



Variability of Alfalfa (*Medicago sativa* L.) Seasonal Forage Production in the Southwest of Uruguay

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Summary

Weather conditions determine seasonal forage production. Air temperature, solar radiation, and soil water availability are the main variables affecting alfalfa growth. This study analyzed the relationship between alfalfa growth (*Medicago sativa* L.) and some climatic variables along 15 years (1997 to 2011) of production and climate data, collected in the southwest of Uruguay. The results highlighted that alfalfa growth rate (GR) presented significant differences among seasons and varied with pasture age. The alfalfa growth rate increased in autumn when the accumulated radiation was less than or equal to 1095 MJ m⁻² period⁻¹ and the difference between atmospheric demand and rainfall (cWB) was close to 0 mm. In winter, the GR increased with minimum temperatures up to 8.4 °C and daily average radiation higher than 11 MJ m⁻² day⁻¹. In spring the GR was higher during the years with daily radiation higher than 16 MJ m⁻² day⁻¹. Maximum air temperatures above 27.5 °C affected negatively summer GR. The highest GR (62.5 kg ha⁻¹ day⁻¹) was achieved in summer when the ETa:ETm ratio was close to one. This result suggests the implementation of field techniques that increase water-use efficiency, as well as summer irrigation as a management practice to achieve alfalfa forage potential.

Keywords: water deficit, growth rate

Variabilidad de la producción estacional de forraje de alfalfa (*Medicago sativa* L.) en el suroeste de Uruguay

Resumen

La producción estacional de forraje de pasturas está determinada por las condiciones climáticas. La temperatura del aire, la radiación solar y la disponibilidad de agua en el suelo son las principales variables que afectan el crecimiento de la alfalfa. En este trabajo se analizó la relación entre el crecimiento de la alfalfa (*Medicago sativa* L.) y algunas variables climáticas usando 15 años (1997-2011) de datos productivos y climáticos recolectados en el suroeste de Uruguay. Los resultados mostraron que la tasa de crecimiento (TC) de la alfalfa presenta diferencias significativas entre las estaciones del año y varía con la edad de la pastura. La TC fue favorecida cuando la radiación solar acumulada en otoño fue menor o igual a 1095 MJ m⁻² período⁻¹ y la diferencia entre la demanda atmosférica y la precipitación (BHc) fue cercana a 0 mm. En invierno la TC se incrementó hasta temperaturas mínimas de 8,4 °C y la radiación solar diaria promedio fue mayor a 11 MJ m⁻² día⁻¹. En primavera la TC fue mayor en años con radiación diaria superior a 16 MJ m⁻² día⁻¹. Temperaturas máximas del aire mayores a 27,5 °C afectaron negativamente la TC estival. Las mayores TC (62,5 kg ha⁻¹ día⁻¹) se lograron en verano, cuando la relación ETa:ETm fue cercana a uno, resultado que podría justificar medidas de manejo que mejoraran la eficiencia del uso del agua en el suelo, así como la incorporación de riego suplementario en verano para alcanzar el potencial forrajero de la alfalfa.

Palabras clave: déficit hídrico, tasa de crecimiento

Introduction

Alfalfa (*Medicago sativa* L.) is a summer legume that presents high forage yield potential, good persistence and great tolerance to drought and frost, associated with the accumulated reserves in its crown⁽¹⁾⁽²⁾. The extensive genetic and phenotypic variation found in alfalfa allows its cultivation in diverse climates⁽³⁾. These characteristics make alfalfa a great alternative among pastures grown in Uruguay⁽⁴⁾⁽⁵⁾⁽⁶⁾.

The most important climatic variables influencing alfalfa growth are temperature⁽⁷⁾⁽⁸⁾, solar radiation⁽⁹⁾, evapotranspiration⁽¹⁰⁾⁽¹¹⁾ and rainfall regime. The latter two variables are strongly associated with alfalfa productivity concerning deficit and excess of soil water. Uruguay has a sub-humid temperate subtropical climate⁽¹²⁾, presenting a relatively stable thermal regime between years. These climatic conditions allow the favorable development of alfalfa⁽⁴⁾, with optimal growth temperatures between 25-30 °C⁽⁷⁾, considering a wide range of temperatures for growth from 5 to 30 °C, with an optimal daytime temperature of 15 to 25 °C, and 10 to 20 °C optimal night temperature⁽¹³⁾⁽¹⁴⁾⁽¹⁵⁾. On the other hand, frosts in winter and early spring, as well as the intensity and frequency of water deficit in spring and summer, also limit alfalfa growth⁽⁶⁾. Annual alfalfa production varies widely in Uruguay; the contribution of each growing period to the annual yield is influenced by current climatic conditions and by pasture age, particularly in the first year after installation⁽¹⁶⁾. In this regard, climatic variables influence alfalfa growth differently according to the season of the year⁽¹⁷⁾.

This study aims to estimate the relative contribution of rainfall, solar radiation, temperature, evapotranspiration and water balance in seasonal alfalfa production in southwestern Uruguayan conditions. These findings will allow to estimate forage production based on climate forecasts and to take appropriate management measures when facing climate change scenarios.

Material and Methods

Alfalfa forage production

Analyzed data correspond to experimental results from rainfed forage alfalfa production in the framework of the National Cultivar Evaluation, INASE-INIA agreement, between 1997 and 2011 at INIA La Estanzuela (34° 20' 48", 46" S; 57° 43' 48". 74" W), Uruguay⁽¹⁸⁾⁽¹⁹⁾⁽²⁰⁾⁽²¹⁾⁽²²⁾⁽²³⁾⁽²⁴⁾⁽²⁵⁾⁽²⁶⁾⁽²⁷⁾⁽²⁸⁾⁽²⁹⁾⁽³⁰⁾⁽³¹⁾⁽³²⁾. The

