



FORAGE PRODUCTION BASED ON TRITICALE (*X. triticosecale* Wittmack) IN LIXIC FERRALIC NITISOL SOIL WITH VARYING NITROGEN DOSES AND ARBUSCULAR MYCORRHIZAL FUNGAL INOCULATION

Producción de forraje a base de triticale (*X. triticosecale* Wittmack) en suelo nitisol ferrálico lúxico, con dosis variables de nitrógeno e inoculación con hongos micorrízicos arbusculares

Rodolfo R. Plana Llerena[✉], Pedro J. González Cañizares, Ramón Rivera Espinosa, Mario Varela Nualles and Marta A. Álvarez Gil

ABSTRACT. An experimental work was carried out at "Niña Bonita" Pasture and Forage Station of Bauta, in order to achieve high forage production based on triticale by using minimum nitrogen doses. It was made on a Lixic Ferralic Nitisol with arbuscular mycorrhizal fungal inoculations (AMF). Treatments consisted of AMF inoculation and non-inoculation as well as varying N doses (0, 50, 100, 150, 200, 250 and 300 kg ha⁻¹), with a fixed bottom of 54 and 70 kg ha⁻¹ of phosphorus and potassium, respectively. Evaluations were performed to leaf rates (% N, % P and % K), fungal variables, N, P and K extraction (kg ha⁻¹), raw protein (%), mycorrhizal efficiency (%), dry forage mass (kg ha⁻¹), apparent recovery efficiency (kg kg⁻¹) and partial factor productivity, besides using ANOVA statistical analyses with one-way classification model to the original data. In every case, the best fitting models (dose-yield) were chosen to estimate optimal N doses with and without AMF, followed by the first derivative criteria to find the respective optimum ones. Results showed that AMF application allows reducing N doses to 50 and 100 kg ha⁻¹, achieving a dry mass production of 6185 kg ha⁻¹ with 11,75 % raw protein; leaf rates of 1,94 % N, 0,23 % P and 30 % K, indicating the adequate forage quality produced.

RESUMEN. Con el objetivo de lograr producciones altas de forraje a base de triticale, empleando dosis mínimas de Nitrógeno, se desarrolló un trabajo experimental en la Estación de Pastos y Forrajes "Niña Bonita", Bauta, en suelo Nitisol Ferrálico Lúxico, basado en inoculaciones con hongos micorrízicos arbusculares (HMA). Los tratamientos fueron con y sin inoculación de HMA y dosis variables de nitrógeno (0, 50, 100, 150, 200, 250 y 300 kg ha⁻¹), con fondos fijos de 54 y 70 kg ha⁻¹ de fósforo y potasio, respectivamente. Se evaluaron los índices foliares (% N, % P y % K), variables fúngicas, extracción de N, P y K (kg ha⁻¹), proteína bruta (%), eficiencia micorrízica (%), masa seca forraje (MS) en kg ha⁻¹, Eficiencia Aparente de Recuperación (ER_N) (kg kg⁻¹) y el Factor Parcial de Productividad (FPP). Los análisis estadísticos se realizaron mediante ANOVA, con modelo de clasificación simple al dato original. Para la estimación de las dosis óptimas de nitrógeno con y sin HMA se escogieron los modelos de mejor ajuste (dosis-rendimiento) en cada caso y seguidamente, se utilizó el criterio de la primera derivada para encontrar los óptimos respectivos. Los resultados mostraron que la aplicación de HMA permite reducir las dosis de nitrógeno a 50 y 100 kg ha⁻¹, lográndose una producción de masa seca de 6185 kg ha⁻¹, con proteína bruta de 11,75 %; índices foliares de 1,94 % N, 0,23 % P y 30 % K, lo cual indica la adecuada calidad, del forraje producido.

Key words: forage, vesicular arbuscular mycorrhizae, nutrition, cereals

Palabras clave: forraje, micorrizas arbusculares vesiculares, nutrición, cereales

INTRODUCTION

Animal food production in Cuba is among staple livestock priorities to achieve higher milk and meat production for human consumption. In this sense, forage production is highly significant, especially from

Instituto Nacional de Ciencias Agrícolas (INCA), gaveta postal 1, San José de las Lajas, Mayabeque, Cuba, CP 32 700.

✉ plana@inca.edu.cu

herbaceous crops, since pasture food is scarce for cattle during the dry period, because of the weather conditions of this season.

In this regard, temporary cereal crops growing and developing favorably at this time is a nice choice for poorly-rainy period for Cuban conditions. These crops are able to yield high amounts of biomass, which can be used both for forage (green and dry mass) and grain production of aggregates (fodder, flour and other compounds).

In Cuba, there are genetic materials of different cereal genus and species, which are important for human and animal nutrition (1). Among the species studied are wheat and triticale that enable to achieve high yields and quality of grain and biomass (2). Triticale is considered a valuable choice, because of its high biomass production and grain yield for animal food production (3, 4); besides, it surpasses wheat in biomass production, leaf disease resistance and marginal production conditions (5).

Concerning wheat production, nitrogen fertilization is a common practice, since it is one of the most important macroelements that significantly increase its growth and yield (6). However, its indiscriminate global use, which is observed in the rise of synthetic N (7), causes environmental damage and makes production more expensive. This could be avoided by obtaining local information, in order to define the minimum dose of this nutrient that reaches the maximum economic benefit (8).

Arbuscular mycorrhizal fungi (AMF) are highly-evolved mutualistic associations between soil fungi and most vascular plant roots; when they colonize host plant roots, they encourage growth and a better nutrient absorption, also improving yield of a wide range of agricultural crops (9). While the effects of phosphorus uptake by AMF associated with plants are well known, their relevance for other nutrient absorption (N, K, Ca, Mg, Fe, Mn, Cu and Zn) has been less investigated (10). Other studies report the positive effect of AMF on growth, productivity and grain quality in wheat crop related to non-inoculated treatments (11). In turn, there are

reports of economically viable agronomic response of forage and grain production of triticale growing in Mediterranean areas, with low doses of mineral fertilizers using AMF (3).

