

Forage production and leaf proportion of lucerne (*Medicago sativa* L.) in subtropical environments: fall dormancy, cutting frequency and canopy effects

Producción de forraje y proporción de hojas de alfalfa (*Medicago sativa* L.) en ambientes subtropicales: efectos del grado de latencia, la frecuencia de corte y la canopia

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ABSTRACT

In subtropical and humid environments, winter-active cultivars of lucerne usually produce more forage with lower leaf proportion (leaf to stem ratio) than winter-dormant ones. The aim of present research was to analyze i) forage production of cultivars contrasting in fall dormancy under contrasting cutting frequencies, and (ii) the origin of differences between cultivars in the leaf proportion trait. In each of two subtropical locations of Argentina, an experiment including three lucerne cultivars (FD4= winter-dormant, FD6=semi winter-dormant, FD9=winter-active) and three cutting frequencies ('high': defoliated when intercept 0.50 of incident radiation, 'intermediate': when intercept 0.95 of incident radiation, 'low': when intercept 0.95 of incident radiation plus 150 growing degree days) was established. A significant cultivar*cutting frequency interaction was detected. In treatments where the cutting interval was longer (*e.g.* 'high' vs. 'low' cutting frequency) the more winter-active cultivars were more productive than the more winter-dormant ones (FD9>FD6>FD4), mainly due to a higher stem production and without major differences in leaf production. In turn, in treatments where the cutting interval was shorter, the cultivars showed similar forage production (FD9=FD6=FD4). Compared at similar canopy height, the differences between cultivars in leaf proportion were practically irrelevant. We confirm that (i) in subtropical and humid environments, the differences in forage production between cultivars contrasting in their fall dormancy depend on the cutting frequency, and that (ii) leaf proportion in aerial biomass of lucerne pastures is governed mainly by plant morphology, especially canopy height.

Keywords

lucerne • fall dormancy • cutting frequency • forage production • leaf to stem ratio

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RESUMEN

En ambientes subtropicales húmedos los cultivares de alfalfa sin latencia invernal suelen mostrar mayor producción de forraje y menor proporción de hoja que los cultivares con latencia invernal. El objetivo del trabajo fue analizar i) la producción de forraje de cultivares de alfalfa de distinto grado de latencia invernal bajo diferentes frecuencias de corte, y ii) el origen de las diferencias observadas entre cultivares en la proporción de hoja. En dos localidades subtropicales de Argentina, se estableció un experimento que incluía tres cultivares de alfalfa (FD4= con latencia invernal, FD6= con latencia invernal intermedia, FD9= sin latencia invernal) y tres frecuencias de corte ('alta': defoliados cuando se interceptó el 50% de la radiación incidente, 'intermedia': cuando se interceptó el 95% de la radiación incidente, 'baja': cuando se acumularon 150 grados días de crecimiento a partir del momento en que se llegó al 95% de intercepción de la radiación incidente). Se detectó una interacción significativa entre frecuencia de corte y cultivar. En los tratamientos con largos intervalos entre cortes (*e.g.* 'alta' frecuencia vs. 'baja' frecuencia), los cultivares con menor latencia invernal produjeron más forraje (FD9>FD6>FD4), debido principalmente a una mayor producción de tallos y sin mayores diferencias en la producción de hojas. A su vez, las diferencias entre cultivares desaparecieron (FD9=FD6=FD4) en los tratamientos con menores intervalos entre cortes. Comparados a similar altura de canopia, las diferencias entre cultivares en la variable proporción de hoja fueron irrelevantes. Este trabajo confirma que (i) en ambientes subtropicales, las diferencias en producción de forraje entre cultivares depende de la frecuencia de corte, y que (ii) la proporción de hoja depende principalmente de la morfología de las plantas, especialmente de la altura de la canopia.

