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Increase in forage yield in narrowleaf birdsfoot trefoil (*Lotus tenuis* Waldst & Kit ex Willd) in a permanent pasture with foliar applied gibberellic acid (GA_3), and phosphorus

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Abstract

Lotus tenuis is a perennial legume with a good adaptation to infertile, heavy and waterlogging soils. It can replace alfalfa in these sites with a similar feeding value. An important constraint is its weak competitive capacity with other graminae and weed species in permanent pastures, having consequently a poor forage yield. The objective of the present research is to overcome this disadvantage, enhancing its competitive ability with foliar applications of GA_3 (GA) and phosphorus (P), increasing *L. tenuis* forage yield. Field experiments were conducted during 1994 with foliar application of GA (50 mg.l^{-1}) and during 1995 with foliar application of GA (25 and 50 mg.l^{-1}), phosphorus (8 kg.ha^{-1}, as P_2O_5) and their combinations, in permanent pastures with *L. tenuis* and other companion grasses. In 1994 GA 50 increased significantly *L. tenuis* dry matter (DML) in 64.6% but not the dry matter of graminae fraction (DMG) and in consequence the total dry matter of the pasture (TDMP) was increased. In 1995 all GA treatments and their combinations with phosphorus enhanced DML but not DMG. In this sense GA 25 + P was the most effective treatment with a 151% increment of DML. Consequently TDMP was significantly increased due to a larger participation of *L. tenuis* in the forage yield. This increase was achieved due to a greater length and diameter of *L. tenuis* branches, with a logical modification in leaf:stem ratio. Moreover GA treatments reduced *L. tenuis* flower number. Phosphorus treatment, applied alone, showed an increase in the DML. GA treatments did not modify the feeding value of the forage in *L. tenuis* and graminae fractions, except GA 50 and GA 50 + P in acid detergent fiber (ADF), neutral detergent fiber (NDF) and crude protein (CP), respect to the control. The total crude protein (CP.m^{-2}) was enhanced in all GA and GA + P treatments. Foliar GA_3 and phosphorus spray applications increased the competitiveness of trefoil for light, on account of morphological changes in the spatial disposition of *L. tenuis* stems reaching faster the top of the pasture canopy. This practice can be an adequate alternative to increase the forage yield and total crude protein in permanent and cultivated pastures with a low cost-benefit ratio.

1. Introduction

Narrowleaf birdsfoot trefoil is a perennial forage legume of mediterranean origin. It is very adaptable to waterlogged, heavy and infertile soils of the low-lying soils areas, in permanent and cultivated pastures of Canada, New Zealand, USA and South America [23].

In Argentina, in the Salado river basin (province of Buenos Aires) *L. tenuis* became naturalized and is a valuable forage because it has a good adaptation in an area with graminae predominance [17] and a poor presence of other legumes [1]. Therefore *L. tenuis* is the more convenient legume to enhance the nitrogen content and digestibility property of the forage in this and other regions where alfalfa and other legumes do not grow. Because of this capacity its use is being increased.

Lotus tenuis grows from Spring to Autumn and flowers from November to March. Plants of *L. tenuis* are generally less vigorous than alfalfa and red clover and stands may be lost due to competition from other companion graminae species and weeds with a similar growth cycle [11] due to a strong competition for mineral nutrients, water and light. Although it is well known that phosphorus fertilization improves competitive ability of legumes, *L. tenuis* demonstrated to response to lower doses than other legume species [8, 18, 22]. In this sense *L. tenuis* showed a greater dry matter increase than *Trifolium pratense* to low rates of phosphorus [2, 3]. Foliar fertilizer trials with 6 $kg.ha^{-1}$ phosphorus indicated that *L. tenuis* also responded to improvements of phosphorus by foliar fertilization [10].

