Original article

Response of Yacare (*Urochloa hibrido* cv. CIAT BR02/1752) pasture to biofertilization with arbuscular mycorrhizal fungi

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ABSTRACT

With the aim to evaluate the biofertilization effectiveness with arbuscular mycorrhizal fungi in Yacaré pasture (*Urochloa* hybrid) cultivated in Lixiviated Ferrallitic Red soil, an experiment was carried out at Niña Bonita Genetic Cattle Enterprise. Four treatments were evaluated: three species of arbuscular mycorrhizal fungi (AMF), *Glomus cubense*, *Funneliformis mosseae*, and *Rizhophagus irregularis*, in addition to a control without inoculating, distributed in a Latin square design. Different species inoculation of AMF was carried out at sowing time. The indicators evaluated were the frequency and intensity of mycorrhizal colonization, AMF spore number in the rhizosphere, the NPK biomass concentrations, the dry mass yield of the pasture and its nutritive value, as well as the efficiency index and AMF strains influence in pasture nutrition. All the strains produced significant increases in the evaluated variables; nevertheless, when *G. cubense* was inoculated, increases in the efficiency index and in pasture nutrition were obtained. It is concluded that biofertilization with *G. cubense* can be an effective agronomic practice to improve the yield and nutritive value of the Yacare pasture cultivated in Lixiviated Ferrallitic Red soil.

Key words: productivity, rhizosphere, glomus, nutritional value

INTRODUCTION

The cultivation of more productive species and pasture varieties, adapted to the different edaphoclimatic conditions in which the livestock activity takes place, is one of the ways to improve livestock food base [1]. In the specific case of Cuba, it acquires great importance, since only between

15 and 18 % of the land area dedicated to livestock is occupied by improved pastures, and the rest is made up of natural pasturelands of very low productivity and little nutritional value [2].

In recent years, new species and pasture and forage cultivars have been introduced in the country, mainly obtained from the International Center for Tropical Agriculture, including Yacaré pasture (*Urochloa* hybrid cv. CIAT BR 02/1752). It has been resulted from the two clones cross of this genus, which is by its high resistance to humidity, rapid soil cover and high productive potential and nutritional value characterized. To produce high yields with high nutritional value, this crop requires an adequate supply of nutrients [3].

However, the soil low fertility used for livestock in Cuba and the fertilizer shortage in this sector due to its high prices in the international market, leads to the search for alternatives to achieve its sustainable management [4].

In Cuba, in recent years there has been progress in research on the use and management of mycorrhizal biofertilizers in pastures, demonstrating their potential to increase the yield and biomass nutritional value and, at the same time, decrease between 30 and 50 % its requirements for mineral or organic fertilizers [5-7].

However, in the aforementioned investigations it has also been possible to verify that the biofertilization effectiveness depends on AMF inoculated strain, the species or cultivar of pasture and the edaphic conditions where its cultivation takes place, as well as its management. Thus, evaluating the response of the new species that will form part of the varietal structure of the country's pastures is essential to achieve the productive response that livestock needs and, at the same time, make a rational use of such inputs.

Based on the aforementioned, this research was carried out with the objectives of evaluating the inoculation influence with arbuscular mycorrhizal fungi species for Yacaré pasture (*Urochloa* hybrid cv. CIAT BR 02-1752) cultivated on a leached Red Ferrallitic soil, and selecting the most effective for use as a biofertilizer.

MATERIALS AND METHODS

The experiment was carried out at the Pastures and Forages microstation of Niña Bonita Genetic Livestock Company, located in Bauta municipality, Artemisa province, on a leached Red Ferrallitic soil [8], whose main chemical characteristics are presented in Table 1.

Table 1. Chemical characteristics of the soil (depth: 0 – 20 cm)

pH	OM	P_2O_5	Ca^{2+}	Mg^{2+}	Na^+	K^+	ECB
H_2O	(%)	(mg 100 g^{-1})	($cmol_c$ kg^{-1})				
6.4	3.34	2.0	8.9	2.1	0.19	0.35	11.54
0.2	0.33	0.6	0.8	0.3	0.02	0.03	0.76

OM: organic matter, ECB: Exchange capacity of base, CI: confidence interval ($\alpha = 0.05$)

The soil had an acid pH, medium content of organic matter and exchangeable potassium (K), as well as Cationic exchange capacity (CEC) according to its type. The assimilable phosphorus (P_2O_5) presented average contents. For soil analysis, in soil laboratory, organic fertilizers and plant tissue of Biofertilizers and Plant Nutrition Department from the National Institute of Agricultural Sciences (INCA) were used the methods established [9].

