



Evaluation of yield in alfalfa cultivation in response to the application of **Mist Prado.**

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Introduction:

Argentina has 3.8 million hectares planted with alfalfa, used in increasingly intensified production systems. To achieve productive efficiency, high profitability, and sustainability, proper agronomic management of fields destined for alfalfa is necessary, aiming for high yields in dry matter, and more importantly, in kilograms of protein and megacalories of harvested plant energy, either through grazing with high stocking rates or as conserved forages in the form of hay or haylage.

In alfalfa, as in any other crop, potential yield is defined as that which can be obtained without controllable biotic and abiotic limitations (pests, diseases, water and nutrient deficiencies), but under the specific conditions of temperature, radiation, and photoperiod of each environment.

Soil-supplied nutrients, biological processes, and fertilization play a predominant role in determining production. Among all nutrients consumed, Nitrogen (N), Phosphorus (P), Potassium (K), and to a lesser extent Sulfur (S) and Boron (B) are the elements that most limit production. After nitrogen, which is covered through biological fixation, Potassium and Calcium (Ca) are the nutrients most demanded by alfalfa production, with reported values of 28, 22, and 12 kg/t, respectively, of forage produced. Studies show that in fields with an agricultural history, Ca content decreased by 20% compared to soil profiles described more than 35 years ago. In this sense, to achieve a balance between forage produced and sustainable systems over time, it is essential to return to the soil the nutrients extracted by the crop.

Objectives of the Trial:

1. To evaluate biomass production in both quantity and quality in alfalfa cultivation under the application of MIST-PRD "MIST Prado".
2. To evaluate the effect of MIST-PRD "MIST Prado" application on light interception.
3. To analyze variations in basic indicators of alfalfa crop profitability under the application of MIST-PRD "MIST Prado".

Materials and Methods:**Experimental Period and Site**

The trial was conducted at the INTA Manfredi Experimental Agricultural Station, Córdoba, Argentina (31.5° S, 63.5° W, 292 masl), from October 2021 to June 2022.

Experimental Site Characterization

The trial was carried out on group 9 alfalfa, variety Traful PV INTA Palo Verde, established in March 2021 in a 10 ha field belonging to the Demonstrative Unit for High-Quality Hay (UPEX alfalfa) of INTA Manfredi.

The soil is an Entic Haplustoll, Oncativo series, deep, well-drained, developed on loam-silt materials, with an available water storage capacity of 307 mm up to 200 cm depth (INTA, 1987). It presents pH 7.1, 12 ppm phosphorus, 2.6% organic matter, and 0.4 dS m⁻¹ electrical conductivity.

The average annual rainfall (series 1931-2010) is 757 mm, with 80% concentrated between October and March and the remaining 20% between April and September (Severina et al., 2020). The mean annual temperature is 16.8 °C, with a mean minimum of 9.5 °C in July and a mean maximum of 23.4 °C in January (Severina et al., 2020).

Experimental Design

A randomized complete block design (RCBD) with four replications was used. Each plot measured 15 m wide x 600 m long (Figure 1). Two treatments were applied:

- Control: alfalfa without fertilization
- MIST Prado: alfalfa with fertilization

The formulation "MIST Prado" was applied at a rate of 2 L/ha after the second, fourth, and sixth cut.

TO	N
T2	
TO	N
T2	
TO	N
T2	
T2	N
TO	



Figure 1. Trial layout. TO: unfertilized alfalfa. T2: alfalfa with fertilizer. Formulation "MIST Prado" applied at 2 L/ha after the second, fourth, and sixth cut. Plot size: 15 m wide x 600 m long.

Agronomic management

During the crop cycle, monitoring was carried out to prevent the incidence of undesirable factors (weeds, insects, diseases) that could affect the results.

Measurements

Above-ground biomass (AGB)

Harvests to estimate crop yield were carried out every 450-500 accumulated growing degree days (GDD) or at the phenological stage of 10% flowering (Kalu & Fick 1981; Moot et al. 2001). Yield was determined by harvesting, at each cutting date and experimental unit, the above-ground biomass (AGB).