Palabras clave

alfalfa • latencia invernal • frecuencia de corte • producción de forraje • relación hoja:tallo

INTRODUCTION

Lucerne cultivars are grouped according to fall dormancy (or winter activity) based on shoot elongation during autumn (19, 22). In temperate latitudes, lucerne cultivars contrasting in fall dormancy showed similar annual forage production, regardless of the cutting frequency (9, 19, 21, 23). In subtropical locations with humid and mild winters, winter-active cultivars produce more forage than winter-dormant ones (12, 13, 15), but such differences tend to disappear as the cutting interval decreases (6).

In turn, most of the literature indicates that winter-dormant cultivars had higher leaf proportion in their aerial biomass than winter-active ones (8, 14, 24). Leaves had better nutritive quality than stems (5) and positive relationships between leaf proportion and animal performance, were reported (4). Therefore, in lucerne cultivars, a typical trade-off between forage production (higher in 'winter-active' cultivars) and nutritive forage quality (lower in 'winter-active' cultivars) is frequently observed (8, 14, 24).

It was demonstrated that, as plants got bigger, biomass allocation in support tissues (stems) increases relatively more than in metabolic tissues (leaves) (11). A recent study in a temperate location showed that the relationship between yield and quality was independent of genotype and changes in leaf/stem ratio were associated with changes in canopy biomass and canopy height (21). These findings suggest that the origin of the above-mentioned differences between cultivars in leaf proportion may be not intrinsic (*i.e.* they were not due to a cultivar effect). We hypothesized that in lucerne, the variability in leaf proportion could be simply explained by the variability in aerial biomass or in canopy height.

The aim of the present research was to analyze i) forage production of cultivars contrasting in fall dormancy under contrasting cutting frequencies, and (ii) the origin of differences between cultivars in the leaf proportion trait.

MATERIAL AND METHODS

Experimental conditions, design and measurements

Two similar trials were carried out in two locations of the Humid Pampas of Argentina: Paraná (31°44' S, 60°31' W, Exp. 1, irrigated) and Marcos Juárez (32°42' S, 62°06' W, Exp. 2, rainfed). The climate is sub-tropical humid (16). Monthly mean temperature ranges from 10.7°C in July to 24.6°C in January. Average annual rainfalls are 1069 and 900 mm for Paraná and Marcos Juárez, respectively. Treatments were established on Aquic Argiudoll (Paraná) and Typic Argiudoll (Marcos Juárez) soils (20). In both locations, soils showed no physical restriction to root growth and adequate levels of P availability (> 20mg/kg P Bray) and organic matter content (~3.0-3.2%).

Cultivars were sown, at a seeding rate of 24 kg/ha, with 15 cm row spacing on 20th April 2010 (Paraná) and on 24th September 2009 (Marcos Juárez) in plots of 1.5 x 6.0 m size in a randomized complete block with three replicates. At sowing, calcium triple superphosphate was surface broadcasted at a rate of 40 kg/ha of P to provide non-limiting P availability. Plots were hand-weeded approximately every 20 days; and chemical control of insects was done as necessary. Irrigation at Paraná was provided by drip-irrigation (driplines spaced 0.60 m apart bearing 1 L/h emitters every 0.30 m) every time soil water content was near 30 mm below the drained upper limit (*i.e.* 210 mm for 1 m soil depth) estimated using evapotranspiration and rainfall as described by Allen *et al.* (1998).

In each plot, a factorial combination of cultivar and cutting frequency was established. Cultivars contrasting in fall dormancy were: a winter-dormant cultivar (FD4), a semi-dormant cultivar (FD6), and a winter-active cultivar (FD9). Each cultivar was defoliated at 'high', 'intermediate' and 'low' frequency. The cutting frequency was based in radiation capture and thermal time accumulation. Plots of 'high' and 'intermediate' cutting frequency were defoliated each time they intercepted 0.50 ('high' frequency) and 0.95 ('intermediate' frequency) of incident radiation. In turn, plots of 'low' cutting frequency had to spend 150 growing degree days (GDD) once 0.95 of incident radiation, was intercepted. In consequence, the interval between cuttings was not fixed to a number of days.