seeding of the experiments was carried out in April, except in 1997, 1998 and 2007, which took place in May, and in June in 2002. The experimental unit in each trial was a plot of six furrows of 5 m long spaced 0.16 m from each other (total area 4.8 m²). Seeding density was 20 kg seeds ha⁻¹, corrected by the germination percentage. Fertilization was applied at seeding time according to soil analysis; phosphorus was added to a final concentration of 17 ppm of P₂O₅, and nitrogen to a maximum of 20 kg N ha⁻¹ to ensure implantation. Every time the average of the alfalfa genotypes reached a height of 20 cm and/or regrowths from the crown were observed, the evaluation of forage production was carried out in the three central furrows corresponding to an area of 2.65 m². Forage harvest was conducted with a mechanical cutter with a collection bag, and field fresh weight was obtained. A forage sample was oven-dried at 60 °C to obtain dry matter percentage, to finally express the yield in kg ha⁻¹ of dry matter. The seeding year was considered the first year of pasture life; and the following, as the second and third year of pasture life. In the latter, the test evaluation finished at the end of November by the protocol; therefore, the production of the third summer of pasture life was not recorded. Alfalfa trials were refertilized with phosphorus at the beginning of their second and third autumn. A randomized incomplete block design was conducted by triplicates. Information was extracted from the database for two cultivars used as test controls, Estanzuela Chaná (E_CHANA) and Crioula (CRIOU), both of intermediate latency. The dry matter production per hectare (kg DM ha⁻¹) was calculated between each season cutting dates, for each cultivar, season and year. The cutting date information was used to estimate the length of the period between cuttings. Daily GR between cuttings (kg DM ha⁻¹ day⁻¹) was calculated from the forage yield (kg DM ha⁻¹) and the duration of each period between cuttings. Cuttings were grouped and averaged according to seasons: autumn (A) from March 1st to May 31st, winter (W) from June 1st to August 31st, spring (Sp) from September 1st to November 30th, summer (Su) from December 1st to February 28th for each particular year, so that the GR of a particular season and year could be constituted by one, two or three cuttings.

The climatic data used, covering the period 1997-2011, were obtained from INIA La Estanzuela meteorological station, 4 km from where the experiments were carried out. The climatic variables analyzed were: minimum (Tmin), medium (Tmed) and maximum (Tmax) daily air temperature (°C); daily incident radiation (Rs_RADIAT)

(MJ m⁻² day⁻¹); accumulated incident radiation (RADIAT_Accu), period addition between cuttings, (MJ m⁻² period⁻¹); accumulated rainfall in the period between cuttings (RAIN) (mm); effective accumulated rainfall (Pe) (mm)⁽³³⁾ and reference evapotranspiration (ETo, mm)⁽³⁴⁾. Maximum pasture evapotranspiration (ETm) corresponded to the reference evapotranspiration (ETo) times the cultivation coefficient (Kc) between cuttings⁽³⁴⁾. Average values of 0.40; 0.95; and 0.90 for the initial Kc, medium Kc, and final Kc were used; when pasture cuttings within the season occurred, values of 0.40; 1.20 and 1.15 were used respectively. These Kc coefficient values were applied immediately after cutting; in full coverage; and immediately before cutting, respectively. The growing season was defined as a set of individual cutting periods⁽³⁴⁾. The actual evapotranspiration (ETa) was estimated through the soil water balance Winsareg model⁽³⁵⁾. This model, due to its simplicity and versatility, allows estimating, on daily basis, water content of the soil for a particular year or group of years, as well as water requirements and deficits magnitude according to the pre-established cultivation criteria or different irrigation strategies based on soil variables, potential evapotranspiration and rainfall or irrigation, and crop parameters, including simple Kc⁽³⁵⁾. This procedure allowed to obtain the ETa:ETm ratio and the difference between rainfall and maximum evapotranspiration (climatic water balance, cWB, mm). The average or accumulated value of the direct or derived meteorological variables for each period between pasture cuttings was calculated, obtaining for each period the pasture GR associated with an average or accumulated value of the climatic variable or a calculated variable.

Parameters of the Winsareg model

The daily soil water balance was estimated during the evaluation period 1997-2011. To this goal, the same INIA-

LE climate database was used, together with the water retention characteristics of the soil profile where the pasture was planted, as well as the Kc, following the respective phenology and cuttings. The crop Kc was taken from the FAO guide No. 56⁽³⁴⁾, following the cutting dates and growth stages adjusted to each season dates in each particular year. The soil was classified as a *Brunosol Eutricto Tipico* LAc V (Table 1). The intended effective depth for the root system was 60 cm with a threshold (p) = 0.50, which represents the maximum depletion fraction of available water in the soil at root depth, to which the pasture growth is not affected by the reduction of water content in the soil. The contribution of water coming from lower layers and the effect of soil salinity were considered null due to their low significance in local conditions.

Statistical analysis

To study the magnitude of variability between years and between seasons within the years of climatic variables, regardless of a particular year or season, an analysis of the variance components was carried out, adjusting a Mixed Linear Model MLM⁽³⁶⁾ with a random effect of the years and seasons. The variables used in this analysis were: Tmin, Tmed, Tmax, Rs_RADIAT, RADIAT_Accu, RAIN, Pe, ETa, ETm, ETa:ETm, and cWB. To evaluate the effect of the season of the year, the pasture age, the cultivar and its interactions on the GR, the ANOVA was carried out. The mean comparison was made using Fisher's least significant difference test (LSD) for a significance level of $\alpha = 0.05$. To relate the effect of climatic variables on GR, linear regression analysis, two-section non-linear regression and multivariate analysis using regression trees (CART)⁽³⁷⁾ were performed. All analyses were performed using the INFOSTAT⁽³⁸⁾ software and its interface with the R⁽³⁹⁾ software.

Table 1. Gravimetric soil water content (g. g⁻¹). Brunosol Eutricto Tipico LAc V. INIA LE.

Depth	Field Capacity (0.01 Mpa)	Permanent Wilting Point (1.5 Mpa)	Bulk Density
0-30 cm	0.34	0.16	1.2
30-42 cm	0.35	0.22	1.3
42-72 cm	0.36	0.23	1.3
72-110 cm	0.32	0.21	1.4

Results and Discussion

Climate variables

High dispersion of values found for the main meteorological variables in southwestern Uruguay is mainly explained by the dispersion within the seasons and the differences between seasons (Figure 1). These

climatic characteristics are frequent in temperate sub-humid climates of similar latitudes⁽¹²⁾. This strong climatic seasonality conditions the growth of most pastures, both natural and cultivated (Figure 1). During the annual growth cycle, temperature, radiation and water availability will determine the seasonality of alfalfa forage production⁽⁷⁾⁽¹³⁾⁽¹⁴⁾. At the same time we found an important variation in the