Thus, it indicates that AMF application could be a complement to the use of mineral fertilizers for forage production in tropical areas during the dry period, in order to reduce mineral fertilizer doses without affecting biomass production and quality for animal feeding. In addition, it decreases production costs, improves soil biota and agricultural ecosystem sustainability (9).

Therefore, this investigation was carried out with the objective of determining the minimum fertilizer dose with AMF inoculation that allows obtaining high forage yields with nutritional quality, by using triticale cultivars adapted to produce under tropical conditions.

MATERIALS AND METHODS

Two experiments were seeded in December (2008 and 2009) and harvested in March (2009 and 2010), respectively. For this purpose, Cuban triticale cultivar (*X. triticosecale* Wittmack) INCA TT-7 (12) was used.

The experiments were performed at "Niña Bonita" Genetic Livestock Enterprise of Bauta, Artemisa province. The experimental site was at the small pasture and forage station located on a Lixiviated Red Ferralitic soil (13), corresponding to a Rhodic Eutric Lixic Ferralic Nitisol, according to the World Reference Base (14).

Table I shows the main soil chemical characteristics of the arable horizon (0-20 cm) prior to sowing. The amount of AMF spores dwelling in the experimental area (15) was determined: 80,75 per 50 g of soil. For the chemical characterization of soil pH, organic matter, P_2O_5 , exchangeable cations (Ca^{2+} , Mg^{2+} , Na^+ and K^+) and base exchange capacity, the analytical techniques established by the Soil Laboratory from the National Institute of Agricultural Sciences (INCA) were followed (16).

Rainfall at "Niña Bonita" Genetic Livestock Enterprise was 1261 mm: 83,1 % during the rainy season and 16,9 % (November to April) in the dry season, when both experiments were developed. An average temperature of 21,77 °C and average relative humidity of 73,91 % were observed (17).

Table I. Chemical characteristics and content of AMF spores dwelling in the soil

pH	OM (%)	P ₂ O ₅ (mg kg ⁻¹)	Exchangeable cations (cmol _c kg ⁻¹)				CCB (cmol ₍₊₎ kg ⁻¹)	No. AMF spores 50 g ⁻¹ soil
			Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺		
6,7	3,16	13	10,6	3,3	0,16	0,22	14,28	80,75

Experimental soil was prepared by a farming sequence that consisted of plowing, harrowing, cross plowing and harrowing, approximately every 20 days between each of them. The experiment was sprinkler irrigated following a pattern of 350 m³ ha⁻¹, applied immediately after seeding and every 15 days during crop growing season.

The recently reclassified AMF *Glomus cubense* sp. was used in the experiments (18), whose agricultural effectiveness has been tested several times, especially in Ferralitic soils (19). This species was inoculated by EcoMic® solid inoculant using seed coating technique at a dose of 20 spores g⁻¹ substrate (20).

Coated seeds were drilled following a pattern of 150 kg ha⁻¹ seeds in 3-m-long x 4,2-m-wide experimental plots (six rows spaced at 0,70 m) from a 12,6 m² area per plot and a 11,2 m² calculation area. A randomized block design with 14 treatments and four replications were used. Table II describes 14 treatments.

Table II. Description of experimental treatments by combining AMF application and different N doses of the mineral fertilizer with a fixed bottom of 54 kg ha⁻¹ P₂O₅ and 70 kg ha⁻¹ K₂O

Treatment	AMF	Nitrogen doses (kg ha ⁻¹)
1	yes	0
2	yes	50
3	yes	100
4	yes	150
5	yes	200
6	yes	250
7	yes	300
8	-	0
9	-	50
10	-	100
11	-	150
12	-	200
13	-	250
14	-	300

For every treatment, a complete formula fertilizer was applied to furrow bottom at seeding time whereas urea 41 days after seed germination at Z. 2,4 phase (main shoot and four tillers) of Zadocks' scale (21). The production control (T.11) of the experiment was the recommended dose of 150 kg ha⁻¹ N, 54 kg ha⁻¹ P₂O₅ and 70 kg ha⁻¹ K₂O (22). Doses were delivered along with NPK (9-13-17) complete formula fertilizer at the rate of 412 kg ha⁻¹ plus urea (46-0-0) at the rate of 137 kg ha⁻¹. The rest of the treatments received a fixed bottom of P₂O₅ and K₂O, like the one used in treatment 11, but adding one or several doses of 50 kg ha⁻¹ N, which was obtained by applying urea at the rate of 109 kg ha⁻¹, so as to achieve treatments from 50 to 300 kg ha⁻¹ N.

Samplings to evaluate leaf indexes were performed at Z. 3,9 phase of Zadoks' scale (21), when flag leaf was fully emerged, 71 days after sowing. Root samples from eight plants to assess fungal variables were equidistantly distributed from plant stem and spaced 10 cm from the rows, following the methodology used for determining mycorrhizal structures in grass species (23). Likewise, triticale was cut for forage at Z. 7,3 phase of Zadoks' scale (early milky stage) 89 days after sowing (21).

The following variables were analyzed:

- ♦ **Plant leaf indexes:** leaf percentage contents of nitrogen (N), phosphorus (P) and potassium (K) were determined according to the methodology described by the Laboratory handbook of INCA (16).
- ♦ **Fungal variables:** colonization frequency and intensity were determined in root samples dried at 70 °C until reaching constant and stained weights, according to the methodology described for these variables (24); AMF spore number 50 g soil⁻¹ was also evaluated (15).
- ♦ **Forage dry mass yield (kg ha⁻¹):** it was estimated from dry mass percentage and green mass yield of each plot. The aerial green mass (GM) was cut from plants of the calculation area of each plot and weighed by means of a 0,25-kg precision balance; then, a sample of 200 g was taken and further put in an air circulation stove at 70 °C until reaching a constant mass to determine dry mass (DM) percentage, according to the following formula:

DM (%)= [sample DM (g)/sample GM (g)] x 100

DM yield was estimated from the formula:

DM (kg ha⁻¹)= [GM (kg plot⁻¹) x DM (%)/100] x f

where:

f= a factor to convert DM yield from kg plot⁻¹ to kg ha⁻¹ (0,43 for the calculation area of plots, 11,2 m²)

- N, P or K extraction (kg ha⁻¹): [aerial part DM (kg ha⁻¹) x % element of aerial part DM]/100.
- Raw protein content (%): % protein= % N x 5,27 (wheat conversion factor) (25).
- Mycorrhizal efficiency index (EI) (%): EI (%)= [(DM yield (t ha⁻¹) of inoculated treatment-DM yield (t ha⁻¹) of the control]/DM yield (t ha⁻¹) of the control] x 100 (26).
- Agronomic indexes of N use efficiency: apparent recovery efficiency (kg increased absorption kg⁻¹ applied nutrient) and partial productivity factor (kg yield kg⁻¹ applied nutrient) (27).