Climate during the experimental period was measured daily at 1.5 m height with a portable meteorological station (LI-1200S, Li-Cor Inc., Lincoln, NE). Main data are shown in figure 1 (page 82). Briefly, mean day temperature was similar to historical records and between locations. In both locations, rainfall was lower than historical records (data not shown) and in Marcos Juárez (Exp. 2, rainfed) a strong water deficit was observed from June 2010 to late January 2011 (figure 1d, page 82).

In Paraná (Exp. 1), treatments started on 16/11/2010 and ended, for 'high', 'intermediate' and 'low' cutting frequencies on the 27/12/2011 (407 days), 29/12/2011 (409 days) and 28/12/2011 (408 days), respectively. In Marcos Juárez (Exp. 2), they all started on 15/3/2010 and ended on 17/3/2011 (368 days), 28/3/2011 (379 days) and 1/4/2011 (383 days) also for 'high', 'intermediate' and 'low' cutting frequencies, respectively.

Radiation interception was measured, on sunny days, at midday every 3-5 days using a tube solarimeter type TSL (Delta-T Devices Ltd., Burwell, Cambridge, UK). In each plot, one above-canopy measurement (Ia) was followed by three below-canopy measurements (Ib). The proportion of radiation intercepted by the canopy was estimated by the following equation: $1 - Ib/Ia$. Growing degree days (GDD) were calculated as the sum of daily mean temperatures above the base temperature of 5°C (3, 10).

Prior to cutting, canopy height was measured in fifteen representative sites of each plot. Forage dry matter (kg DM/ha) was estimated by harvesting all strips of 5 m length and 1 m wide (5 m² in the center of the plot) with a motor mower (cutting height: 5 cm above ground level). Fresh forage was weighted at field. After that, two sub-samples (~ 200 g) were extracted in order to determine moisture content and components (leaves and stems). Leaves (blades, petiole, stipules and 3 cm of stem tip) and stems were hand separated. All samples were dried at 60°C for 72 h.