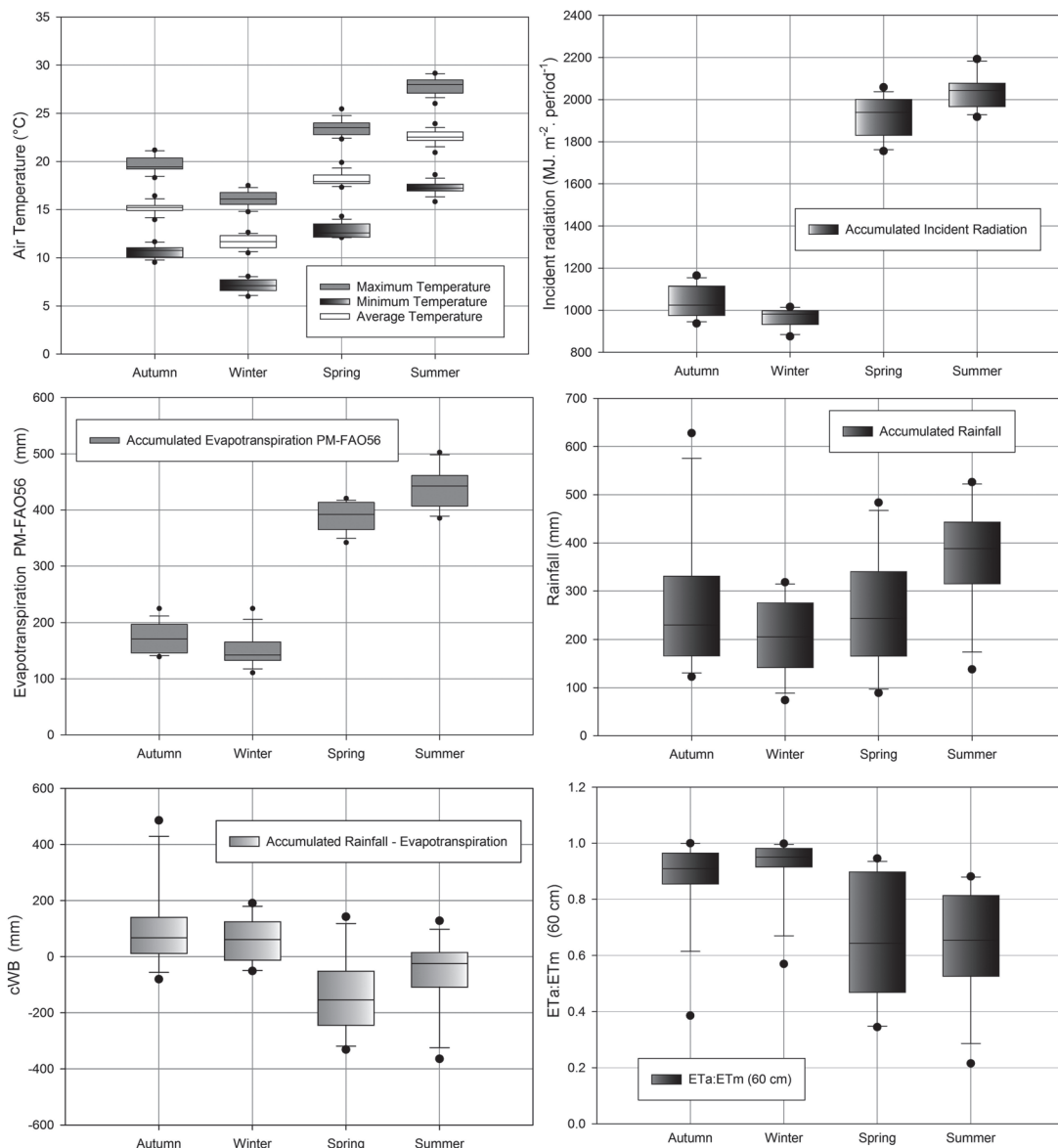


Figure 1. Air temperature (°C), incident solar radiation (MJ m⁻²period⁻¹), accumulated reference evapotranspiration (ETo) (PM FAO 56), accumulated rainfall (mm), accumulated rainfall - ETo difference (cWB) and ETa:ETm ratio for INIA La Estanzuela climatic station, 1997-2011. The central line of the box (box plot) represents the median; superior and inferior values represent percentiles 75 and 25, whereas the ends of the bars percentiles 90 and 10. The black dots represent extreme values of the series.

Table 2. Average growth rate (kg DM ha⁻¹ day⁻¹) by growth season and pasture age INIA LE (1997-2011)..

GROWING SEASON	ALFALFA AGE			ADJUSTED MEAN	
	1st year	2nd year	3rd year		
Autumn	n/p	42.33	35.71	39.02	b
Winter	16.69	22.55	16.93	18.72	c
Spring	40.43	47.79	32.50	40.24	b
Summer	57.80	49.82	n/d	53.81	a
ADJUSTED MEAN	38.3 A	40.54 A	28.38 B		

Adjusted means with a capital letter in the row or lowercase in the column are significantly different ($p < 0.05$ and $p < 0.01$, respectively). n/p: No forage production. n/d: No data, the forage production was not registered in the 3rd summer.

alfalfa growth in the same growing season among years (Table 2), not only explained by the climatic conditions prevailing in that season but also by the possible influence of the physiological state of alfalfa from the previous growing season⁽⁴⁰⁾⁽⁴¹⁾.

The interdependence with the individual meteorological variables and with their associated environmental variables is relatively large⁽⁴²⁾, in such a way that the influence of one of them on the growth factors is almost always associated in greater or lesser degree with another climatic variable⁽⁸⁾⁽⁴²⁾⁽⁴³⁾, except for extreme weather events.

Alfalfa growth rate

The alfalfa GR (kg DM ha⁻¹ day⁻¹) changed significantly between seasons of the year ($p < 0.0001$) and with pasture age ($p = 0.0307$). No significant differences were found in the GR between cultivars E_CHANA and CRIOU ($p = 0.9376$), both of intermediate latency, through crop age, seasons and years. The possible combinations of interactions between season, age and cultivar were not significant in any of the cases, allowing the evaluation of the main effects of season and pasture age separately.

Recorded average annual GR between 1997-2011 (37.94 kg DM ha⁻¹ day⁻¹) was similar to that obtained in other areas of similar climatic characteristics⁽⁴⁴⁾⁽⁴⁵⁾. High seasonality of climatic variables (Figure 1) determines, as in other regions, the seasonality of the GR for an alfalfa cultivar⁽⁴³⁾⁽⁴⁵⁾.