L. tenuis showed a negative gravitropic response of plagiotropic stems treated with GA_3, like prostrated branches of *Arachis spp*. [14], lateral branches of *Cupressus arizonica* [5] and stolons of *Trifolium fragiferum* [4, 15]. In other experiments with foliar application of GA_3 *L. tenuis* showed, not only an erect growth of branches, but also an enhancement of internodes and stem length and an increase in the diameter of them, to low GA_3 rates [9].

Therefore, the purpose of this work was to enhance *L. tenuis* competitiveness over companion graminae species in permanent pastures, inducing an increase in its growth rate and forage yield, with foliar applications of GA_3 and phosphorus.

2. Materials and methods

Two field experiments were carried out at a permanent pasture established in La Plata (s.l. 34°54′), during 1994 and 1995. Research plots were located at a typical natracualf soil. Prior to the beginning of each experiment and thirty days after the applications, when the harvesting was done the qualitative botanical composition of the pasture was determined using the method described by Braun-Blanquet [6] and expressed on a percentage dry weight basis. Four 0.25 m^2 randomly quadrats for each replicate were harvested randomly by hand-clipping to a stubble height of 7 cm. The fresh forage samples harvested were hand-separated into *L. tenuis* and graminae fractions and were oven-dried at 60 °C for a week and weighed for dry matter determination of *L. tenuis* fraction (DML, $g.m^{-2}$), percentage by weight of *L. tenuis* in the total dry matter production of the pasture (DML,%), dry matter of the graminae fraction (DMG, $g.m^{-2}$) and total dry matter production of the pasture (TDMP, $g.m^{-2}$).

Leguminosae fraction was approximately 56% in the first year, represented mainly by *Lotus tenuis* (99%). Graminae fraction for the same growing season (approximately 44%) was dominated by *Cynodon dactylon, Paspalum dilatatum, Lolium multiflorum* (together 70%), *Setaria geniculata* and *Bothriochloa laguroides.*

In 1995 Leguminosae fraction was approximately 39% with a similar percentage of *L. tenuis* respect to the first year. Graminae fraction showed a similar composition and abundance of species respect to Experiment 1.

Experiment 1:

Research plots were located at a pasture grazed 15 days before the beginning of the experiment, to a stubble height of 8 cm. Plots 6 m long and 4 m width were used in a complete randomized design with 6 replications of each treatment. On 3 October 1994 gibberellic acid (GA) was applied with a high volume machine at a rate of 50 $mg.l^{-1}$ GA_3 (GA 50). Control plot was sprayed with water. Water volume per plot was equivalent to 500 $l.ha^{-1}$. Tween 20 (0.005%) was used as tensioactive in all treatments, even in the control.

Experiment 2:

In this trial research plots were located at a pasture grazed 5 days before the beginning of the experiment, to a stubble height of 8 cm. The same size and number of replicates mentioned in Experiment 1 were used. On 22 November, at the beginning of blossoming, plant growth regulator and phophorus were applied with a high volume machine at the following rates: a) phosphorus (P) as PO_4H_2K 36.82 g per plot (equivalent to 8 $kg.ha^{-1}$ as P_2O_5), b) GA_3 25 $mg.l^{-1}$ (GA 25), c) GA_3 50 $mg.l^{-1}$ (GA 50), d) GA 25 + P, e) GA 50 + P, f) control sprayed with water. Water volume per plot and the tensioactive concentration were the same as Experiment 1.

Thirty days after the beginning the number of branches per plant, length and diameter of branches were determined as well as leaf:stem ratio in dry weigth on 10 random selected plants. Number of inflorescences and infructescences were counted in four

Table 1. Effect of foliar spray treatments of GA 50 (50 mg.l^{-1} GA_3), in the dry matter of *L. tenuis* (DML) percentage of *L. tenuis* in the total dry matter production of the pasture (DML, %), dry matter of the graminae fraction (DMG) and total dry matter production of the pasture (TDMP), at the beggining of the trial (i) and 30 days after (f), in 1994.