Statistics was performed through an analysis of variance, according to a one-way classification model of the original data. Duncan's procedure was applied to compare means with 5 % significance, in cases where ANOVA was significant. To estimate optimal N doses with or without AMF, the best fitting models (dose-yield) were identified in each case and then the first derivative criterion was used to find the optimum respective ones. The statistical program used was IBM SPSS for Windows version 21 (28).

RESULTS AND DISCUSSION

Table III presents the values of mycorrhizal variables studied in triticale crop during the experiment. Frequency percentages of mycorrhizal colonization, its intensity and AMF spore number g⁻¹ soil showed their top values when applying N fertilization from 50

to 150 kg ha⁻¹, its maximum value being at the dose of 100 kg ha⁻¹. Such variable response to higher N doses (200 to 300 kg ha⁻¹) was observed when it decreased significantly compared to lower N doses (100 and 150 kg ha⁻¹).

Results found in mycorrhizal variables with the most effective N doses (from 50 to 150 kg ha⁻¹) could be attributed to the fact that inoculated AMF germinated in the soil, surpassing AMF residents, giving rise to fungal hyphae, which, in turn, interacted with triticale root system, developing profitable interactions that favored the responses of variables analyzed; it was achieved due to a significant rise at guest-host level of the treatments applied.

In this regard, results found a good AMF ability in wheat colonization when there is an adequate nutrient supply (29). Meanwhile other studies show that mycorrhizal symbiosis needs an initial nutrient supply, specifically N, to have a promising wheat crop response.

On the other hand, when N increases, the symbiosis decreases significantly, so that host-fungus mutualism is depressed by applying seemingly excessive doses (> 200 kg ha⁻¹), which proves that mycorrhizal symbiosis between the host and symbiont declines notably, as a result of an increased nutrient supply surpassing the requirements needed for an effective plant-fungus mutualism; consequently, there is a progressive decrease of guest-host interaction. Thus, some authors have said that high doses of fertilizers cause less root mycorrhization (7, 29).

Table III. Behavior of colonization frequency and intensity as well as triticale spore number g⁻¹ soil

Arbuscular mycorrhizae	Nitrogen doses (kg ha ⁻¹)	Colonization frequency (%)	Colonization intensity (%)	Number of AMF spores g ⁻¹ soil
With mycorrhizae	0	40,05 c	1,6075 e	129,50 c
With mycorrhizae	50	54,00 ab	2,2725 b	180,50 b
With mycorrhizae	100	60,78 a	2,6425 a	227,00 a
With mycorrhizae	150	56,95 a	2,61 a	224,00 a
With mycorrhizae	200	48,33 b	2,175c	121,50 c
With mycorrhizae	250	38,28 cd	2,0725 d	104,00 d
With mycorrhizae	300	32,70 d	1,3525 f	88,75 e
Without mycorrhizae	0	31,28 d	1,27 g	81,30 e
Es x		0,8125**	0,0079**	1,43**

Equal letters per columns are not significantly different according to Duncan's test (p≤0,05)

It should be noted that values recorded in percentages of mycorrhizal colonization frequency (60 %) and intensity (2,64 %) as well as a high spore content (200 g soil⁻¹) shows that triticale crop responds favorably to mycorrhizal symbiosis after AMF application.

AMF applied to other two wheat cultivars also showed a high impact on plant root colonization and spore production, which was directly related to cultivar productivity (11).

Moreover, inoculants applied in a solid or liquid medium enhance root mycorrhizal colonization of durum wheat (*Triticum durum* L.), compared to roots without such application, which were only colonized by AMF residents in the soil^A.

Spore number g⁻¹ soil was shown like mycorrhizal colonization frequency and intensity in different treatments, it being higher when N doses increased up to 100 and 150 kg ha⁻¹ and significantly lower for N doses up to 300 kg ha⁻¹, whose value did not differ from the absolute control. This result made evident an AMF sporulation phase matching a high mycorrhizal colonization frequency and intensity, although they do not often have such behavior; however, it behaved the same for the three fungal variables evaluated in this study.

Table IV shows N, P and K leaf contents (percentages), indicating triticale nutritional status with regard to different treatments applied in the study. As it can be observed, AMF applications were able to reduce

N doses (100 and 150 kg ha⁻¹) in relation to higher ones (200 and 250 kg ha⁻¹) without AMF application, in order to achieve significant leaf N contents, thus saving the chemical fertilizer applied, compared to doses without AMF supply.

What is above mentioned could be justified due to AMF behavior in plants through mycorrhizal symbiotic mechanism, for allowing a higher nutrient supply to the host, since N absorption is also favored by mycorrhization (29); it is clearly explained in crop leaf N contents recorded with different N doses applied.

These contents are found in the adequate indexes reported for wheat crop (11) in forage production. It is not the case of higher N doses, due to its low values recorded, which could be evident as a result of a mycorrhizal symbiotic decrease of such doses with AMF, because high N doses decrease mycorrhizal symbiosis significantly with harmful effects on crop production and the environment (30).

Likewise, leaf N percentage values registered for the best treatments with and without AMF application correspond to those reported for bread wheat (*Triticum aestivum* L.) productions of 4000 kg ha⁻¹ (29), which were suitable under warm and humid tropical conditions (ME5A) of the experimental work (31).

Results from leaf P percentage showed that the fixed dose of 54 kg ha⁻¹ P₂O₅ had no significant differences for any treatment with and without AMF application. This response could be only explained due to this soil content plus the dose applied were enough to supply crop requirements; that is, satisfactory leaf P rates are achieved in triticale.