The GR in summer was significantly higher than the GR in autumn and spring, when radiation and temperature are higher and consequently, greater photosynthesis⁽⁴⁰⁾ and radiation use efficiency (RUE) (g DM MJ⁻¹ incident radiation)⁽⁴⁶⁾ are achieved. The lowest GR occurred in

winter, influenced by the interactions due to environmental conditions (low temperature and radiation) and defoliation intensity, affecting carbohydrates partition⁽⁴⁵⁾ and consequently reducing the GR.

The evolution of the GR according to pasture age and season, showed a wide variation of the values within the seasons for all the years analyzed (Table 2). On an annual basis, the calculated variation coefficients (VC) were closely comparable to those obtained in other producing areas in Argentina⁽⁴⁷⁾.

The average GR during the first year of growth in winter and summer, as well as in the third year in autumn and winter showed variation coefficients greater than 45 % (Table 2). This greater variability between years could indicate a stronger influence of meteorological factors in the GR during these growth periods, given that crop management was similar between years in the plots.

Relation between growth rate and climatic variables by season

Autumn

In autumn, GR was similar to spring (Table 2). As pasture ages, the dispersion of GR mean values increased at this time of the year, shown by VC above 50 % (Table 2). Air temperature in autumn decreased significantly compared to summer (Figure 1), reducing alfalfa GR, however, the temperature range remained within favorable growth thresholds, between 8 and 20 °C⁽⁴³⁾⁽⁴⁶⁾ with no extreme temperatures (Figure 1). Despite this global relationship with temperature, no direct relationship between GR and autumn temperature between years ($r^2 = 0.05$) was found to explain the variability of GR between years. Furthermore, the results of CART analysis allowed us to

discriminate four GR response groups concerning cWB (Table 3a), integrating in this index the relationship between rainfall and atmospheric demand.

In autumn, the maximum GR ($56.3 \text{ kg DM ha}^{-1} \text{ day}^{-1}$) was associated with intermediate water conditions; cWB in autumn ranged between -18 and 140 mm , with an average of 48 mm in 16 out of 46 periods analyzed. Water conditions in autumn higher or lower than these cWB values, significantly reduced alfalfa GR, in 30 out of 46 autumn periods (Table 3a). This relation was found regardless of alfalfa age. This higher GR was associated with cWB values close to 48 mm (Table 3a) when the crop's accumulated evapotranspiration was slightly lower than rainfall. On the other hand, the lowest GR ($23.8 \text{ kg DM ha}^{-1} \text{ day}^{-1}$) were found during autumn periods with significant high rainfall compared to ET_a, creating excess water situations that did not enhance alfalfa growth. Growth periods with extremely positive cWB would indicate agronomic conditions for possible soil waterlogging, which induce hypoxia, influenced by the internal soil drainage where planting takes place. Waterlogging decreases the growth of shoots and roots, increases mortality and decreases alfalfa vigor⁽⁴⁸⁾⁽⁴⁹⁾⁽⁵⁰⁾; on the other hand, higher temperatures increment damage produced by root asphyxia⁽⁴⁹⁾, combining lack of oxygen at root level, modulation reduction and a partial increase in plant metabolism⁽⁵¹⁾. In general, these climatic conditions also favor fungal diseases development in foliage and root, which usually reduce yield and compromise its persistence⁽⁵²⁾⁽⁵³⁾. The dispersion of the rainfall in autumn was higher in relation to other growth seasons (Figure 1), which could explain the very positive cWB frequency and autumn GR variability.

Winter

Given that E_CHANA and CRIOU cultivars are of intermediate latency, growth stops in response to short and cold days, then lower GR are expected in winter than in other seasons. GR in winter was the lowest among all seasons (Table 2). Analyzing GR in the set of two alfalfa cultivars through CART, showed that high T_{min} in winter ($> 8.5 \text{ }^\circ\text{C}$) produced lower GR (Table 3b), possibly due to the increase in respiration rate and decrease in carbohydrates availability⁽⁵⁴⁾. This relationship is not linear and enabled the identification of three groups according to the relationship between T_{min} and GR in winter. The GR was highest in winter ($26.1 \text{ kg ha}^{-1} \text{ day}^{-1}$) when air T_{min} ranged between 7.8 and 8.5°C ; outside this range, GR

decreased significantly. Although T_{min} showed greater capacity of group separation in GR through CART; T_{min}, T_{max}, and T_{med} were autocorrelated in this period for the same groups (Table 3b).

Daily average radiation during winter was not significantly different between GR groups, or from relative parameters to soil water conditions, shown by high values of ET_a:ET_m ratio, suggesting GR are independent of environmental factors related to water dynamics in winter (Table 3b).

Spring

The GR in spring was higher than in winter, and there was a relatively low average variation of the GR between years (Table 2). The GR increased when Rs_RADIAT was greater than $16.03 \text{ MJ m}^{-2} \text{ day}^{-1}$ and when the air temperature rose (Table 3c); causing a RUE increase⁽⁴⁶⁾, thus showing better conditions for photosynthesis and nodulation⁽⁴⁰⁾ and consequently more biomass. As temperature increased the GR increased too, producing a direct effect on alfalfa RUE⁽⁴⁶⁾. Incident radiation and water well-being (ET_a:ET_m > 0.6) were significantly associated with GR during spring (Table 3c), where good water availability conditions interacted with radiation and temperature rise, favoring a GR increase, possibly through the rise of the stomatal conductance of the canopy⁽⁴⁴⁾.

(1) Spring GR = $-61.77 + 4.1 (\text{Rs_RADIAT}) + 36.87 (\text{ETa:ETm})$, $r^2 = 0.32^{**}$, $^{**}p < 0.01$

The relation between water well-being and alfalfa GR was different according to average incident radiation during spring (Table 3c). Three GR groups discriminated by CART showed three different relations between GR and ET_a:ET_m. For Rs_RADIAT lower than $16.03 \text{ MJ m}^{-2} \text{ day}^{-1}$, there was no direct relationship between GR and water well-being ($r^2 = 0.0018$, $p < 0.6$, $n = 13$); when Rs_RADIAT was between 16.3 and $50.54 \text{ MJ m}^{-2} \text{ day}^{-1}$, GR slightly began to increase when ET_a:ET_m increased ($r^2 = 0.24$, $p < 0.039$, $n = 34$). Finally, when Rs_RADIAT was greater than $50.54 \text{ MJ m}^{-2} \text{ day}^{-1}$ the possibility of achieving the highest GR was related to high values of ET_a:ET_m ($r^2 = 0.91$, $p < 0.001$, $n = 9$). These three scenarios defined spring periods where radiation and temperature can limit GR. On the other hand, when the highest GR were expected due to maximum radiation ($> 50.54 \text{ MJ m}^{-2} \text{ day}^{-1}$), GR were limited by rainfall when the maximum atmospheric demand was not satisfied (ET_a:ET_m).