Cuts were performed over a surface of 2 linear meters of row at 5 cm above soil level, along a transect across each experimental plot (8 subsamples per plot). Samples were weighed fresh, oven-dried at 45 °C for 72 hours, and then dry weight was obtained. From these values, dry matter percentage (%DM) was calculated. Yield was then determined from AGB and %DM.

Radiation interception

In the regrowth following-fertilizer application, the fraction of incident solar radiation (RFAinc MJ m⁻²) above and below the canopy was measured in four sectors of each plot using a ceptometer (Bar-Rad) covering one meter width. The fraction of radiation intercepted (f) was calculated as the ratio between RFAinc above and below the canopy.

Forage quality parameters

Determinations of crude protein (CP), neutral detergent fiber (NDF), and in vitro dry matter digestibility (IVTDMS) were carried out twice, using composite samples of 200 g per plot, separated into stem and leaf, and oven-dried at 45 °C to constant weight. Samples were analyzed according to the Association of Official Analytical Chemists (AOAC, 1990), following methods of the Cornell Net Carbohydrate and Protein System (CNCPS):

- DM (method 930.15)
- Ash (method 924.05)
- CP Kjeldahl (method 984.13), using a Kjeltec Sampler 8420 automatic analyzer (FOSS).

Stem density and size

Stems used for forage quality parameters were weighed and counted to obtain stem density (stems/m²) and average stem size (g). Leaves and stems were weighed separately to calculate the leaf/stem ratio.

Statistical analysis

The evaluated variables were statistically analyzed with Infostat software, and multiple post hoc comparisons were adjusted using Tukey's HSD test, with a significance level set at $\alpha = 0.05$; 0.10.

Results and Discussion

Treatment definition

The growth cycle evaluated extended from November 18, 2021, to June 30, 2022. Determinations were carried out on an alfalfa crop sown in March 2021, which had an establishment period allowing for the accumulation of the necessary reserves in root and crown.

Thus, in October (200 days after sowing) the first of seven cuts was performed. Cuts were carried out every 35-40 days on average, or ~520 GDD. The fourth cut was performed at 768 GDD (Table 1), due to rainfall occurring during the optimal cutting period. Since the UPEX is destined for hay roll production, it was considered appropriate to delay the cut to avoid nutrient losses or leaching in the windrow.

Foliar fertilizations were performed at three stages: after the second, fourth, and sixth cut.

SOWING - ESTABLISHMENT			CUTS						
			1	2	3	4	5	6	7
Date	18/3 a 20/10		20/10	18/11	20/12	27/1	4/3	18/4	20/6
DEC		-		28	32	37	37	44	62
TT (°CDA)		-		441	570	768	580	560	430
Mean T (°C)				15.8	20	23	22	18	11
PP (mm)				122	96	139	66	64	20,5

Table 1. Days between cuts (DEC), Thermal Time (TT, °CDA), Mean Temperature (°C), and Precipitation (PP, mm) corresponding to the experimental evaluation period. Manfredi. 2021-2022.

Above-ground biomass production

The accumulated above-ground biomass production during the evaluation period (i.e., from the beginning of the experiment) was 16% higher in the foliar fertilization treatment (hereafter MIST Prado) compared with the treatment without foliar fertilization (hereafter Control). On average, MIST Prado reached 24,000 kg DM ha⁻¹, while the Control reached 20,500 kg DM ha⁻¹.

When production was analyzed per cut, it was found that MIST Prado achieved higher yields (Figure 3). Specifically, during the growth periods following product application (cuts three, five, and seven), an average 30% higher production was obtained ($p < 0.10$), whereas in cuts four and six, yields were similar between treatments ($p > 0.10$) (Figure 3).

