 | 459ab | 189 | 3 | 939a | 918 |
| | HF+SC | 1 | 664b | 184 | 1 | 1884b | 919 |

[†]DM, dry matter; HF, high frequency; LF, low frequency; SC, summer closing.

and therefore did not increase white clover performance. However, our results suggest that with irrigation, or probably in a wet summer, the SC of tall fescue—white clover pasture may reduce the harvestable yield of clover during the summer and will promote tall fescue growth that may depress clover growth in the future.

The Effects of Cultivars

Cultivars presented different seasonal patterns of forage production especially when limiting factors were removed as in the irrigated plots, but annual yields were similar between both cultivars except in the second year when Kanopus yielded more than Zapicán (Tables 1 and 4). On a seasonal basis, Kanopus outyielded Zapicán in the spring and summer of 1997 and Zapicán produced more in the winter of 1998. This different seasonal pattern of growth may be responsible for the irrigation × cultivar interactions observed in the winter and summer of 1997 and 1998 (Tables 2 and 8). During the winters of both years, the differences between cultivars (Zapicán > Kanopus) were higher under irrigation than in the rainfed pastures. Since no water was applied during the winter, this interaction can be attributed to the higher number of stolons of the irrigated plots (Fig. 2) as well as to the greater winter potential of Zapicán. Similarly, during the summers of 1997 and 1998 when Kanopus outyielded Zapicán,

the differences between cultivars were also higher under irrigation. Clover percentages in the pastures also varied between cultivars in a similar way to forage yields since Kanopus swards had higher clover percentages in the spring-summer of 1997 and Zapicán in the winter of 1998 (Table 5). In spite of the different yield seasonality, the stolon numbers of both cultivars presented small differences during the experiment (Table 3). Kanopus had on average more stolons in the spring-summer of 1997, but the differences were small in magnitude (1105 vs. 1044 stolons m⁻²). Zapicán showed higher stolon number than Kanopus (800 vs. 590 stolons m⁻²) only at the end of the experiment in the autumn-winter of 1999. After the second summer, both cultivars declined in the nonirrigated swards regardless of defoliation management and produced very low yields in the third year in contrast with the irrigated plots where yields were maintained (Table 8).

Tall fescue growth was affected by the accompanying white clover cultivar (Table 1). With irrigation, tall fescue yields were higher in swards with Zapicán than in swards with Kanopus in all years, while in the nonirrigated swards significant differences were observed only in the second year (Table 10). The distinct seasonal growth pattern of each clover cultivar explain these results: Zapicán has earlier flowering and less potential for spring—summer growth

[‡]Within columns and for each year, means with different letters are significant at $P \le 0.05$.

than Kanopus and consequently favors the tall fescue growth during spring-summer especially under irrigation.

CONCLUSIONS

Our study allowed the evaluation of the yield and demographic evolution of two white clover cultivars sown with tall fescue under different water status and defoliation regimes in a scarcely studied environment of South America. Low volume irrigation during the warmer months can drastically improve white clover stolon production, increasing yield and persistence. Defoliation frequency was not an important factor conditioning white clover production or persistence but higher frequencies may increase yields under well-watered conditions. Stolons showed a clear seasonal pattern of growth, increasing during autumn and winter and decreasing during spring and summer regardless of any treatment applied and probably determined by environmental conditions. Summer closing decreased white clover yields during the closing periods and had no effect on stolon production but increased tall fescue yields, thus did not represent a good alternative for increasing white clover production and persistence. The two white clover cultivars analyzed presented different seasonal growth patterns, with Zapicán being more productive in the winter and Kanopus in the summer, that affected the accompanying tall fescue growth. White clover permanence appears to be based mainly on persistence of stolons, and seedling recruitment does not seem to be a reliable mechanism for persistence in most years but can be important after severe drought followed by wet seasons.

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Table 10. The effect of the white clover cultivar with and without irrigation (average of the three cutting regimes) on the tall fescue yield in each year.[†]

| White clover cultivar | 1996–1997 | 1997–1998 | 1998-1999 |
|-----------------------|-----------|----------------------------|-----------|
| | | – kg DM ha ⁻¹ – | |
| Zapicán + irrigation | 886a‡ | 3329a | 4536a |
| Zapicán | 438c | 2667b | 2281c |
| Kanopus + irrigation | 754b | 2425b | 3960b |
| Kanopus | 401c | 2143c | 2069c |

†DM, dry matter.

[‡]Within years means with different letters are significant at $P \le 0.05$.

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