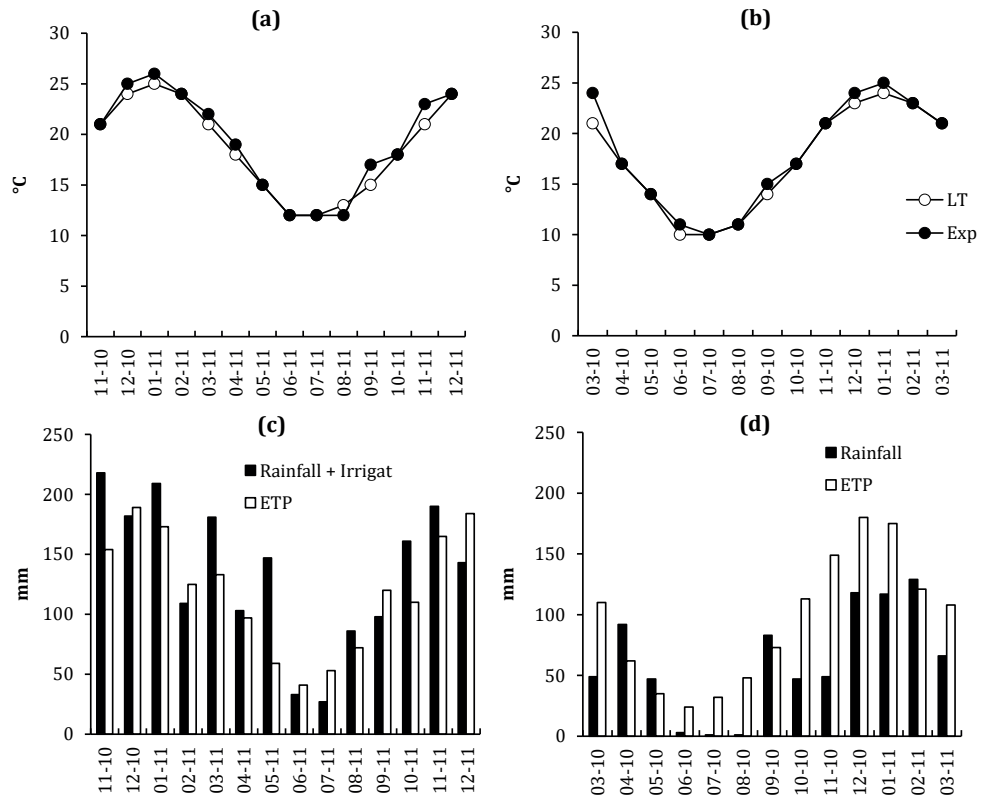


Figure 1. Mean air temperature (°C), rainfall (mm), irrigation (mm) and evapotranspiration (ETP, mm) observed in the experimental period (Exp) and for the long term (LT) in Paraná (a, c) and Marcos Juárez (b, d). 2010-2011.

Figura 1. Temperatura media del aire (°C), precipitaciones (mm), irrigación (mm) y evapotranspiración (ETP, mm) durante el período experimental (Exp) y los datos históricos (LT) en Paraná (a, c) y Marcos Juárez (b, d). 2010-2011.

Statistical analysis

Both experiments were analyzed separately. All data was checked for normality and homogeneity of variances. For total forage and total leaf production (forage mass and leaf mass produced in approximately one year) analysis of variance (ANOVA) was used. When significant effects resulted from ANOVA ($P < 0.05$), Fisher's least significant difference was used to identify treatment differences. In order to distinguish intrinsic (cultivar differences) from size-mediated effects, we plotted, for each defoliation treatment, leaf proportion *versus* canopy size traits (aerial biomass and canopy height). After that, data within similar size range, were selected. Range of aerial biomass and canopy height in data selected for Paraná (Exp. 1) were: 'high' frequency= 10-25 cm and 600-1700 kg DM/ha; 'intermediate' frequency= 20-45 cm and 1600-4900 kg DM/ha; 'low' frequency= 25-65 cm 2000-5700 kg DM/ha. Range of aerial biomass and canopy height in data selected for Marcos Juárez (Exp. 2) were: 'high' frequency= 10-25 cm and 400-1600 kg DM/ha; 'intermediate' frequency= 10-50 cm and 900-4000 kg DM/ha; 'low' frequency= 15-55 cm 1000-4200 kg DM/ha. With this dataset, a statistical comparison (t-test) was made (2, 17) within each defoliation frequency treatment, using only data sharing the same size trajectory. Regression functions were fitted between explanatory (aerial biomass, canopy height and thermal time between cuttings) and dependent (leaf proportion) variables using Genstat version 18 (VSN, International Ltd).

RESULTS AND DISCUSSION

In both experiments, significant cultivar*cutting frequency interactions were detected. In irrigated conditions (Exp. 1: Paraná), forage production was similar ($p > 0.05$) between cultivars at 'high' cutting frequency but differences were detected at lower cutting frequencies (figure 2a, page 84). At 'intermediate' cutting frequency, the winter-active and semi-dormant cultivars (FD6 and FD9) produced more forage ($p < 0.05$) than the winter-dormant cultivar (FD4). At 'low' cutting frequency, FD9 was more productive, FD4 was less productive and FD6 was intermediate but not significantly different from FD4 and FD9 (figure 2a, page 84). Under rainfed conditions (Exp. 2: Marcos Juárez), the cultivars showed similar forage production at 'high' and 'intermediate' cutting frequencies, but at 'low' cutting frequency, FD9 and FD6 resulted significantly more productive than FD4 (figure 2b, page 84).

Results of the present research were similar to that observed in subtropical latitudes (6) but differed from those observed at temperate latitudes where interaction between cultivar and cutting frequency was not detected (9, 14, 19, 21, 23). Thus, in subtropical latitudes, cultivar choice must consider the cutting frequency of the productive system. However, leaf yield was quite similar between cultivars (figure 2c, d, page 84). In fact, significant differences between cultivars were detected only at 'low' cutting frequency under rainfed conditions of Marcos Juárez (figure 2d, page 84). Such results imply that the higher forage productivity of more winter-active cultivars (FD6 and FD9) observed at 'intermediate' and 'low' cutting frequencies, was mainly due to a higher production of stems (figure 2e, f, page 84), the less nutritive fraction of aerial biomass (5).