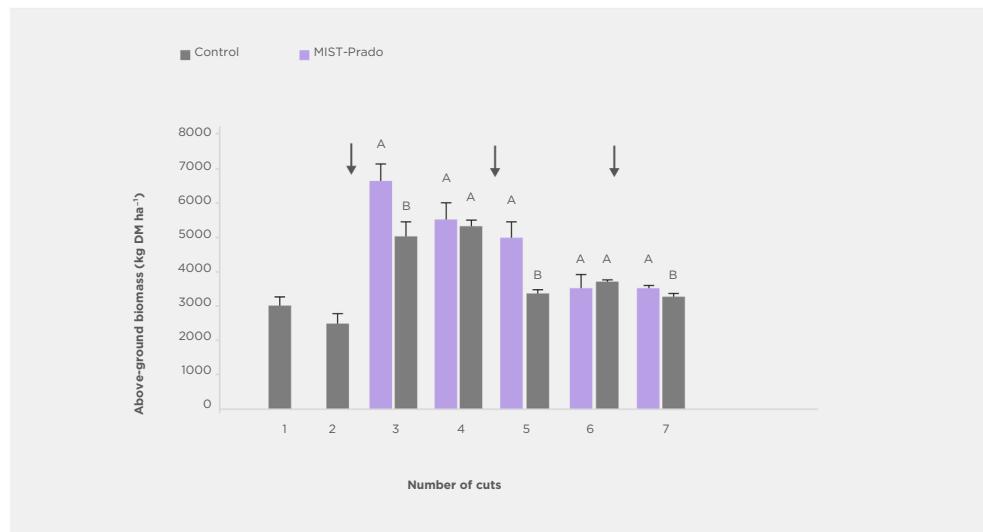


Figure 3. Above-ground biomass (kg DM ha⁻¹) per cut for an alfalfa GR9 cultivar during the first growth cycle under two treatments: without fertilization (Control) and with foliar fertilization (MIST Prado). Trial conducted at INTA Manfredi Experimental Station, 2021-2022. Vertical bars represent mean values \pm standard error of the mean. Different uppercase letters vertically indicate significant differences between treatments at each cut ($p < 0.10$). Black arrows indicate the timing of MIST Prado application, i.e., after the second, fourth, and sixth cut.

Yield components

One way to evaluate biomass production is through yield components: stem density per unit area and stem weight (Volenc et al., 1987). In this regard, during cuts three, four, and seven, corresponding to the growth period after fertilizer application, both treatments (Control and MIST Prado) were evaluated for stem density and stem weight; in addition, leaf percentage, leaf-to-stem ratio, and plant height at cutting were quantified.

Stem density (m^2) and stem weight

In most cuts after the application of MIST Prado, a higher stem density was observed (Figure 4a) ($p < 0.10$). At the third cut, values reached nearly 2500 stems/ m^2 , representing almost 50% more than the Control. This trend was similar at the fifth cut, although with a lower overall stem density. However, in this cut, MIST Prado achieved almost 30% more stems than the Control (1206 vs. 810 stems/ m^2).

Stem weight (Figure 4b) averaged 0.40 and 0.35 g/stem for MIST Prado and Control, respectively. Except for the third cut, in the remaining evaluated periods, MIST Prado consistently achieved higher stem weight (20%) than the Control ($p < 0.05$).

The higher biomass production observed in the third cut under the MIST Prado treatment (Figure 3) could be explained by the greater stem density (Figure 4a), since stem weight in both treatments was similar (Figure 4b). On the other hand, when comparing the third cut with the fifth and seventh, it was observed that in MIST Prado, stem density decreased by more than 30%, while stem weight increased by nearly 50%, explaining the higher production achieved in subsequent cuts.

Although the Control treatment showed a similar compensatory behavior, the lower stem proportion (Figure 4a) and stem weight (Figure 4b) ultimately resulted in lower yield (Figure 3).

Despite the yield differences found, under a given resource availability, the crop exhibited a compensatory effect between stem density and stem weight.

Meanwhile, plant height at cutting averaged 55 cm for MIST Prado and 52 cm for the Control.