^A Domínguez, H. N. *Efectividad de dos inoculantes a base de hongos micorrízicos arbusculares (HMA) y su relación con la nutrición mineral en el cultivo del trigo duro (Triticum durum L.)*. Tesis de Grado, Universidad Agraria de La Habana «Fructuoso Rodríguez Pérez», Mayabeque, 2012.

Table IV. N, P and K leaf index contents (%) of triticale when applying different N doses with and without adding AMF

Nitrogen doses (kg ha ⁻¹)	% leaf N		% leaf P		% leaf K	
	with AMF	without AMF	with AMF	without AMF	with AMF	without AMF
0	1,78 c	1,62 d	0,23	0,22	2,26	2,23
50	1,84bc	1,67 d	0,23	0,22	2,25	2,26
100	1,94 a	1,76 c	0,23	0,23	2,24	2,25
150	1,92 a	1,83 c	0,23	0,23	2,21	2,23
200	1,91 ab	1,91 ab	0,23	0,23	2,25	2,20
250	1,82 c	1,95 a	0,23	0,23	2,25	2,24
300	1,81 c	1,82 c	0,23	0,23	2,27	2,26
Es x	0,0073**		0,001ns		0,0073 ns	

Equal letters per columns are not significantly different according to Duncan's test (p≤0,05)

This is consistent with the results reported for bread wheat crop (29) in experiments where mycorrhizal species from *Glomus* genus were individually inoculated or combined (*Glomus* sp. G1 + *Glomus* sp. G2), applying doses of 40 kg ha⁻¹ P₂O₅ and 100 kg ha⁻¹ N, which showed no leaf differences for wheat P and N contents, since AMF increased assimilation efficiency of both nutrients (29).

Significant differences were recorded in leaf K percentage between the absolute control treatment with AMF (0 N, 0 P₂O₅, 0 K₂O) and without AMF (0N, 0 P₂O₅, 0 K₂O); however, no significant differences were observed for N doses with and without AMF, which proves that such element rate with increasing N doses were enough for an adequate triticale nutrition. The most important interaction is N-K, probably because high N doses along with an insufficient K nutrition makes cereals sensitive to disease and accidents, especially to lodging, besides restricting yields, decreasing its quality and specific weight. For the sake of K, nitrogen productivity can exceed 50 % (32).

Observe that its value was higher with AMF in the control treatments with and without AMF (T1 and T8), which proves mycorrhizal contribution to crop nutrition from soil nutrients carried through mycelium-root system interface.

Results from N, P and K nutrient extraction (kg ha⁻¹) by triticale leaf biomass are shown in Table V. Regarding crop biomass N contents, the highest values were statistically significant compared to the other treatments: treatments of 100 and 150 kg ha⁻¹ N with AMF application and

doses of 150 and 200 kg ha⁻¹ without AMF. Results indicate that by applying 100 kg ha⁻¹ N with AMF, an appropriate forage biomass quality can be achieved in triticale and a viable agronomic response is confirmed in its forage production with low doses of mineral fertilizers besides a better nutrient uptake by means of AMF (3, 33).

Concerning P extraction (kg ha⁻¹) by crop biomass, the highest N values were observed between 100 and 200 kg ha⁻¹ with AMF as well as between 150 and 250 kg ha⁻¹ without AMF, which were significantly higher than the other treatments under study. It is important to point out that by AMF application, the greatest N absorption starts from the dose of 100 kg ha⁻¹.

This study was performed with a fixed bottom of 60 kg ha⁻¹ P₂O₅, reaching relatively high P values by triticale biomass extraction. In this sense, results from wheat growth and yield report that AMF significantly improved biomass yield when applying N (not exceeding 100 kg ha⁻¹) and P (50 kg ha⁻¹) doses that were effective for these nutrient contents in forage production, without the need of additional applications (34).

As for K extraction by foliar biomass (Table V), significantly higher values were recorded with different N doses applied, highlighting N dose of 100 kg ha⁻¹ with AMF, which allowed the crop to achieve the highest extraction; thus, the aforementioned N dosage with AMF was the best for triticale forage production.

Table V. N, P and K extraction (kg ha⁻¹) by triticale leaf biomass in response to different treatments applied to the study

Nitrogen doses (kg ha ⁻¹)	N, P and K extraction (kg ha ⁻¹) by leaf biomass					
	N extraction kg ha ⁻¹		P extraction kg ha ⁻¹		K extraction kg ha ⁻¹	
	with AMF	without AMF	with AMF	without AMF	with AMF	without AMF
0	62,42 f	44,57 g	10,62 e	8,12 f	103,56 f	81,38 g
50	98,0225 d	76,61 e	12,43 bcd	10,32 e	119,98 de	103,75 f
100	120,107 a	94,86 d	14,08 ab	10,89 de	138,63 a	121,29 de
150	118,28 ab	112,17 abc	14,05 ab	14,42 a	135,67 abc	137,16 ab
200	108,44 c	116,67 abc	12,85 abc	14,12 ab	126,81 bcd	136,25 ab
250	93,95 d	110,46 bc	11,87 cde	12,995 abc	116,30 de	125,57 cde
300	93,24 d	92,08 d	11,73 cde	11,67 cde	117,08 de	114,76 e
Es x		0,725**		0,15**		0,8998**

Equal letters per columns are not significantly different according to Duncan's test (p≤0,05)

It should be noted that this element has a special significance for this crop as fodder, because it influences directly on its quality, considering the role in carbohydrate and protein formation of crops. In addition, K is especially important in small grains, as it ensures plant growth, resistance to frost, lodging and diseases, which becomes greater if there is available mineral supply rich in potassium (32).

Table VI shows results from forage dry mass yield (kg ha^{-1}) and raw protein percentage. For the first variable, N doses of 100 and 150 kg ha^{-1} with AMF as well as those of 150 and 200 kg ha^{-1} without AMF reached the highest triticale fodder yields.