In turn, differences in forage production between cutting frequencies (figure 2a, b, page 84) were explained by differences in both leaf and stem production (figure 2c, d, e, f, page 84). However, these differences were always of greater magnitude in stem than in leaf production. In other words, such results also imply that the higher forage productivity observed in the low cutting frequency managements ('low' and 'intermediate'), were mainly explained by differences in stem biomass.

Stem biomass is the less nutritive fraction of aerial biomass (5). Thus, breeding programs focused on traits related to fiber stem digestibility will be highly valuable for systems using winter-active cultivars and/or systems prone to using long intervals between cuttings (7) (*e.g.* conserved forage industry). Of course, this could also be important for grazing systems, since stem biomass accumulation has a negative impact on animal performance (4). Certainly, the choice of a cultivar also depends on other characteristics such as persistence and pest/disease tolerance.

Table 1 (page 85) shows the comparison between cultivars in leaf proportion of harvested aerial biomass (kg DM/ha), among data sharing a range of similar aerial biomass and canopy height at the moment of cutting (17). At high defoliation frequency, FD4 was leafier than FD6 (Paraná and Marcos Juárez) and FD9 (only in Marcos Juárez). At the intermediate defoliation frequency, FD4 was leafier than FD9 (Paraná and Marcos Juárez) and FD6 (only in Marcos Juárez). All cultivars showed the same leaf proportion at low defoliation frequency. FD6 and FD9 showed similar leaf proportion irrespective of the defoliation frequency treatment (table 1, page 85). It is important to note that most differences between cultivars disappeared when leaf proportion was compared at a similar canopy height (table 1, page 85). In fact, at similar canopy height, FD4 was leafier than FD9 and FD6 only at intermediate defoliation frequency (Paraná) and at high defoliation frequency (Marcos Juárez), respectively.

An inverse relationship between forage production (higher in winter-active cultivars) and leaf proportion (lower in winter-active cultivars) is frequently reported (8, 14, 24). Our results indicate that the variability in leaf proportion was mainly explained by variability in both, canopy biomass and canopy height (figure 3a, b, c, d, page 86). Similar findings were also reported in a temperate and humid environment (21). Thus, it is possible to speculate that differences in leaf proportion between cultivars contrasting in fall dormancy are not intrinsic (*i.e.* the differences in leaf proportion are not due to a cultivar effect). One of the most important implications of previous findings is that changes in leaf proportion obtained in isolated plants during breeding programs are hard to be sustained or observed under pasture conditions. In fact, leaf/stem partition in plants growing in dense canopies follows changes in canopy biomass and canopy height, which in turn are driven by the competition among plants by light (11).