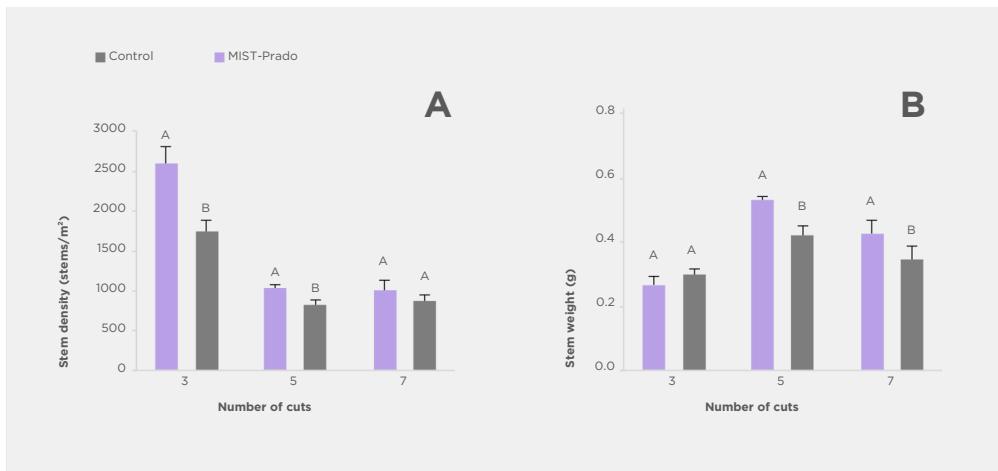


Figure 4. (a) Stem density (stems/ m^2) per cut for an alfalfa GR9 cultivar during the first growth cycle under two evaluation treatments: without fertilization (Control) and with foliar fertilization (MIST Prado). Trial conducted at INTA Manfredi Experimental Station, 2021-2022. Vertical bars represent mean values \pm standard error of the mean. Different uppercase letters vertically indicate significant differences between treatments at each cut ($p < 0.05$). Cuts 3, 5, and 7 correspond to the growth periods with fertilizer application. (b) Stem weight (g) per cut for an alfalfa GR9 cultivar during the first growth cycle under two evaluation treatments: without fertilization (Control) and with foliar fertilization (MIST Prado). Trial conducted at INTA Manfredi Experimental Station, 2021-2022. Vertical bars represent mean values \pm standard error of the mean. Different uppercase letters vertically indicate significant differences between treatments at each cut ($p < 0.05$). Cuts 3, 5, and 7 correspond to the growth periods with fertilizer application.

Leaf Percentage at Cutting Time

Of the total production (leaves + stems), leaves are the component of highest nutritional value and potential intake by animals (Romero et al., 2007). This variable is expressed as a percentage proportion of the total biomass. For both treatments, in cuts three and seven, the leaf percentage accounted for 53% of the produced biomass, with no differences between treatments. In cut five, the trend remained similar, although the leaf proportion was lower (Figure 5).

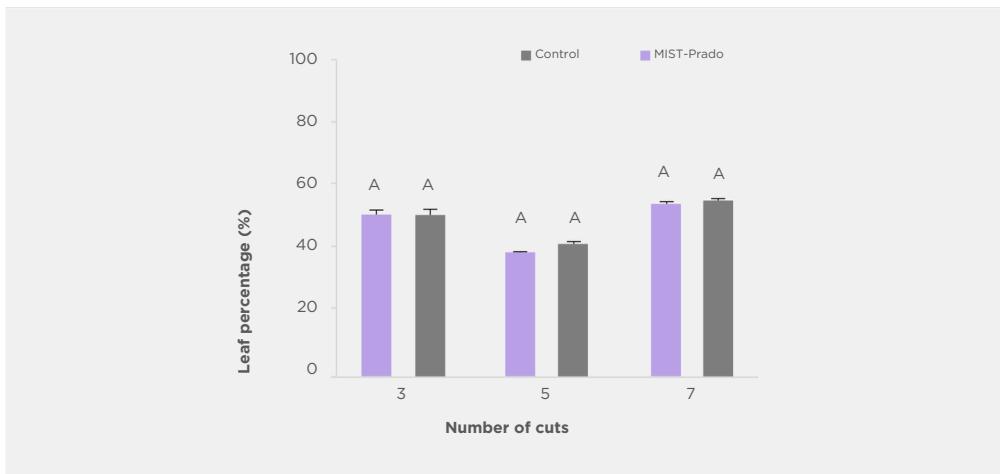


Figure 5. Leaf percentage per cut for an alfalfa GR9 cultivar during the first growth cycle under two evaluation treatments: without fertilization (Control) and with foliar fertilization (MIST Prado). Trial conducted at INTA Manfredi Experimental Station, 2021-2022. Vertical bars represent mean values \pm standard error of the mean. Different uppercase letters vertically indicate significant differences between treatments at each cut ($p < 0.05$). Cuts 3, 5, and 7 correspond to the growth periods with fertilizer application.