According to these results, with AMF and N doses of 100 kg ha^{-1} , high yields are obtained by saving resources due to the high cost of fertilizers and continuous environmental impact concern, particularly considering water quality associated with inadequate use of nutrients, as well as the economic feasibility of fodder production for animal feeding.

In this sense, corn and wheat studies indicate that AMF use possibly reduced crop mineral fertilization, increased biomass yields, nutrient content and forage quality as well as improved mycorrhizal colonization in plant roots (35, 36).

Furthermore, the possibility of transporting N through mycorrhizal symbiosis is confirmed, increasing crop yields with lower fertilizer amounts (29). Such results support that AMF can help plant N uptake (6).

Consequently, it is necessary to continue deepening on the research of N fertilizer application and its relationship to AMF role in plant uptake, related to plant symbiosis and the environment, its influence on crop productivity and N dose reduction to avoid ecosystem contamination.

Table VI shows that forage raw protein content was higher at N doses of 100, 150 and 200 kg ha^{-1} with AMF; however, similar values were obtained just with N doses of 200 and 250 kg ha^{-1} without AMF. Thus, it proves that by applying AMF, at least 50 kg ha^{-1} N can be saved to achieve the same yields and forage quality (raw protein content percentage), which is important since it allows to reduce the negative environmental impact caused by unnecessary N amounts applied to the soil and enhances the efficient plant use of this nutrient (11).

Figure 1 shows the regression analysis between dry mass production (kg ha^{-1}) and N doses applied with and without AMF. Also, significant regression rates were observed, $R^2= 0,987$, $R^2= 0,982$, and regression equations had a positive cubic effect depending on N dose applied, with and without AMF, highlighting that starting from N dose of 71,44 kg ha^{-1} with AMF, forage dry mass yields of 5662 kg ha^{-1} are obtained, achieving top yields of 5751,25 kg ha^{-1} at N doses of 120,4 kg ha^{-1} , which was a significant production increase, compared to treatments without AMF.

In both curves (with and without AMF application), it is observed how N application converted into forage dry mass (kg ha^{-1}) was lower as N doses were higher, after reaching its highest yield. In this sense, precision farming works in wheat highlight that the product increased by adding more inputs become every time lower and has passed the maximum yield point; thus, additional input quantities may have a negative effect on yield (37).

Table VI. Behavior of dry mass (kg ha^{-1}) and raw protein percentage in triticale forage production

Nitrogen doses (kg ha^{-1})	Forage dry mass (kg ha^{-1})		% Raw protein	
	With AMF	Without AMF	With AMF	Without AMF
0	3703 e	2808 f	10,13 de	9,16 f
50	5335 bc	4580 d	10,48 bcd	9,50 f
100	6185 a	5375 bc	11,08 a	10,02 e
150	6158 a	6138 a	10,72 abc	10,42 cde
200	5645 e	6200 a	10,88 ab	10,89 a
250	5163 c	5595 b	10,30 cde	11,09 a
300	5160 c	5073 c	10,38 cde	10,35 cde
Es x \pm		30,33		0,036

Equal letters per columns are not significantly different according to Duncan's test ($p \leq 0,05$)

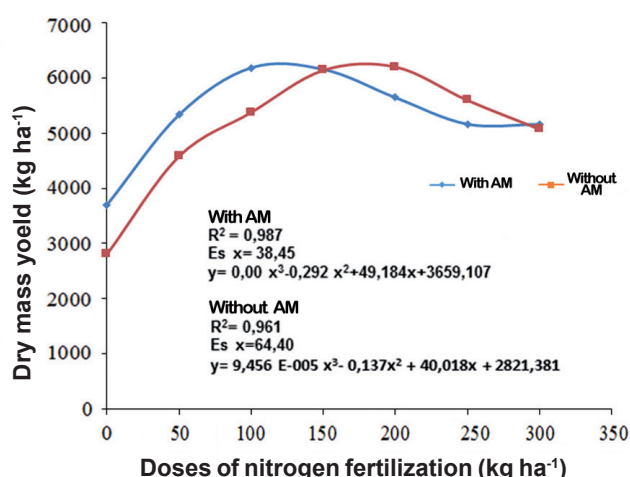


Figure 1. Dry mass variation at different N doses with and without AMF application

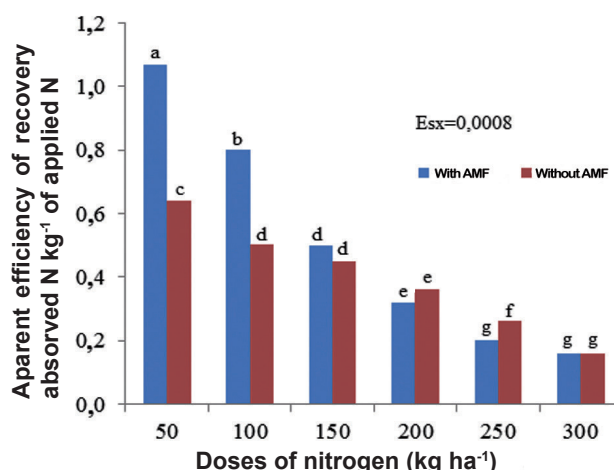
Also, other studies in wheat showed similar results, since a high regression in biomass production (kg ha^{-1}) was attained with different N doses (37). These results agree with those reported on the positive correlation between aerial biomass accumulation and N uptake when applying low amounts of it (38).

Moreover, AMF use had a direct and significant relationship with nutrient absorption and water intake, so that there is a fertilizer and water reduction to be applied to crops (33).

This result made evident that N fertilizer is saved by using AMF in triticale forage production and there was a direct and positive relationship with nutrient absorption by employing AMF, thus allowing the application of lower amounts of mineral fertilizers.

Figure 2 shows apparent recovery efficiency; that is, increased N absorption kg kg^{-1} N applied to triticale crop decreases with increasing N doses applied to the crop, with and without AMF, such differences being significant (with and without AMF) up to N dose of 100 kg ha^{-1} . This result is consistent with that reported for corn crop when studying the efficient N use in this cereal and its response to N fertilization by reducing the apparent recovery efficiency with increasing N doses (39).