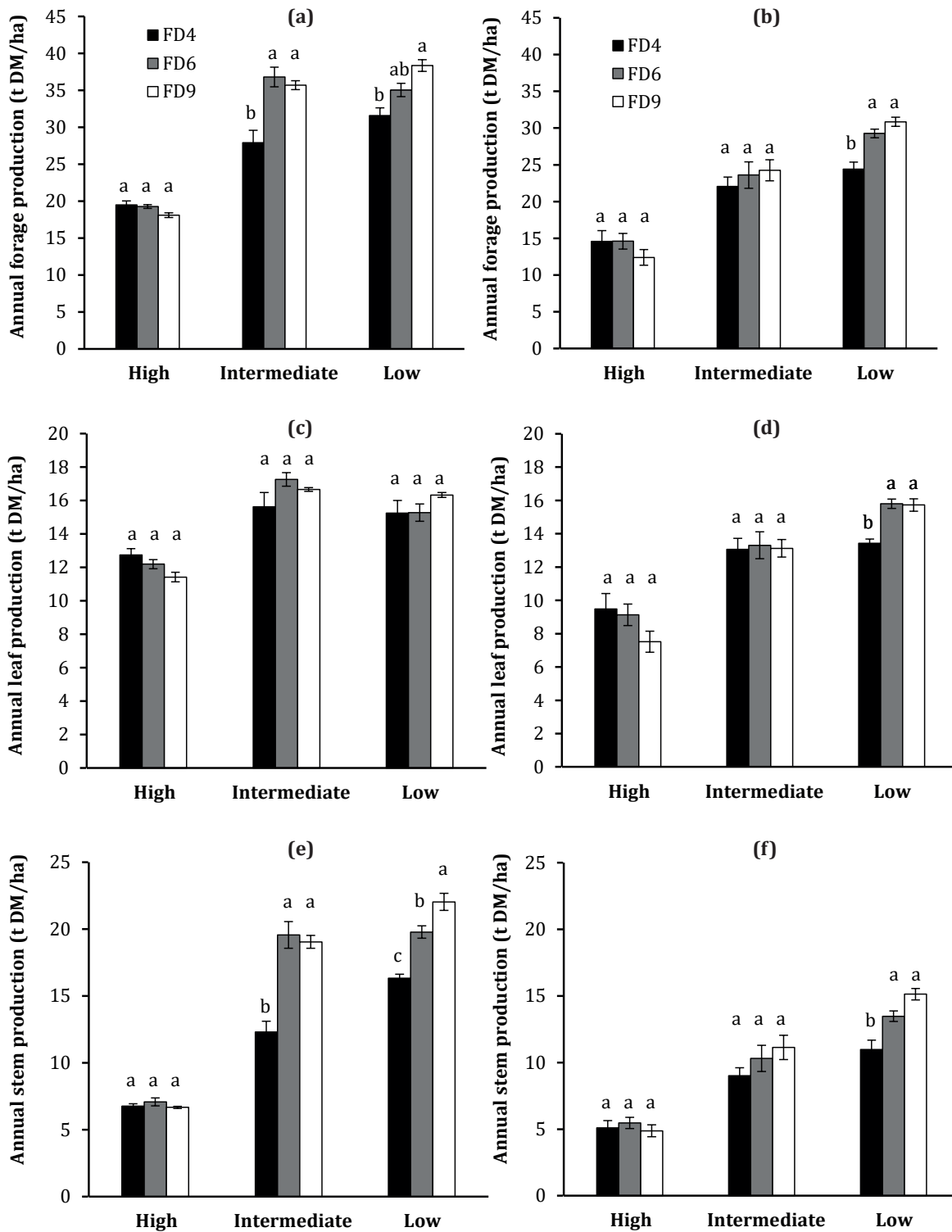


Figure 2. Annual forage (a, b), leaf (c, d) and stem (e, f) production (t DM/ha) for lucerne cultivars differing in fall dormancy (FD4, FD6 and FD9) and subjected to different cutting frequencies (High, Intermediate and Low). Paraná (a, c, e) and Marcos Juárez (b, d, f). Different letters indicate differences between cultivars ($P < 0.05$), within each cutting frequency. Bars are the standard error of each mean. 2010-2011.

Figura 2. Producción anual (t DM/ha) de forraje (a, b), de hojas (c, d) y de tallos (e, f) para cultivares que difieren en su latencia invernal (FD4, FD6, FD9) sometidos a diferentes frecuencias de corte (High, Intermediate y Low). Paraná (a, c, e) y Marcos Juárez (b, d, f). Letras diferentes denotan diferencias entre cultivares ($P < 0,05$), dentro de cada frecuencia de corte. Las barras representan el error estándar de la media. 2010-2011.