According to Cangiano (2005), from the onset of flowering there is a reduction in leaf production, an increase in leaf senescence in the lower canopy, and an increase in stem weight, producing a gradual decrease in the leaf-to-stem ratio. This ratio varies between 1.5 and 3.9 during vegetative stages and drops to 0.5 when plants reach maturity. Table 2 shows the leaf-to-stem ratio recorded for each evaluation moment.

Table 2. Leaf-to-stem ratio per cut for an alfalfa GR9 cultivar during the first growth cycle under two evaluation treatments: without fertilization (Control) and with foliar fertilization (MIST Prado). Trial conducted at INTA Manfredi Experimental Station, 2021-2022. \pm signs represent mean values \pm standard error of the mean. Different uppercase letters vertically indicate significant differences between treatments at each cut ($p < 0.05$). Cuts 3, 5, and 7 correspond to the growth periods with fertilizer application.

Treatment	CUT		
	3	5	7
MIST prado	1,05 \pm 0,07A	0,65 \pm 0,02 A	1,19 \pm 0,06 A
Control	1,04 \pm 0,09A	0,72 \pm 0,04 A	1,24 \pm 0,06 A

Although no significant differences were recorded, it can be observed that the MIST Prado treatment presented, at some points, a lower leaf-to-stem ratio compared to the Control. This could be due to the greater stem weight reached in these treatments, combined with an increase in leaf senescence in the lower canopy, which, although not quantified in this study, Olivo et al. (2018) reported that in longer regrowth periods, senescence can increase by 7-10%.

Nutritional Quality of Total Biomass

From the total biomass produced, leaves are the component of highest nutritional value and potential intake. As maturity advances, quality decreases, with an increasing proportion of stems relative to leaves (Romero et al., 2007).

Table 3 presents the quality parameters of leaves and stems evaluated. The variables analyzed were: Crude Protein (CP), Neutral Detergent Fiber (NDF), and Digestibility (DIG).

As can be observed, leaf quality in terms of CP was similar ($p > 0.05$) in both treatments. However, stem quality was similar in protein content ($p > 0.05$) but different in digestibility and NDF ($p < 0.05$).

Table 3. Nutritional quality expressed as percentage of Crude Protein (CP), Digestibility (DIG), and Neutral Detergent Fiber (NDF) for an alfalfa GR9 cultivar during the first growth cycle under two evaluation treatments: without fertilization (Control) and with foliar fertilization (MIST Prado). Trial conducted at INTA Manfredi Experimental Station, 2021-2022. \pm signs represent mean values \pm standard error of the mean. Different uppercase letters vertically indicate significant differences between treatments at each cut ($p = 0.05$).

NUTRITIONAL QUALITY				
		CP	DIG	NDF
Leaf	MIST prado	27,7 \pm 0,57 A	67,2 \pm 0,91 A	37,1 \pm 1,22A
	Control	28,2 \pm 0,49 A	67,7 \pm 0,75 A	36,6 \pm 0,97A
Stem	MIST prado	13,0 \pm 0,69 A	45,1 \pm 0,63 B	66,6 \pm 0,22A
	Control	14,0 \pm 0,65 A	46,6 \pm 0,89 A	63,3 \pm 0,31B

The quality of stems in the MIST Prado treatment was lower than in the Control (Table 3). This is related to the biomass produced and the yield components that contribute to such biomass (Figure 4a and 4b). In other words, the greater stem weight and plant height achieved in MIST Prado led to a higher proportion of NDF and lower digestibility. This decline in quality with greater accumulation of above-ground biomass is inevitably observed in pastures as plants increase in size and in the amount of supporting tissues (Wilson, 1976).