It stresses that N doses of 50 and 100 kg ha^{-1} with AMF application were significantly higher than without it, so that they are inserted in well-managed systems at low level of N use (35), with a N absorption range between $0,5$ and $0,8 \text{ kg kg}^{-1}$ N applied in cereals (33).



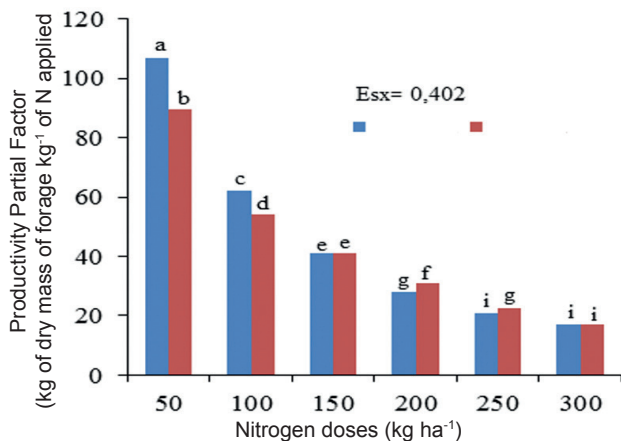
Same letters per column no differ significantly, according of Duncan test $p \leq 0,05$

Figura 2. Apparent efficiency of recuperation (AER_n) of forage to triticale base to different nitrogen doses (50 , 100 , 150 , 200 , 250 and 300 kg ha^{-1}) with and without application of AMF

On the other hand, no differences were recorded in N doses between 150 and 200 kg ha^{-1} , with and without AMF applied to apparent recovery efficiency; however, their values were minimal, showing a low level of N use for cereals (33).

Therefore, the amount of nutrients recovered in forage dry mass biomass when applying AMF is higher than without its application, which can be understood; moreover, as AMF have a positive influence on N used by plants, there is a content decline of this nutrient from N dose 150 kg ha^{-1} applied (Table V).

Figure 3 analyzes partial productivity factor at different N doses applied with and without AMF. It is evident that AMF application enables a greater productive response with lower N doses (50 and 100 kg ha^{-1}); also, higher dry mass values than 60 kg kg^{-1} N applied are achieved, which is considered a well-managed system at low level of N use (33). The result with N dose of 50 kg ha^{-1} confirms what has been stated about the optimal economic dose that allows the maximum benefit for wheat crop (40).



Equal letters per columns are not significantly different according to Duncan's test ($p \leq 0,05$)

Figure 3. Triticale partial productivity factor as a response to different N doses: 50, 100, 150, 200, 250 and 300 kg ha⁻¹ with and without AMF application

It can be considered that mycorrhizal symbiotic functioning is directly related to the highest dry mass production increases, since it enables to reach greater amounts of the product per unit of nutrient applied, significantly compared to that without AMF.

Finally, it should be pointed out that values of partial productivity factor from N dose of 150 kg ha⁻¹ showed a significant decrease with and without AMF; that is, by increasing N doses, the values of mycorrhizal variables begin to decrease (Table III), indicating a continuous host-guest reduction as a result of the possible effect of high N doses applied (11).

CONCLUSIONS

- ◆ AMF application enables to reduce N fertilization doses in triticale crop between 50 and 100 kg ha⁻¹, without decreasing forage production. In addition, adequate forage quality indices were achieved by introducing AMF and applying lower N fertilizer doses.
- ◆ Mycorrhizal structures varied depending on N fertilization, attaining the highest values at doses of 50, 100 and 150 kg ha⁻¹.
- ◆ By applying AMF, a higher N, P and K percentage was recorded in forage biomass with N dose of 100 kg ha⁻¹.

- ◆ Significant regression indexes were evident, $R^2 = 0,987$, $R^2 = 0,982$, and the regression equations that showed a positive cubic effect, depending on N dose applied, with and without AMF, highlighting that from N dose of 71,44 kg ha⁻¹, high forage dry mass yields (5662 kg ha⁻¹) start to be got with AMF application.
- ◆ Concerning the apparent recovery efficiency, it is more evident that N doses of 50 and 100 kg ha⁻¹ with AMF allowed well-managed systems at low level of N use, due to the values registered in this index (0,5 to 0,8 kg N absorbed kg⁻¹ N applied).
- ◆ Partial factor productivity showed that AMF application allows a more productive response with lower N doses (50 and 100 kg ha⁻¹); in addition, higher values than 60 kg dry mass kg⁻¹ nutrient applied are achieved, which is the condition of a well-managed system with low N use.
- ◆ AMF application improved and made triticale forage production more efficient, as N dose was reduced in this crop without diminishing quality indicators.

BIBLIOGRAPHY

1. Pérez, S. "100 años de Trabajos en la Reintroducción del Trigo en Cuba (1904-2003)". En: *V Taller Internacional sobre Recursos Filogenéticos «Fitogen 2003»*, edit. Instituto de Pastos Y Forrajes, Sancti Spiritus, Cuba, 2 de diciembre de 2003, ISBN 959-246-089-2.
2. Plana, L. R.; González, C. P. J.; Marrero, C. Y.; Fundora, S. L. R.; Arzola, B. J. y Ramírez, P. J. F. "INCA TT-7. Primer cultivar cubano de triticale (*X. Triticosecale* Wittmack)". *Cultivos Tropicales*, vol. 34, no. 3, septiembre de 2013, pp. 64-64, ISSN 0258-5936.
3. Estrada-Campuzano, G.; Slafer, G. A. y Miralles, D. J. "Differences in yield, biomass and their components between triticale and wheat grown under contrasting water and nitrogen environments". *Field Crops Research*, vol. 128, 14 de marzo de 2012, pp. 167-179, ISSN 0378-4290, DOI 10.1016/j.fcr.2012.01.003.
4. Bassu, S.; Asseng, S.; Giunta, F. y Motzo, R. "Optimizing triticale sowing densities across the Mediterranean Basin". *Field Crops Research*, vol. 144, 20 de marzo de 2013, pp. 167-178, ISSN 0378-4290, DOI 10.1016/j.fcr.2013.01.014.
5. Ammar, K. "Promoción y mejoramiento genético del triticale". *Revista EnIACE - CIMMYT. Agricultura de Conservación*, vol. 5, no. 16, 2013, pp. 27-29, ISSN en trámite.
6. Miransari, M. "Arbuscular mycorrhizal fungi and nitrogen uptake". *Archives of Microbiology*, vol. 193, no. 2, 7 de diciembre de 2010, pp. 77-81, ISSN 0302-8933, 1432-072X, DOI 10.1007/s00203-010-0657-6.