During the period in which the experiment was carried out, two well-defined seasons were observed, one rainy (May-October), where 77 % of the total precipitation was accumulated, and another with little rain (November-April), where only the 23 %. Between May and October, the highest values of the maximum temperatures (32.4-32.9 ℃), average (27.5-27.7 ℃) and minimum (20.8- 3.7 ℃) were recorded [10].

The sowing was in May 2016 carried out and the experiment lasted one year. The first cut was made at 90 days after sowing and later at approximate intervals of 45 and 90 days during the rainy and dry season, respectively, depending on the production of aerial biomass, until totaling five cuts.

In the experiment, four treatments were evaluated, consisting of AMF inoculation of the species *Glomus cubense* (Y. Rodr. & Dalpé) [11] INCAM-4 strain, *Funneliformis mosseae* (Nicol. & Gerd.) Walker & Schüßler [12] INCAM-2 strain and *Rizhophagus irregularis* (NC Schenck & GS Sm.) Sieverd., GA Silva & Oehl) INCAM-11 strain, and an uninoculated control, which were distributed in a Latin square design. The plots had a surface area of 28 m^2 and a calculation area of 21 m^2.

The soil was prepared in a conventional way, with a plowing sequence (plowing), harrowing, crossing (plowing) and harrowing, at intervals of approximately 20 days between each one. The pasture was in rows separated at 70 cm sown and in a stream, at 1.5 cm depth. For sowing, a dose of 10 kg of total seed ha^{-1} was used to deliver 1 kg of pure germinable seed ha^{-1}. The experiment was conducted under dry conditions and neither chemical fertilizers nor organic fertilizers were applied.

The inoculation was carried out at sowing time by the method of coating the seeds, for which they were immersed in a fluid paste, made by mixing a quantity of solid inoculum equivalent to 10 % of their weight (1kg) and 600 mL of water [13]. Once the seeds had been coated and the inoculum solidified, they proceeded to sowing. For this, the mycorrhizal inoculant EcoMic®, produced in the Department of Biofertilizers and Plant Nutrition of INCA, with a concentration of 35 spores of the AMF species to be evaluated, per gram of inoculant, was used.

In each cut, the green mass (GM) of plant aerial part that were in the calculation area of the plots was harvested. This was weighed with a precision balance of 0.5 kg. A 200 g sample was taken from

it, which was taken to a circulating air oven at 70 ºC until reaching a constant mass, to determine dry mass percentage (DM). The DM yield was calculated from the green mass yield (GM) and DM percentage (10). The crude protein content (CP) = N x 6.25 [14] and organic matter digestibility [15] were determined from the dried samples in the oven. Fiber Neutral Detergent FND [16]. The concentrations of N P K were determined as a percentage of the dry mass [9].

In alternate cuts and at the time of harvesting the green mass, five subsamples of rhizosphere soil were taken from each plot at 0-20 cm depth, using a metal cylinder of 2.5 cm in diameter and 20 cm high. They were distributed in equidistant points and 10 cm apart from the furrows, following the protocol for mycorrhizal structures determination in pasturelands [17]. These were homogenized to form a sample composed per plot, from which 1 g of rootlets was extracted for staining and clarification [18].

The indicators of frequency and intensity of colonization [19] and the number of spores in the rhizosphere [20], modified by other authors [21], were estimated.

The efficiency index (EI) of the inoculated AMF strains and the degree of participation of the same in the nutrition of the pastures were also determined. The IE was calculated using the formula [22].

$$\frac{\text{Yield. DM inoculated treat - Yield. DM control}}{\text{Yield. DM control}} \times 100$$

Where:

Yield. DM inoculated treat = DM yield (t ha^{-1}) of inoculated treatment

Yield. Control DM = DM yield (t ha^{-1}) of control.

To calculate the participation of AMF strains in pasture nutrition, the following formula was used [23].

$$\frac{\text{Conc. N, P, K biom aerial inoc treat.- Conc. N, P, K biom aerial control}}{\text{Conc. N, P, K biom. aerial control}} \times 100$$

Where:

Conc. N, P, K biom. Aerial inoc treat. = concentration of N, P or K (%) of the aerial biomass of the inoculated treatment

Conc. N, P, K biom. Aerial inoc treat. = concentration of N, P or K (%) in the aerial biomass of control treatment

The statistical processing of the data was done through variance analysis, according to the experimental design used, and when significant differences were found between treatments, the means were compared [24]. At the average values of soil analyzes, as well as the efficiency index and AMF strain participation in pasture nutrition, the confidence interval of the means was

estimated at α=0.05 [25]. All the variables fulfilled the assumptions of normality and variance homogeneity, for which the original data were analyzed in all cases [26]. The SPSS Statistics 21 program [27] was used for data analysis.