Table 3. Classification of climatic variables according to CART group separation for autumn, winter, spring and summer (1997-2011).

a AUTUMN		Number of Periods	Growth rate Kg day ⁻¹ ha ⁻¹	Air temperature			ETm mm	Pe mm	ETa:ETm	cWB mm
cWB (Rainfall-ETm)				Maximum (°C)	Minimum (°C)	Medium (°C)				
< -18.1 mm		12	35.2 B	23.6 A	13.8 A	18.7 A	238.2 AB	162.0 C	0.78 B	-61.5 D
-18.1 < cWB ≤ 140.1 mm		16	56.3 A	23.4 A	13.9 A	18.7 A	186.1 B	184.6 C	0.88 AB	47.7 C
140.1 < cWB ≤ 203.6 mm		8	34.7 B	24.0 A	15.0 A	19.5 A	197.7 B	301.2 B	0.93 A	178.0 B
> 203.6 mm		10	23.8 C	22.7 A	14.0 A	18.4 A	266.2 A	379.6 A	0.87 AB	259.9 A
b WINTER		Number of Periods	Growth rate Kg day ⁻¹ ha ⁻¹	Radiation			ETm mm	Pe mm	ETa:ETm	
Minimum Temperature				MJ m ⁻² day ⁻¹						
≤ 7.8 °C		24	16.98 B	9.61 A	15.97 C	6.96 C	11.49 C	123.38 B	153.18 B	0.92 AB
7.8 °C < T_Min ≤ 8.5 °C		18	26.09 A	9.54 A	16.67 B	8.12 B	12.42 B	244.32 A	308.28 A	0.94 A
> 8.5 °C		8	9.12 C	10.25 A	18.24 A	9.03 A	13.66 A	235.98 A	265.90 A	0.86 B
c SPRING		Number of Periods	Growth rate Kg day ⁻¹ ha ⁻¹	Radiation			ETm mm	Pe mm	ETa:ETm	
Daily radiation				MJ m ⁻² day ⁻¹						
≤ 16.03 MJ m ⁻² day ⁻¹		14	27.43 C	14.62 C	19.10 C	9.85 B	14.5 B	362.71 A	386.7 A	0.84 A
16.03 < Radiation ≤ 20.54		46	41.43 B	18.68 B	21.66 B	11.3 A	16.51 A	351.37 A	273.56 B	0.75 AB
> 20.54 MJ m ⁻² day ⁻¹		10	56.84 A	20.95 A	22.49 A	11.36 A	16.95 A	391.18 A	149.38 C	0.63 B
d SUMMER		Number of Periods	Growth rate Kg ha ⁻¹ day ⁻¹	Radiation			ETm mm	Pe mm	ETa:ETm	
ETa:ETm				MJ m ⁻² day ⁻¹						
≤ 0.42		10	19.83 C	24.73 A	28.71 A	17.35 A	23.05 A	486.8 A	165.34 B	0.31 C
0.42 < ETa:ETm ≤ 0.88		36	60.14 B	23.71 A	27.67 B	16.81 AB	22.26 A	463.1 A	308.53 A	0.66 B
> 0.88		2	101.50 A	24.35 A	26.55 C	16.04 B	21.29 B	82.6 B	50.1 C	0.93 A

Means in the column with the same letter are not significantly different (p > 0.05), Duncan Multiple Range Test.

Therefore, GR was constrained in some years, and consequently DM yield.

In Colonia, where these alfalfa trials were planted, periods with water deficit in spring often occurred, shown by the low ratio values between ETa and ETm, especially in those springs where radiation was not limiting. This suggests that some measures, such as supplementary irrigation, could increase alfalfa GR potential through the reduction of water deficit, satisfying the potential demand and achieving the greatest radiation use.

Although alfalfa is considered a relatively drought-tolerant species that can use up to 65-70 % of the available soil water (ASW) before transpiration decreases⁽²⁾, lower soil water content would decrease transpiration and consequently the ETa:ETm ratio⁽³⁴⁾⁽⁵⁵⁾. Alfalfa showed to have a high growth recovering ability under small water deficit conditions (seven days), but a limiting recovery with a deficit of 14 to 21 days⁽⁵⁶⁾. Equation (1) implies that small variations in ETa:ETm ratio, significantly affect GR in spring.

Air temperature was mainly associated with daily Rs_RADIAT; the lowest average temperature was associated with lower radiations ($< 16.03 \text{ MJ m}^{-2} \text{ day}^{-1}$), which decreased GR (Table 3c), regardless of the soil hydric state, reducing RUE⁽⁴⁶⁾. As radiation and temperature increased, GR rise was more related to the hydric regime and water availability, in agreement with Brown and others⁽⁴⁶⁾ and Ward and Micin⁽¹⁰⁾. The effect of temperature increase was reflected in a higher RUE⁽⁴⁶⁾. In Colonia, growth periods with high daily radiation in spring ($> 20.54 \text{ MJ m}^{-2} \text{ day}^{-1}$) were directly associated with periods in which air Tmax average was the highest (22.5 °C), and rainfall was low compared to the atmospheric demand (ETo).

Summer

Alfalfa growth was higher during summer (Table 2), when radiation and mostly temperature, were between the optimum range (20-25 °C) for temperate species growth⁽⁴⁴⁾⁽⁵⁷⁾. These two variables alone did not allow to discriminate GR variability found between years during summer ($r^2 = 0.20$ and 0.01 respectively, $p < 0.60$, $n = 48$). However, GR was discriminated in significantly different groups when analyzing the ratio between ETa and ETm (Table 3d). The GR in summer increased (3x) when ETa:ETm ratio was greater than 0.42 (Table 3d), allowing

to discriminate GR in three groups according to its relationship with water stress level in summer.

Summer growth periods that had high ETa:ETm values (> 0.88) and the highest GR ($101.5 \text{ kg ha}^{-1} \text{ day}^{-1}$) were relatively few in comparison to the total number of summer periods analyzed (two periods out of 48). The ETa:ETm average ratio in summer was 0.66 (36 out of 48) (Table 3d) ($\text{GR} = 60.1 \text{ kg ha}^{-1} \text{ day}^{-1}$), value well below the optimum to achieve high potential yields⁽³⁴⁾⁽⁵⁸⁾. This shows suboptimal conditions for alfalfa growth in most evaluated years, in which neither radiation nor temperature were the limiting factor, but water, in maximum yield. During the analyzed period, the value of 0.88 was exceeded only in few years, in which the GR was the highest.