7. Hirel, B.; Tétu, T.; Lea, P. J. y Dubois, F. "Improving Nitrogen Use Efficiency in Crops for Sustainable Agriculture". *Sustainability*, vol. 3, no. 9, 7 de septiembre de 2011, pp. 1452-1485, ISSN 2071-1050, DOI 10.3390/su3091452.
8. Barbieri, P. A.; Echeverría, H. E. y Sainz, R. H. R. "Dosis óptima económica de nitrógeno en trigo según momento de fertilización en el Sudeste Bonaerense". *Ciencia del Suelo*, vol. 27, no. 1, 2009, ISSN 1850-2067.
9. Verbruggen, E.; Heijden, M. G. A.; Rillig, M. C. y Kiers, E. T. "Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success". *New Phytologist*, vol. 197, no. 4, 1 de marzo de 2013, pp. 1104-1109, ISSN 1469-8137, DOI 10.1111/j.1469-8137.2012.04348.x.
10. Farzaneh, M.; Vierheilig, H.; Lössl, A. y Kaul, H. P. "Arbuscular mycorrhiza enhances nutrient uptake in chickpea". *Plant, Soil and Environment*, vol. 57, no. 10, 2011, pp. 465-470, ISSN 1214-1178, 1805-9368.
11. Talaat, N. B. y Shawky, B. T. "Influence of arbuscular mycorrhizae on root colonization, growth and productivity of two wheat cultivars under salt stress". *Archives of Agronomy and Soil Science*, vol. 58, no. 1, 1 de enero de 2012, pp. 85-100, ISSN 0365-0340, DOI 10.1080/03650340.2010.506481.
12. Plana, R.; Alvarez, M.; Ramirez, A. y Moreno, I. "Triticale (*X triticum secale* Wittmack), a new crop in Cuba. A varietal collection from CIMMYT evaluated under the western conditions of the country". *Cultivos Tropicales*, vol. 24, no. 2, 2003, pp. 51-54, ISSN 1819-4087.
13. Hernández, A.; Pérez, J.; Bosch, D. y Castro, N. *Clasificación de los suelos de Cuba 2015*. edit. Ediciones INCA, Mayabeque, Cuba, 2015, 93 p., ISBN 978-959-7023-77-7.
14. IUSS Working Group WRB. *World Reference Base for soil resources 2014: international soil classification system for naming soils and creating legends for soil maps*. (ser. World Soil Reports, no. ser. 106), edit. Food and Agriculture Organization of the United Nations, Rome, 2014, 191 p., ISBN 978-92-5-108370-3.
15. Gerdemann, J. W. y Nicolson, T. H. "Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting". *Transactions of the British Mycological Society*, vol. 46, no. 2, junio de 1963, pp. 235-244, ISSN 0007-1536, DOI 10.1016/S0007-1536(63)80079-0.
16. Paneque, P. V. M. *Manual de técnicas analíticas para análisis de suelo, foliar, abonos orgánicos y fertilizantes químicos* [en línea]. edit. Ediciones INCA, La Habana, 2010, 157 p., ISBN 978-959-7023-51-7, [Consultado: 27 de enero de 2016], Disponible en: <<http://mst.ama.cu/578/>>.
17. Instituto de Meteorología. "Sistema de vigilancia agrometeorológica y alerta temprana especializados para el desarrollo de la producción ganadera en Cuba". *Boletín Agrometeorológico Nacional*, vol. 28, no. 10, 2009, ISSN 1029-2055.
18. Rodríguez, Y.; Dalpé, Y.; Séguin, S.; Fernández, K.; Fernández, F. y Rivera, R. A. "*Glomus cubense* sp. nov., an arbuscular mycorrhizal fungus from Cuba". *Mycotaxon*, vol. 118, no. 1, 5 de enero de 2012, pp. 337-347, ISSN 0093-4666, 2154-8889, DOI 10.5248/118.337.
19. Rivera, R.; Fernández, F.; Fernández, K.; Ruiz, L.; Sánchez, C. y Riera, M. "Advances in the management of effective arbuscular mycorrhizal symbiosis in tropical ecosystems" [en línea]. En: eds. Hamel C. y Plenchette C., *Mycorrhizae in Crop Production*, edit. Haworth Press, Binghamton, N. Y., 2007, pp. 151-196, ISBN 978-1-56022-306-1, [Consultado: 15 de julio de 2015], Disponible en: <10.13140/RG.2.1.1771.2162>.
20. Fernández, F.; Gómez, R.; Vanegas, L. F.; de la Noval, B. M. y Martínez, M. A. *Producto inoculante micorrizógeno*. no. 22641, Inst. Oficina Nacional de Propiedad Industrial, Cuba, 2001.
21. Zadoks, J. C.; Chang, T. T. y Konzak, C. F. "A decimal code for the growth stages of cereals". *Weed Research*, vol. 14, no. 6, 1 de diciembre de 1974, pp. 415-421, ISSN 1365-3180, DOI 10.1111/j.1365-3180.1974.tb01084.x.
22. Iglesias, L. A. y Pérez, N. "El cultivo del trigo en condiciones tropicales y posibilidades para su siembra en Cuba". *Cultivos Tropicales*, vol. 16, no. 1, 1995, pp. 52-63, ISSN 1819-4087.
23. Johnson, N. C.; Rowland, D. L.; Corkidi, L.; Egerton-Warburton, L. M. y Allen, E. B. "Nitrogen Enrichment Alters Mycorrhizal Allocation at Five Mesic to Semiarid Grasslands". *Ecology*, vol. 84, no. 7, 1 de julio de 2003, pp. 1895-1908, ISSN 1939-9170, DOI .10.1890/0012-9658(2003)084[1895:NEAMAA]2.0.CO;2.
24. Phillips, J. M. y Hayman, D. S. "Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection". *Transactions of the British Mycological Society*, vol. 55, no. 1, agosto de 1970, pp. 158-168, ISSN 0007-1536, DOI 10.1016/S0007-1536(70)80110-3.
25. Kalra, Y. P. *Handbook of Methods for Plant Analysis*. edit. CRC Press, Taylor & Francis Group, Washington, D.C., 1998, ISBN 978-1-57444-124-6.
26. Rivera, R. y Fernández, K. "Bases científico-técnicas para el manejo de los sistemas agrícolas micorrizados eficientemente". En: Rivera R. y Fernández K., *Manejo efectivo de la simbiosis micorrízica, una vía hacia la agricultura sostenible. Estudio de caso: el Caribe*, edit. Ediciones INCA, La Habana, Cuba, 2003, p. 166, ISBN 959-7023-24-5.