RESULTS AND DISCUSSION

Treatment effect on pasture mycorrhizal structures was evaluated through the variables colonization and visual density, which indicate, in that order, the root occupation level of the host plant by the fungus and colonization intensity, as well as by AMF spore number in 50 g of soil. As can be seen in Table 2, during the rainy season, all the strains increased colonization levels, visual density and spore number in the rhizosphere in relation to the uninoculated control, which reflected radical occupation level of resident AMF; however, the highest values of these variables were reached with *G. cubense*.

Table 2. Effect of AMF strains on the fungal structures of grass

AMF strains	Rainy period			Little rainy period		
	Colonization (%)	VD (%)	Spores 50 g^{-1}	Colonization (%)	VD (%)	Spores 50 g^{-1}
Control	24.12 c	1.93c	286.8 c	10.93 b	0.73 b	75.3 b
G. cubense	61.86 a	4.17 a	567.5 a	22.45 a	1.83 a	208.5 a
F.mosseae	38.82 b	2.92b	420.8 b	11.15 b	0.76 b	73.3 b
R. irregularis	40.39 b	2.89b	418.0 b	10.89 b	0.72 b	79.5 b
SE	2.15**	0.24**	45.7**	0.57**	0.10**	19.3**

VD: visual density. Averages with unusual letters in the same column differ significantly at

p<0.05, according to Duncan's test

In the dry season, only *G. cubense* produced significant increases in these variables, since the values reached by the rest of AMF species did not differ from those observed in the control without inoculation. It was found that *F. mosseae* and *R. irregularis* not only reached lower levels of root occupation than *G. cubense*, but that their effect had a shorter duration in time, since it was only observed in the rainy season. In experiments carried out under field conditions, [28,29], they also observed increases in colonization levels, visual density and spores in forage plant rhizosphere inoculated with AMF, although not all strains had the same behavior.

When analyzing indicators results of the fungal variables, it was evident that the introduced strains were more effective than resident AMF, to colonize pasture roots. However, not all did so with the same intensity, since the highest values were obtained with *G. cubense*, whose effect, unlike the others, extended until the dry season.

Several studies show that introduced AMF species can reach higher levels of root occupation than resident ones, in cases where these are not found in adequate quantities or are not effective enough to colonize cultivated plants. Nevertheless, to achieve adequate colonization, the species that is inoculated must be compatible with the environment (host crop, soil type and nutrient levels), as well as having a greater capacity than resident AMF to compete and establish themselves in the environment. This could explain the better behavior of *G. cubense* in pasture mycorrhizal structures, in relation to the rest of the inoculated strains [30].

The absolute values of the colonization percentages, visual density and spore number in the rhizosphere were higher in the rainy season than in the dry one, which could be due to climatic condition influence on biomass production. The rapid growth of pastures during the period, in which the highest levels of rainfall, temperature and luminosity occur, enhances a greater demand for nutrients and consequently, greater amount formation of fungal structures to facilitate the access of mycorrhized plants to ground resources [31].

As in the fungal variables, during the rainy period, all the strains increased the concentrations of N, P and K in the biomass of the aerial part were observed, in relation to the control; however, the highest values of N and K were reached with *G. cubense* (Table 3). No differences were found between the inoculated strains for P concentrations. These also had a different behavior over time, since in the dry season, only *G. cubense* managed to increase nutrient concentrations in the aerial biomass.

**Table 3.** Nutrient concentrations in the biomass of the aerial part (g kg^{-1} MS)

AMF strains	Rainy period			Little rainy period		
	N	P	K	N	P	K
Control	11.9 c	2.0 b	12.2 c	13.5 b	2.1 b	13.9 c
G. cubense	15.7 a	2.3 a	16.3 a	16.1 a	2.4 a	16.7 a
F. mosseae	13.5 b	2.2 a	13.9 b	13.9 b	2.1 b	14.2 b
R. irregularis	13.2 b	2.3 a	14.1 b	14.1 b	2.2 b	13.7 b
SE	0.3**	0.1**	0.3**	0.5**	0.1**	0.4**

Averages with unusual letters in the same column differ significantly at $p<0.05$, according to Duncan's test