Lower GR in summer is also associated with higher Tmax (Table 3d), which corresponded to lower ETa:ETm ratio periods and greater water deficit. The greater the soil water stress was (ETa:ETm ratio decrease), the lower GR was during summer periods ($\text{GR} = 84.79 \text{ ETa:ETm} + 2.75$, $r = 0.72$, $p < 0.01$, $n = 48$). This ratio slope showed that small ETa:ETm ratio increase indicates significant alfalfa GR increase. The closer ETa:ETm ratio is to one, the greater transpiration is compared to the atmospheric demand adjusted to the phenological stage (between cuttings) the lower its water stress and the higher its productivity⁽³⁴⁾. The individual analysis of evapotranspiration and rainfall showed no sources of discrimination of productive alfalfa behavior.

During summer in Colonia, variables associated with important GR changes were mainly related to atmospheric demand and water dynamics (Table 3d) under equal solar radiation conditions. Radiation and temperature directly affect RUE, and consequently growth⁽⁴⁶⁾⁽⁵⁹⁾; however, the effect of these variables on GR is directly limited to plant water balance⁽⁶⁰⁾⁽⁶¹⁾. These observations coincide with Henry⁽⁶²⁾ revealing that in our country there are periods of climatic water deficiency, especially from December to March. Depending on the storage capacity of soils and pasture tolerance, this period could entail plant water deficit and could extend in some years from spring (October) to autumn (April). Water stress is the most limiting environmental factor behind productivity and plant growth stability⁽⁶³⁾, and the impact on yield due to water stress is the main limiting factor behind summer crop production in Uruguay⁽⁶⁴⁾. On the other hand, it is the main reason for crop variability yields between years⁽⁶⁴⁾⁽⁶⁵⁾⁽⁶⁶⁾.

Conclusions

Alfalfa GR presented significant differences between seasons of the year and according to pasture age in the southwest of Uruguay. Variability of GR according to pasture age, expressed through changes in the variation coefficient, could be associated to a greater meteorological variables influence in the most vulnerable pasture stages: the initial alfalfa installation stage (first year of life), and the stage when it can begin to affect pasture persistence by plant death, root and crown diseases, insect attack, etc. (third year of life).

Climatic variables affected alfalfa GR differentially according to the season of the year considered. In autumn, water deficits and excesses were limiting to GR, showing maximum GR values between -18 and 140 mm of cWB, which correspond to 35 % of the evaluated autumn growth periods. In winter, GR was favored by the increase of minimum temperatures in periods of no water excess. The GR was maximum in winter ($26.1 \text{ kg ha}^{-1} \text{ day}^{-1}$) when the minimum air temperature ranged between 7.8 and 8.5 °C; outside this range, GR decreased significantly, which only occurred in 36 % of the winters considered. In spring and according to pasture age, the higher the solar radiation and ETa:ETm (lower water deficit), the higher alfalfa GR. Summer was the greatest growth period of this summer legume. The greater the water well-being of the pasture, denoted by the ETa:ETm ratio closer to one, the higher GR, provided that the maximum air temperatures were not limiting. Only in 6 % of the evaluated summers, ETa:ETm ratio exceeded 0.80; showing GR restrictions due to water deficiency during growth. These water restrictions could be alleviated by the incorporation of management measures that improve water use efficiency, more drought tolerant cultivars, or supplementary irrigation technologies during summer; especially during evident water deficit years. Consequently, growth rates would be high and therefore alfalfa forage yield maximized.

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Author's contribution

All the authors contributed equally to the content.

References

- 1) McKenzie JS, Paquin R, Duke SH. Cold and heat tolerance. In: Hanson A, Bames DK, Hill RR Jr, editors. Alfalfa and Alfalfa Improvement. Madison, WI: ASA; 1988. p. 259–301. (Agronomy Monograph; 29).
- 2) Sheaffer CC, Tanner CB, Kirkham MB. Alfalfa water relations and irrigation. In: Hanson A, Bames DK, Hill RR Jr, editors. Alfalfa and alfalfa improvement. Madison, WI: ASA; 1988. p. 373-409. (Agronomy Monograph; 29)
- 3) Bula RJ. Morphological characteristics of alfalfa plants grown at several temperatures. *Crop Sci.* 1972;12(5):683–6.
- 4) Rebuffo M. Adopción de variedades en Uruguay. In: Rebuffo M, Risso D, Restaino E, editors. Tecnología en Alfalfa. Montevideo: INIA; 2000. p. 5-16. (Boletín de Divulgación; 69).
- 5) Formoso F. Manejo de alfalfa para producción de forraje. In: Rebuffo M, Risso D, Restaino E. editors. Tecnología en Alfalfa. Montevideo: INIA; 2000. p. 53-74. (Boletín de Divulgación; 69).
- 6) Formoso F. Manejo de mezclas forrajeras y leguminosas puras: Producción y calidad del forraje: Efectos del estrés ambiental e interferencia de gramilla [*Cynodon dactylon*, (L) Pers]. Montevideo: INIA; 2011. 302 p. (Serie Técnica N° 188).
- 7) Brown RH, Radcliffe DE. A comparison of apparent photosynthesis in *Sericae lespedeza* and alfalfa. *Crop Sci.* 1986; 26:1208-11.
- 8) Sharratt BS, Sheaffer CC, Baker DG. Base temperature for the application of the growing-degree-day model to field-grown Alfalfa. *Field Crop Res.* 1989; 21:95-102.
- 9) Cooper CS, Qualls M. Morphology and chlorophyll content of shade and sun leaves of two legumes. *Crop Sci.* 1967;7:672-3.
- 10) Ward PR, Micin SF. The capacity of dryland lucerne for groundwater uptake. *Aust J Agric Res.* 2006;57(5):483-7.
- 11) Durigon A, de Jong var Lier Q. Canopy temperature versus soil water pressure head for the prediction of crop water stress. *Agric Water Manag.* 2013;127:1-6.
- 12) Castaño J, Giménez A, Ceroni M, Furest J, Aunchayna R. Caracterización Agroclimática del Uruguay: 1980-2009. Montevideo: INIA; 2011. 34 p. (Serie Técnica; 193).
- 13) Sharratt BS, Baker DG, Sheaffer CC. Climatic effect on alfalfa dry matter production: Part I. Spring harvest. *Agric Forest Meteorol.* 1986;37:123-31.
- 14) Sharratt BS, Baker DG, Sheaffer CC. Climatic effect on alfalfa dry matter production: Part II. Summer harvest. *Agric Forest Meteorol.* 1987;39:121-9.
- 15) Wolf DD, Blaser RE. Leaf development of alfalfa at several temperatures. *Crop Sci.* 1971; 11:479–82.
- 16) Labandera M. Comportamiento de cultivares. In: Rebuffo M, Risso D, Restaino E, editors. Tecnología en Alfalfa. Montevideo: INIA; 2000. p. 17-26. (Boletín de Divulgación; 69).
- 17) Quiroga Garza HM. Tasa de acumulación de materia seca de alfalfa en respuesta a las variables climáticas. *Rev Mex De Cienc Agric.* 2013;4(4):503-16.