27. Dobermann, A.; Krauss, A.; Isherwood, K. y Heffer, P. "Nutrient use efficiency-measurement and management" [en línea]. En: *Fertilizer best management practices: general principles, strategy for their adoption and voluntary initiatives vs regulations. Papers presented at the IFA International Workshop on Fertilizer Best Management Practices*, edit. International Fertilizer Industry Association, Brussels, Belgium, 2007, pp. 1-28, ISBN 978-2-9523139-2-6, [Consultado: 27 de enero de 2016], Disponible en: <<http://www.cabdirect.org/abstracts/20083154896.html;jsessionid=FDDB76ECCE587EA330911A0637D26371>>.
28. IBM Corporation. *IBM SPSS Statistics* [en línea]. versión 21, [Windows], U.S, 2012, Disponible en: <<http://www.ibm.com>>.
29. Asghari, H. R. y Cavagnaro, T. R. "Arbuscular mycorrhizas enhance plant interception of leached nutrients". *Functional Plant Biology*, vol. 38, no. 3, 2011, pp. 219-226, ISSN 1445-4408, DOI <http://dx.doi.org/10.1071/FP10180>.
30. Garg, N. y Chandel, S. "Arbuscular mycorrhizal networks: process and functions. A review". *Agronomy for Sustainable Development*, vol. 30, no. 3, septiembre de 2010, pp. 581-599, ISSN 1774-0746, 1773-0155, DOI 10.1051/agro/2009054.
31. Centro Internacional de Mejoramiento de Maíz y Trigo. *Diversity to heal the Earth and feed its people* [en línea]. Annual Report 2001-2002, Inst. CIMMYT, México, D.F., 2002, p. 64, [Consultado: 27 de enero de 2016], Disponible en: <<http://repository.cimmyt.org/xmlui/bitstream/handle/10883/610/448358.pdf>>.
32. López, B. L. "Abonado de los cereales de invierno: trigo y cebada" [en línea]. En: *Guía práctica de la fertilización racional de los cultivos en España*, edit. Ministerio de Medio Ambiente y Medio Rural y Marino, España, 2010, pp. 123-133, ISBN 978-84-491-0997-3, [Consultado: 27 de enero de 2016], Disponible en: <<http://dialnet.unirioja.es/servlet/articulo?codigo=3311006>>.
33. Razouk, R. y Kajji, A. "Effect of Arbuscular Mycorrhizal Fungi on Water Relations and Growth of Young Plum Trees under Severe Water Stress Conditions". *International Journal of Plant & Soil Science*, vol. 5, no. 5, 10 de enero de 2015, pp. 300-312, ISSN 23207035, DOI 10.9734/IJPSS/2015/15408.
34. Minaxi; Saxena, J.; Chandra, S. y Nain, L. "Synergistic effect of phosphate solubilizing rhizobacteria and arbuscular mycorrhiza on growth and yield of wheat plants". *Journal of soil science and plant nutrition*, vol. 13, no. 2, junio de 2013, pp. 511-525, ISSN 0718-9516, DOI 10.4067/S0718-95162013005000040.
35. Ortas, I. "The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions". *Field Crops Research*, vol. 125, 18 de enero de 2012, pp.35-48, ISSN 0378-4290, DOI 10.1016/j.fcr.2011.08.005.
36. Sharma, R. C. y Banik, P. "Arbuscular Mycorrhiza, *Azospirillum* and Chemical Fertilizers Application to Baby Corn (*Zea mays* L.): Effects on Productivity, Nutrients Use Efficiency, Economic Feasibility and Soil Fertility". *Journal of Plant Nutrition*, vol. 37, no. 2, 28 de enero de 2014, pp. 209-223, ISSN 0190-4167, DOI 10.1080/01904167.2013.859692.
37. Landriscini, M. R.; Galantini, J. A. y Martínez, J. M. "Estrategias de fertilización con nitrógeno en trigo en la región pampeana". *Revista AAPRESID*, no. esp, 2013, pp. 50-57, ISSN 1850-0633.
38. Kamiji, Y.; Pang, J.; Milroy, S. P. y Palta, J. A. "Shoot biomass in wheat is the driver for nitrogen uptake under low nitrogen supply, but not under high nitrogen supply". *Field Crops Research*, vol. 165, 15 de agosto de 2014, (ser. Crop root system behaviour and yield), pp. 92-98, ISSN 0378-4290, DOI 10.1016/j.fcr.2014.04.009.
39. Silvana, A. M.; Degioanni, A. J. y Bonadeo, E. "Recuperación del nitrógeno residual de la producción de leche". *European Scientific Journal*, vol. 10, no. 27, 2014, pp. 50-68, ISSN 1857-7881, 1857-7431.
40. Sáez, C. Á.; Cantón, F. R.; Forde, B. G.; Kirma, M.; Araújo, W. L.; Fernie, A. R.; Galili, G.; Moschou, P. N.; Wu, J. y Cona, A. "Nitrogen use efficiency in plants". *Journal of Experimental Botany*, vol. 63, no. 14, 2012, p. 4993, ISSN 1460-2431, 0022-0957.

Received: August 7th, 2014

Accepted: October 29th, 2015