Strain influence on pasture nutrition, which was evaluated through the percentage increase in the concentrations of the primary macronutrients in the aerial biomass in relation to the non-inoculated treatment, is shown in Figure 1. In the rainy season, *G. cubense* had a greater participation than the rest of the strains, with increases of around 25% in the levels of N and K. The increases of both nutrients reached by *F. mosseae* and *R. irregularis* exhibited values that did not exceed 13 %. The participation of all strains in phosphoric nutrition was similar, with values around 12 %. During

the dry season, only *G. cubense* participated in pasture nutrition, with increases in N and K concentrations in the biomass between 15 and 16 %, and of 8 % in the concentrations of P.
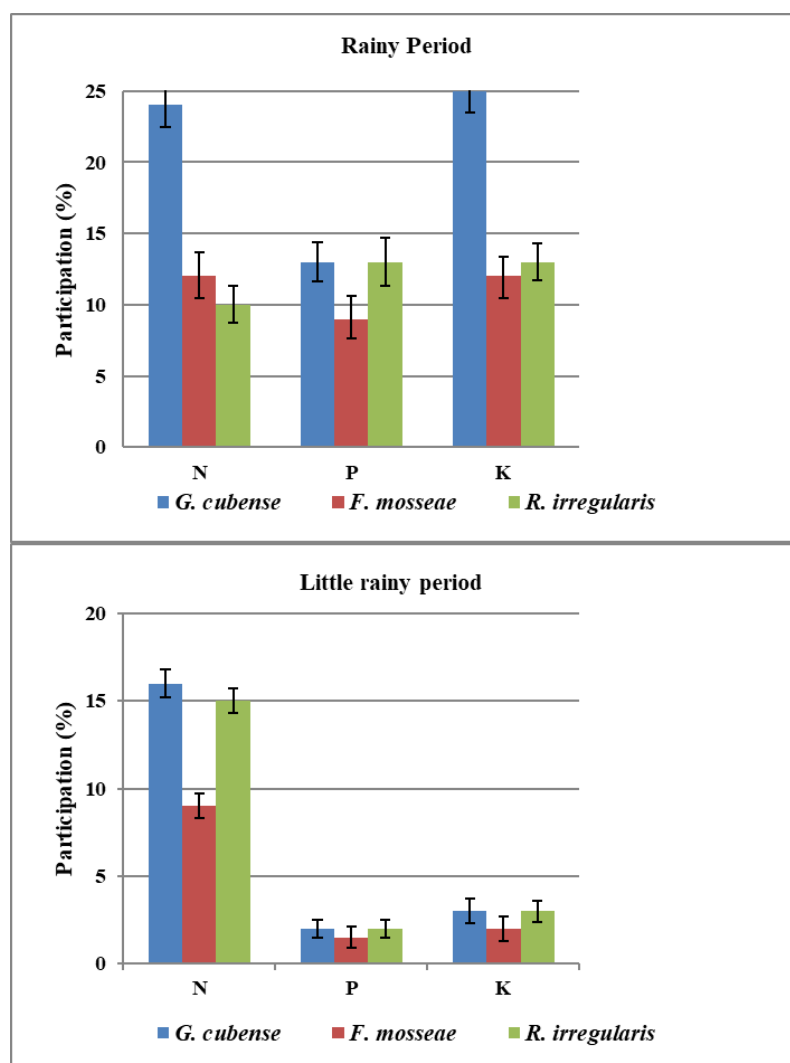
The vertical bars show the confidence interval ($\alpha = 0.05$)

Figure 1. Participation of AMF strains in the increase of the concentrations of N, P and K in the biomass of the aerial part of the pastures

AMF increases the absorption and translocation of nutrients, based on the morphological and physiological modifications that they produce in host plant roots, which increase the contact surface with the soil [32]. However, AMF effect on nutrient absorption depends on strain effectiveness, mycorrhizal dependence degree of the host plant, and soil fertility, among other factors [33].

In poaceae perennial plants, it has been found that higher levels of mycorrhizal colonization are generally associated with an increase in nutrient absorption [34,6]. This could explain the higher concentrations of N and K in pasture aerial biomass inoculated with *G. cubense*, as well as their

greater participation in the nitrogen and potassium nutrition of the pasture, since their root occupation levels were significantly higher than the achieved ones, by the rest of the strains as could be seen in Table 2.

The fact that even with *G. cubense* AMF strain participation in phosphoric nutrition has been low, in relation to nitrogen and potassium, could be a consequence of host plant characteristics, although other factors are not ruled out. The pastureland species of the genus *Urochloa* respond to nitrogen and potassium fertilization [35,36] and judging by the organic matter content and the assimilable K content of the soil where the experiment was carried out, these do not seem sufficient to guarantee plant adequate nutrition. In this sense, mycorrhizae were able to make an important contribution to pasture nutrition, by facilitating the absorption of both nutrients from the soil.