- 18) Castro M, Condón F, Altier N. Leguminosas: Alfalfa. In: Resultados experimentales de Evaluación de Cultivares: Especies forrajeras. Montevideo: INIA; 1998. p. 39-46.
- 19) Labandera M, Altier N. Alfalfa. In: Resultados Experimentales de Evaluación de Especies forrajeras Bianaues y Perennes para el Registro Nacional de Cultivares. Montevideo: INASE; 1999. p. 6-22.
- 20) Labandera M, Altier N. Alfalfa. In: Resultados Experimentales de Evaluación de Especies forrajeras Bianaues y Perennes para el Registro Nacional de Cultivares. Montevideo: INASE; 2000. p. 19-35.
- 21) Labandera M, Altier N. Alfalfa. In: Resultados Experimentales de Evaluación de Especies forrajeras Bianaues y Perennes para el Registro Nacional de Cultivares. Montevideo: INASE; 2001. p. 18-33.
- 22) Labandera M, Altier N. Alfalfa. In: Resultados Experimentales de Evaluación de Especies forrajeras Bianaues y Perennes para el Registro Nacional de Cultivares. Montevideo: INASE; 2002. p. 16-33.
- 23) Vilaró D, Altier N. Comportamiento de cultivares de alfalfa en Uruguay. In: Resultados Experimentales de Evaluación de Especies forrajeras para el Registro Nacional de Cultivares. Anuales, Bianaues y Perennes, período 2002. Montevideo: INASE; 2003. p. 34-42.
- 24) Castro M, Vilaró D, Altier N. Comportamiento de cultivares de Alfalfa en Uruguay. In: Resultados Experimentales de Evaluación de Especies forrajeras para el Registro Nacional de Cultivares. Anuales, Bianaues y Perennes, período 2003. Montevideo: INASE; 2004. p. 32-9.
- 25) Castro M, Altier N, Stewart S. Comportamiento de cultivares de Alfalfa en Uruguay. In: Resultados Experimentales de Evaluación de Especies forrajeras para el Registro Nacional de Cultivares. Anuales, Bianaues y Perennes, período 2004. Montevideo: INASE; 2005. p. 33-40.
- 26) Castro M, Astor D, Altier N, Stewart S. Comportamiento de cultivares de Alfalfa en Uruguay. In: Resultados Experimentales de Evaluación de Especies forrajeras para el Registro Nacional de Cultivares. Anuales, Bianaues y Perennes, período 2005. Montevideo: INASE; 2006. p. 32-7.
- 27) Castro M, Altier N, Stewart S. Comportamiento de cultivares de Alfalfa en Uruguay. In: Resultados Experimentales de Evaluación de Especies forrajeras para el Registro Nacional de Cultivares. Anuales, Bianaues y Perennes, período 2006. Montevideo: INASE; 2007. p. 47-54.
- 28) Castro M, Altier N, Pereyra S. Alfalfa: cultivares evaluados en Uruguay durante 2007. In: Resultados Experimentales de Evaluación de Cultivares de Especies forrajeras. Anuales, Bianaues y Perennes, período 2007. Montevideo: INASE; 2008. p. 57-63.
- 29) Castro M, Altier N, Pereyra S. Alfalfa: cultivares evaluados en Uruguay durante 2008. In: Resultados Experimentales de Evaluación de Cultivares de Especies forrajeras. Anuales, Bianaues y Perennes, período 2008. Montevideo: INASE; 2009. p. 50-9.
- 30) Castro M, Altier N, Pereyra S. Alfalfa: cultivares evaluados en Uruguay durante 2009. In: Resultados Experimentales de Evaluación de Cultivares de Especies forrajeras. Anuales, Bianaues y Perennes, período 2009. Montevideo: INASE; 2010. p. 49-54.
- 31) Castro M, Altier N, Pereyra S. Alfalfa: cultivares evaluados en Uruguay durante 2010. In: Resultados Experimentales de Evaluación de Cultivares de Especies forrajeras. Anuales, Bianaues y Perennes, período 2010. Montevideo: INASE; 2011. p. 51-7.
- 32) Castro M, Altier N, Pereyra S, Stewart S. Alfalfa (*Medicago sativa*): Cultivares evaluados en La Estanzuela, Uruguay durante 2011. In: Resultados Experimentales de la Evaluación Nacional de Cultivares de Especies forrajeras. Anuales, Bianaues y Perennes, período 2011. Montevideo: INASE; 2012. p. 52-7.
- 33) Shaw E. Hydrology in practice. 3rd ed. London: Taylor & Francis; 1994. 546 p.
- 34) Allen RG, Pereira LS, Raes D, Smith M. Evapotranspiración del cultivo: Guías para la determinación de los requerimientos de agua de los cultivos. Rome: FAO; 2006. 298 p. (Cuadernos de Riego y Drenaje; 56).
- 35) Pereira LS, Teodoro PR, Rodrigues PN, Teixeira JL. Irrigation scheduling simulation: The model ISAREG. In: Rossi G, Cancelliere A, Pereira LS, Oweis T, Shatanawi M, Zairi, A, editors. Tools for Drought Mitigation in Mediterranean Regions. Dordrecht: Kluwer; 2003. p. 161-80.
- 36) West BT, Welch KB, Galecki AT. Linear-Mixed Models: A Practical Guide Using Statistical Software. 2nd ed. Boca Raton, (FL): CRC Press; 2015. 440 p.
- 37) Breiman L. Random forests. Mach Learn. 2001;45(1):5-32.
- 38) Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. Infostat Versión 2014 [Internet]. Córdoba: Grupo InfoStat, FCA, Universidad Nacional de Córdoba; 2013. [cited 2019 Jan 21]. Available from: <https://www.infostat.com.ar/index.php>.
- 39) R Core Team. R: A language and environment for statistical computing [Internet]. Vienna: R Foundation for Statistical Computing; 2014. [cited 2019 Jan 21]. Available from: <http://www.R-project.org/>.
- 40) Iker A, Irigoyen JJ, Perez P, Martinez-Carrasco R. Response of nodulated alfalfa to water supply, temperature and elevated CO₂: Productivity and water relations. Environ Exp Bot. 2006; 55:130-41.
- 41) Rimi F, Macolino S, Leinauer B, Laurialt LM, Ziliotto U. Alfalfa yield and morphology of three fall-dormancy categories harvested at two phenological stages in a subtropical climate. Agron J. 2010;102(6):1578-85.
- 42) Wilks DS. Statistical methods in the atmospheric sciences. 2nd ed. Amsterdam: Elsevier; 2006. 627 p.