Defoliation	Cultivars	Paraná		Marcos Juarez	
		Similar biomass	Similar height	Similar biomass	Similar height
		Leaf prop	Leaf prop	Leaf prop	Leaf prop
High	FD4 vs. FD6	0.67 vs. 0.63*	0.66 vs. 0.64	0.66 vs. 0.62*	0.64 vs. 0.62*
	FD4 vs. FD9	0.67 vs. 0.64	0.66 vs. 0.64	0.66 vs. 0.61*	0.64 vs. 0.63
	FD6 vs. FD9	0.63 vs. 0.64	0.64 vs. 0.64	0.62 vs. 0.61	0.62 vs. 0.63
Intermediate	FD4 v. FD6	0.56 vs. 0.48*	0.56 vs. 0.53	0.60 vs. 0.57	0.58 vs. 0.58
	FD4 vs. FD9	0.56 vs. 0.47*	0.56 vs. 0.50*	0.60 vs. 0.56*	0.58 vs. 0.56
	FD6 vs. FD9	0.48 vs. 0.47	0.53 vs. 0.50	0.57 vs. 0.56	0.58 vs. 0.56
Low	FD4 v. FD6	0.50 vs. 0.45	0.48 vs. 0.44	0.56 vs. 0.57	0.56 vs. 0.57
	FD4 vs. FD9	0.50 vs. 0.44	0.48 vs. 0.44	0.56 vs. 0.53	0.56 vs. 0.53
	FD6 vs. FD9	0.45 vs. 0.44	0.44 vs. 0.44	0.57 vs. 0.53	0.57 vs. 0.53

Table 1. Leaf proportion for cultivars differing in fall dormancy (FD4, FD6 and FD9) and subjected to different cutting frequencies (High, Intermediate and Low) in Paraná and Marcos Juarez. Statistical comparison (t-test) was made using data sharing the same size (*i.e.* similar range of aerial biomass or similar range of canopy height). *Denote differences between cultivars ($P < 0.05$). 2010-2011.

Tabla 1. Proporción de hoja para cultivares de distinta latencia inverna (FD4, FD6 y FD9) sometidos a diferentes frecuencias de corte (High, Intermediate y Low) en Paraná y Marcos Juarez. La comparación estadística (test-t) se realizó usando solo datos que compartían un rango similar de tamaño (*i.e.* similar rango de biomasa aérea y de altura de canopia). *Denota diferencias entre cultivares ($P < 0,05$). 2010-2011.

Our work shows that variability on leaf proportion was better explained (higher R^2) by changes in canopy height than by changes in aerial biomass (figure 3a, b, c, d, page 86). In addition, variability in leaf proportion was poorly correlated with thermal time (figure 3e, f, page 86). Previous findings confirm that the observed differences in leaf proportion (leaf to stem ratio) between cultivars and defoliation frequencies were simply different points on a common allometric trajectory. Therefore, in lucerne pastures, biomass allocation among leaves and stems (*i.e.* the nutritive value of lucerne) can be modeled and predicted, for very contrasting lucerne genotypes and under a huge range of defoliation managements (3) using biomass based relations (18) or, even better, using plant height (21).

Temperature is the main driver of phenology and development (10, 11). The poor correlation observed between thermal time and leaf proportion (figure 3e, f, page 86) indicates that traditional criteria based on phenological development (*i.e.* flowering, growing degree-days, node number in stem) seems inappropriate for precise and quality-based defoliation management guidelines, for lucerne pastures (11, 21). Furthermore, the well-known differences in the aerial biomass/canopy height ratio of lucerne cultivars contrasting in fall dormancy (21, 23) are also an issue to be considered.