Treatment	DML (g.m^{-2})		DML (%)		DMG (g.m^{-2})		TDMP (g.m^{-2})	
	(i)	(f)	(i)	(f)	(i)	(f)	(i)	(f)
Control	40.8 a[1]	258.8 b	55.7 a	60.3 b	32.4 a	170.1 a	73.2 a	428.8 b
GA 50	38.8 a	426.0 a	56.7 a	72.6 a	29.6 a	160.8 a	68.4 a	586.8 a

[1] Averages followed by the same letter, in the same column, are not significantly different (P: 0.05).

0.25 m^2 randomly quadrats per plot. The samples were harvested and processed as in Experiment 1.

Forage nutritive value of *L. tenuis* and graminae fractions was determined in the whole plant, leaves and stems together, through acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin (LIG), using methods described by Goering and Van Soest [13] and crude protein (CP) by semi-micro Kjeldahl. Results are presented on a dry matter basis. Likewise it was calculated the total crude protein (CP.m^{-2}) addend for *L. tenuis* and graminae fractions. Digestible dry matter (DDM) was estimated by linear regression equation described by Minson [19] ($y = 110.5 - 1.32.$ ADF).

Data were subjected to analysis of variance (ANOVA). In the second experiment, means of each treatment were compared by Tukey's test at a 0.05 and 0.01 probability level when ANOVA showed a significant treatment effect.

Table 2. Effect of foliar spray treatments of GA 25 (25 mg.l^{-1} GA_3), GA50 (50 mg.l^{-1} GA_3), P (8 kg.ha^{-1} phosphorus expressed as P_2O_5,) and their combinations, in the dry matter of *L. tenuis* (DML), percentage of *L. tenuis* in the total dry matter production of the pasture (DML, %), dry matter of the graminae fraction (DMG) and total dry matter production of the pasture (TDMP), at the harvest, in 1995.

Treatment	DML (g.m^{-2})	DML (%)	DMG (g.m^{-2})	TDMP (g.m^{-2})
Control	78.36	35.44	142.72	221.08
P	109.32	44.93	134.01	243.33
GA 25	145.84	51.44	137.64	283.48
GA 25 + P	196.60	58.26	140.88	337.48
GA 50	140.88	48.20	151.40	292.28
GA 50 + P	155.04	51.55	145.68	300.72
Tukey 5%	28.08	3.82	NS †	52.40
Tukey 1%	34.52	4.70		64.48

† NS, not significant at 0.05 level of probability.

3. Results

In Experiment 1 GA 50 increased significatively DML (g.m^{-2}) about 64.6% respect to the control without differences in DMG, so the percent of *L. tenuis* in the total dry matter production of the pasture (DML, %) was larger. In consequence GA 50 increased TDMP (Table 1).

In the Experiment 2, P, GA 25, GA 50 and their combinations increased DML (g.m^{-2}) without differences in DMG and consequently narrowleaf birdsfoot trefoil contribution to TDMP was greater than graminae fraction, as can be observed in DML (%) values. In this sense GA 25 + P showed the greatest differences enhancing DML (g.m^{-2}) in 151% (Table 2).

In the forage quality GA 50 and GA 50 + P enhanced ADF and NDF and reduced CP and DDM, respect to the control, without significant differences in LIG, while GA 25, GA 25 + P and P, did not show significant differences with the control in none of the mentioned variables. Graminae fraction did not show differences in nutritive forage values (Table 3).

In morphological parameters P increased the branch number of *L. tenuis* but all GA_3 treatments alone and with phosphorus showed a reduction in this variable. In opposition GA_3 alone and its combinations with phosphorus, in both concentrations, enhanced the length and the diameter of the branches. Leaf: stem ratio was reduced by all treatments, but GA 50 showed differences more significant than the other treatments. Moreover all GA treatments decreased severely the number of inflorescences but not infructescences and

Table 3. Effect of foliar spray treatments of GA 25 (25 mg.l^{-1} GA_3), GA 50 (50 mg.l^{-1} GA_3), P (8 kg.ha^{-1} expressed as P_2O_5) and their combinations, in the forage nutritive values (%) of *L. tenuis* (L) and graminae fractions (G): Acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), lignin (LIG), digestible dry matter (DDM) and Total crude protein (CP.m^{-2}), in 1995.