- 43) Christian KR. Effects of the environment on the growth of alfalfa. *Adv Agron* 1977;29:183–227.
- 44) Brown HE, Jamieson PD, Moot DJ. Predicting the transpiration of lucerne. *Eur J Agron*. 2012; 43:9–17.
- 45) Smith AP, Moore AD, Boschma SP, Hayes RC, Nie Z, Pembleton KG. Modelling of lucerne (*Medicago sativa* L.) for livestock production in diverse environments. *Crop Pasture Sci*. 2017;68:74–91.
- 46) Brown HE, Moot DJ, Teixeira EI. Radiation use efficiency and biomass partitioning of lucerne (*Medicago sativa*) in a temperate climate. *Eur J Agron*. 2006;25:319–27.
- 47) Ojeda JJ, Caviglia OP, Irisarri JGN, Agnusdei, MG. Modelling inter-annual variation in dry matter yield and precipitation use efficiency of perennial pastures and annual forage crops sequences. *Agric For Meteorol*. 2018;259:1–10.
- 48) Letey J, Stolzy LH, Blank GB. Effect of duration and timing of low oxygen content on shoot and root growth. *Agron J*. 1962; 54:34-7.
- 49) Thompson TE, Fick GW. Growth response of alfalfa to duration of soil flooding and to temperature. *Agron J*. 1981; 73:329-32.
- 50) Wahab HA, Chamblee DS. Influence of irrigation on the yield and persistence of forage legumes. *Agron J*. 1972; 64:713-6.
- 51) Roberts DM, Choi WG, Hwang JH. Strategies for adaptation to waterlogging and hypoxia in nitrogen fixing nodules of legumes. In: Mancuso S, Shadala S, editors. *Waterlogging Signalling and Tolerance in Plants*. Berlin: Springer-Verlag; 2010. p. 37-59.
- 52) Teutsch CD, Sulc RM. Influence of Seedling Growth Stage on Flooding Injury in Alfalfa. *Agron J*. 1977; 89:970-5.
- 53) Altier N. Enfermedades de pasturas. In: Altier N, Rebuffo M, Cabrera K, editors. *Enfermedades y plagas en pasturas*. Montevideo: INIA; 2010. p. 19-35. (Serie Técnica; 183)
- 54) Lambers H, Atkin OK, Millenaar FF. Respiratory patterns in roots in relation to their functioning. In: Waisel Y, Eshe, A, Kafkafi U, editors. *Plant roots: The hidden half*. New York: Marcel Dekker; 1996. p. 323-62.
- 55) Raza A, Friedela JK, Moghaddamb A, Ardakanic MR, Loiskandld W, Himmelbauerd M, Bodnere G. Modeling growth of different lucerne cultivars and their effect on soil water dynamics. *Agric Water Manag*. 2013;119:100–10.
- 56) Erice G, Louahlia S, Irigoyen JJ, Sánchez-Díaz M, Alami IT, Avice JC. Water use efficiency, transpiration and net CO₂ exchange of four alfalfa genotypes submitted to progressive drought and subsequent recovery. *Environ Exp Bot*. 2011;72(2):123–30.
- 57) McKenzie BA, Kemp PD, Moot DJ, Matthew C, Lucas RJ. Environmental effects on plant growth and development. In: White J, Hodgson J, editors. *New Zealand pasture and crop science*. Oxford: Oxford University Press; 2011. p. 29-44.
- 58) Shen Y, Li L, Chen W, Robertson M, Unkovich M, Bellott Wi, Probert M. Soil water, soil nitrogen and productivity of lucerne–wheat sequences on deep silt loams in a summer dominant rainfall environment. *Field Crops Res*. 2009;111:97–108.
- 59) Brown HE, Moot DJ, Jamieson P, Fletcher A. A framework for quantifying water extraction and water stress responses of perennial lucerne. *Crop Pasture Sci*. 2009; 60:785–94.
- 60) Greenwood KL, Lawson AR, Kelly KB. The water balance of irrigated forages in northern Victoria, Australia. *Agric Water Manag*. 2009;96(5):847–58.
- 61) Annicchiarico P, Pecetti L, Abdelguerfi A, Bouizgaren A, Carroni AM, Hayek T, M'Hammadi Bouzina M, Mezni M. Adaptation of landrace and variety germplasm and selection strategies for lucerne in the Mediterranean basin. *Field Crops Res*. 2011; 120:283–91.
- 62) Henry J. *Uruguay Evapotranspiration studies*. Paris: Unesco; 1973. 140 p.
- 63) Araus JL, Slafer GA, Reynolds MP, Royo C. Plant breeding and water relations in C3 cereals: What to breed for? *Ann Bot*. 2002; 89:925–40.
- 64) Sawchik J, Ceretta S. Consumo de agua por sojas de distintos grupos de madurez en diferentes ambientes de producción. In: *Jornada técnica de cultivos de verano*. Montevideo: INIA; 2005. p. 41-5. (Serie Actividades de Difusión; 417).
- 65) Andersen J, Alagarswamy G, Rotz C, Ritchie J, LeBaron A. Weather impacts on maize, soybean and alfalfa production in the great lakes region. *Agron J*. 2001;93:1059-70.
- 66) Giménez L, García M. Evapotranspiración de cultivos de verano para dos regiones climáticamente contrastantes de Uruguay. *Agrociencia*. 2011;15:100-8.