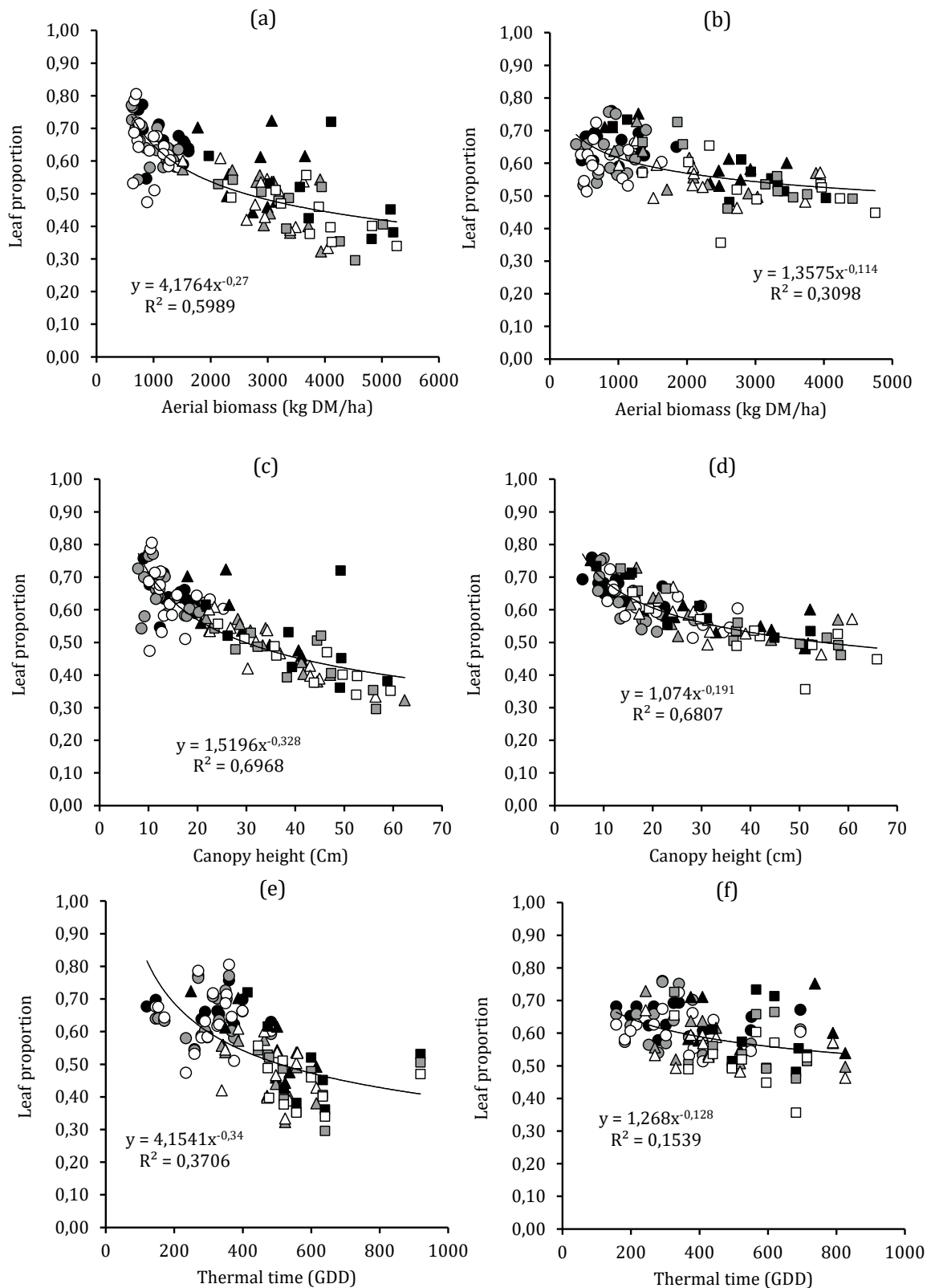


Figure 3. Leaf proportion *versus* aerial biomass (a, b), canopy height (c, d) and thermal time (e, f) for cultivars differing in fall dormancy (FD4: black symbols, FD6: grey symbols, FD9: white symbols) subjected to different cutting frequencies (High: circles, Intermediate: triangles, Low: quadrats) in Paraná (a, c, e) and Marcos Juárez (b, d, f). 2010-2011.

Figura 3. Relación de la proporción de hoja con la biomasa aérea (a, b), la altura de la canopia (c, d) y la suma térmica (e, f) para cultivares que difieren en latencia invernal (FD4: símbolos negros, FD6: símbolos grises, FD9: símbolos blancos) sometidos a diferentes frecuencias de corte (High: círculos, Intermediate: triángulos, Low: cuadrados) en Paraná (a, c, e) y Marcos Juárez (b, d, f). 2010-2011.

CONCLUSIONS

This work confirms that in subtropical and humid environments, differences in forage production between cultivars contrasting in winter activity depend on the cutting frequency. Moreover, the results obtained demonstrate that the variability in leaf proportion was mainly explained by the variability in canopy height.

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