Treatment	ADF		NDF		CP		LIG		DDM		CP.m^{-2}
	%										
	L	G	L	G	L	G	L	G	L	G	
Control	29.34	37.04	39.46	62.65	11.97	6.98	7.54	5.20	71.77	61.61	19.34
P	29.82	35.43	41.12	61.15	11.58	7.16	7.85	5.63	71.04	63.73	22.26
GA 25	31.43	39.24	43.54	64.26	11.27	7.33	8.15	5.73	69.01	58.70	26.29
GA 25 + P	31.08	37.56	43.05	62.56	11.04	6.93	7.63	5.56	69.47	60.92	31.47
GA 50	34.83	34.87	45.75	59.81	9.65	7.52	7.25	5.74	64.52	64.47	24.98
GA 50 + P	35.12	36.06	46.55	61.44	9.56	7.75	7.83	5.18	64.14	62.90	26.11
Tukey 5%	4.78	NS †	5.88	NS	1.86	NS	NS	NS	6.31	NS	4.63
Tukey 1%	5.87		7.23		2.28				7.76		5.68

† NS, not significant at 0.05 level of probability.

Table 4. Effect of foliar spray treatments of GA 25 (25 mg.l^{-1} GA_3), GA 50 (50 mg.l^{-1} GA_3), P (8 kg.ha^{-1} expressed as P_2O_5), and their combinations, in the number, length and diameter of branches, leaf:stem ratio, number of inflorescences and infructescences of *L. tenuis*, in 1995.

Treatment	Branch number	Branch length(cm)	Branch diameter(mm)	Leaf:stem ratio	Inflorescences number.m^{-2}	Infructescences number.m^{-2}
Control	34.6	16.3	1.01	0.926	248.00	44.68
P	40.3	17.4	1.02	0.870	293.20	55.32
GA 25	21.4	32.8	2.05	0.503	27.44	48.68
GA 25 + P	23.3	33.1	2.10	0.437	28.40	44.02
GA 50	14.6	38.2	2.51	0.429	26.40	47.44
GA 50 + P	15.8	36.6	2.54	0.465	20.01	37.36
Tukey 5%	6.98	7.45	0.33	0.34	50.1	NS †
Tukey 1%	8.58	9.17	0.42	0.42	63.6	–

† NS, not significant at 0.05 level of probability.

P did not show differences in this variable respect to the control (Table 4).

4. Discussion

The results obtained in Experiment 1 showed that it was possible to reach the purpose of this work, on account of the great response of *L. tenuis* to foliar applications of GA_3 to very low rates. In Experiment 2 it was confirmed that the enhancement of yield forage was reached even with low rates of GA (25 mg.l^{-1}) and combinations with foliar fertilization of phosphorus. The increment was function of a greater length and diameter of stems and consequently a minor leaf:stem ratio in GA_3 treated plants. This response is opposite to that found in alfalfa by Montaldi and Sanchez [21], where the increase in the dry weight with GA_3 treatments to rates of 60 g.ha^{-1} was due to a significant increase in the length of branches and foliar area of the plant, and not because of an increase in stem diameter and a minor relationship leaf:stem ratio as it is shown in *L. tenuis* in this work.

Moreover in Experiment 2 it was observed that GA 50 and its combinations with phosphorus in spite of having increased the forage yield of *Lotus tenuis* respect to the control, reduced the same variable respect to GA 25 + P, perhaps on account of the significant reduction of the number of branches, as observed in foliar application of GA_3 in decapitated plants of

Lotus tenuis [9]. This response could be explainable in function of the gibberellic acid property to increase the photoassimilates phloem flow [24] and this would make GA_3 responsable for sucrose translocation out from the shoot base towards the apical part, thus diminishing the hydrocarbon resources for basal bud activity.