Fraction of Intercepted Radiation

Another way of analyzing growth is through resource capture, among them light. Biomass production depends on the amount of intercepted radiation and the efficiency with which this radiation is used to generate above-ground dry matter (leaves + stems) (Monteith, 1977). In this sense, when analyzing the fraction of intercepted radiation (Figure 6) for each of the treatments, it was observed that the greater biomass production (Figure 3) can also be explained by the higher light capture of the MIST Prado treatment.

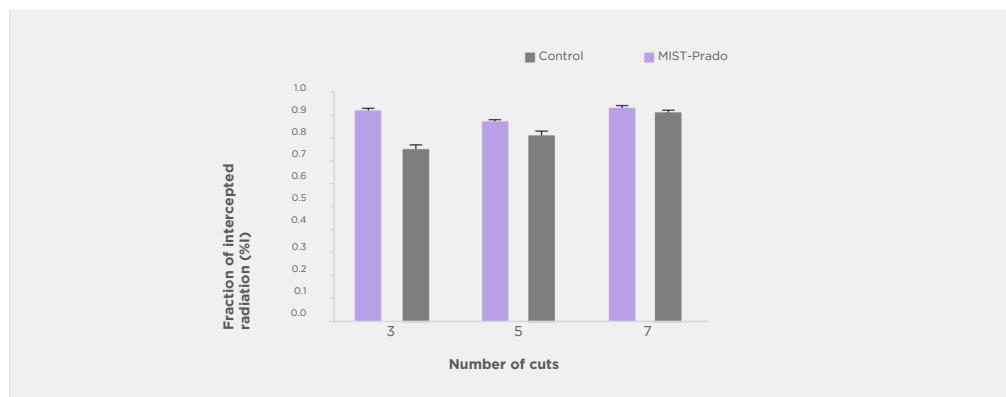


Figure 6. Fraction of Intercepted Radiation (%) for an alfalfa cultivar GR9 during the first growth cycle and two evaluation treatments; without fertilization: Control and with foliar fertilization: MIST Prado. Trial conducted at EEA INTA Manfredi 201-2022. Vertical bars are mean values \pm standard error of the mean. Different uppercase letters in the vertical direction denote significant differences between treatments at each cut ($p<0.05$). Cuts 3, 5, and 7 correspond to the growth period with fertilizer application.

Conclusions:

Under the experimental conditions in which the trial was conducted, it can be concluded that:

- The application of MIST Prado generated an average increase of 16% in biomass production and 30% in regrowth after product application.
- In regrowth following the application of MIST Prado, the higher biomass production at the third cut was due to a greater stem density (stems/m²), while in cuts five and seven it was the result of a higher stem weight.
- In regrowth after the application of MIST Prado, the leaf percentage was similar in both treatments.
- In regrowth after the application of MIST Prado, the nutritional quality of the leaf was similar in both treatments.
- In regrowth after the application of MIST Prado, the nutritional quality of the stem showed similar CP values, while in the MIST Prado treatment digestibility was lower and NDF higher.
- In regrowth after the application of MIST Prado, the fraction of intercepted radiation was higher in the MIST Prado treatments.

In general, most of the alfalfa area destined for haymaking in our country is located in humid, sub-humid, and semi-arid zones, where the pasture growth period coincides with the occurrence of rainfall. One of the major challenges for this activity is achieving high-quality hay, since windrows of cut alfalfa are exposed to rainfall, which occurs with varying frequency during haymaking.

Although throughout the experiment the application of MIST Prado promoted greater biomass production, the nutritional quality was lower. This is related to the fact that in many cuts, due to the probability of rain before harvesting, the cut was postponed.

Therefore, it is important to highlight that under fertilization schemes, it would be advisable to conduct evaluations with shorter cutting intervals (i.e., at the pre-bud stage), aiming for a balance between biomass production and forage quality, positively impacting meat or milk production.

It would also be appropriate to evaluate product application after the last winter cut or at the beginning of spring, to analyze its impact on yield changes at those cuts.

Annex:



Figure 1. Timing of product application and subsequent field sampling



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