In addition GA 50 and GA 50 + P reduced the CP and DDM perhaps due to an increase in the structural polysaccharides of cell wall but not with increments in lignin content. Reduction of CP would not produce a significant reduction in the feeding value because $CP.m^{-2}$ was greater on account of the significant increase in dry matter (Table 3).

Also the GA treatments and their combinations with phosphorus produced significant changes in the relative biomass composition of the pasture, with an increment of *L. tenuis* vs Graminae fraction. Probably the exogenous GA_3 enhanced the competitive ability of *L. tenuis* on account of a faster growth rate of the stems and in this way it reaches faster than the graminae fraction the canopy top. As it was also observed in plagiotropic organs of other dicotiledoneus species as *Arachis spp*. [14], lateral branches of *Cupressus arizonica* [5] and stolons of *Trifolium fragiferum* [15], exogenous GA_3 induced in *L. tenuis* a change in growth habit of stems towards a negative gravitropism that could contribute to a succesful competition for light.

In *L. tenuis* low photosynthetic photon flux density induced an ortotropic growth of stems with greater synthesis of GA_1 and GA_3 [9]. In this sense the exogenous applications of GA_3 should promote an early escape from shady environments caused by companion grasses.

GA_3 treatments, even in the highest concentration used in this trial, did not increase graminea dry matter. This response is coincident with preeceding experiences in forage graminae as *Cynodon dactylon* and *Poa pratensis*, where it was shown a dry matter increase with GA_3 exogenous applications in the order of 100 a 1000 $mg.l^{-1}$ [25].

Phosphorus foliar applications, alone or with GA_3, also showed an increase of *L. tenuis* dry matter. This response is consistent with other experiences of edaphic phosphorus fertilization in *L. tenuis*, that showed other kind of response different from other legume and graminae forage with a significant response to lower doses [3, 20] and it is also consistent with former experiences with foliar phosphorus fertilization in *L. tenuis* [10]. The poor competitive ability of legumes in phosphorus absorption in natural conditions, could explain the great response of this species to phosphorus fertilization [16].

Graminae presents a larger capacity of soil exploration, due to its root system morphology that enhaces its possibility of absorbing a little mobile nutrient as phosphorus in the soil [7]. This situation involves a legume disadvantage to compete for this element in low soils-P as in this trial, with 5 ppm, that could be reversed with phosphorus foliar fertilization with the advantage of avoiding adverse edaphic conditions about availability and absorption of phosphorus and, besides, using lower doses than the usually used in edaphic fertilization.

Both concentrations of GA_3 tested reduced the number of flowers in Experiment 2, due to an inhibition of further bottom flowers but not the fructification of those already formed, this observation is consistent with preceeding works in *L. tenuis* [12].

It is concluded that the use of foliar spray application of GA_3 and phosphorus showed to produce morphological changes and also a different spatial disposition of *L. tenuis* stems and a succesful competition for light on account of a faster reaching to pasture canopy top. In consequence the forage composition of the pasture respect to TDMP was modified enhancing the percentage of *L. tenuis* biomass. In this sense the use of GA_3 and phosphorus could be an adequate alternative to increase the forage yield and $CP.m^{-2}$, in permanent and cultivated pastures with *L. tenuis,* enriching in nitrogen the feeding value of the forage.

For practical purpose this technique could reduce the cost-benefit ratio of the farmer. The treatments with GA_3 (25 $mg.l^{-1}$) and phosphorus (8 $kg.ha^{-1}$) imply an aditional cost of 20% per hectarea, in function of the product and application cost, but the farmer could obtain an aditional benefit of 200% per hectarea due to a greater average daily liveweigth gain per animal.

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