However, pastures of this genus have the ability to access less mobile P forms in the soil, so their response to phosphoric fertilizer application is usually low, even in soils with low levels of this element [37,38]. This could explain the low relative participation of *G. cubense* in the pasture phosphoric nutrition, despite the fact that it reached high levels of colonization, since; apparently, the plants not only depended on an effective mycorrhizal inoculation to guarantee adequate phosphoric nutrition. In fact, the participation of *G. cubense* in the phosphoric nutrition did not differ from that reached by *F. mosseae* and *R. irregularis*, which had a less marked participation in the nutrition of the pasture.

According to recent studies, when evaluating AMF strain contribution to different crop nutrition, it can be observed that symbiosis, rather than favoring the absorption of one or another element, behaved as a mechanism that allowed plants to obtain their nutritional requirements, depending on their own needs and their availability in the soil [39].

Table 4 shows the inoculation influence on the biomass yield of pasture aerial part, as well as the efficiency index of the AMF strains, which expresses, in percentage terms, its effect on the productivity increase with respect to the treatment without inoculation (control). During the rainy season, all the strains increased their yield; however, the greatest effect was obtained with *G. cubense*, which differed significantly from the rest of the treatments. This strain also showed the highest efficiency index.

Table 4. Effect of AMF strains on pasture yield (t DM ha^{-1})

AMF strains	Rainy period	Little rainy period	Total	EI
Control	7.85 c	2.98 b	10.93 c	-
G. cubense	10.98 a	3.85 a	14.83 a	35
F. mosseae	9.45 b	2.97 b	12.42 b	14
R. irregularis	9.58 b	2.90 b	12.48 b	14
SE	0.34**	0.10**	0.38**	

EI: efficiency index of AMF strains. Averages with unusual letters in the same column differ significantly at $p<0.05$, according to Duncan's test

In the dry season, no effect of *F*. *mosseae* or *R. irregularis* was found on the yield, whose values were similar to those reached in the control without inoculation, and this was also reflected in the low efficiency indices reached by both strains. At this time, only *G. cubense* was able to increase performance and consequently exhibit the highest efficiency index, demonstrating not only greater effectiveness, but also greater permanence over time.

These results indicated that the introduced strains (especially *G. cubense*) were more effective than the resident AMF was, in promoting pasture yield and consequently, that biofertilization with AMF could be assumed, at least under the conditions in which this study was carried out, as a promising agronomic practice to improve the Yacaré pasture productivity. Similar results were obtained by other authors when inoculating AMF efficient strains, in pastures of the genera *Megathyrsus* and *Urochloa*, respectively [28,40].

Regarding inoculation effect with AMF, although it is recognized that pasture response tends to vary depending on many factors, because of the ecology of these microorganisms in pastureland agroecosystems is quite complex. Some authors suggest that the introduction of selected strains can be a desirable and even necessary management option, in cases where resident AMF are not effective enough to produce an important agronomic response in crops [34].

As in the fungal variables, it was observed that the yield during the little rainy season was reduced, on average, by 70 % with respect to the rainy season, which is the result of the seasonal nature of pasture biomass production, given by the behavior of meteorological variables throughout the year. At the site where the experiment was conducted, notable variations in rainfall levels and temperatures were observed from one season to another, which undoubtedly could have had an impact on performance. The biomass production of tropical pastures is closely related to rainfall behavior, temperature and light, which have a marked influence on their growth [41].

However, the most interesting thing was to verify that biofertilization with AMF can increase pasture yield, even in the dry season, a period during which biomass production is significantly depressed and, therefore, a greater quantity needs to be produced of food for animals.

Biofertilization with AMF influenced the nutritional value of the pasture, although its effect also depended on the inoculated strain; it was observed that all increased the crude protein content and the biomass digestibility. *G. cubense*, in addition to producing the greatest effect on both variables, decreased its content of neutral detergent fiber (Table 5).

The improvement in the nutritional value of the biomass was in correspondence with the positive effect that biofertilization produced on the nutritional status of the pasture. As could be seen, with the inoculation higher concentrations of N in the biomass of the aerial part were obtained than in the control, which not only meant an increase in the crude protein content, but also, apparently,

had an impact on the rest of the variables. Precisely, the strain with which the highest N concentrations were obtained was the one that exhibited the highest levels of the evaluated nutritional value indicators.

Table 5. Effect of treatments on indicators of the nutritional value of the pasture

AMF strains	CP (%)	NDF (%)	DOM (%)
Control	7.94 c	70.8 b	62.2 c
G. cubense	9.94 a	68.1 a	64.9 a
F. mosseae	8.56 b	70.9 b	61.9 b
R. irregularis	8.53 b	71.1 b	62.1 b
SE	0.16**	0.28**	0.34**

CP: crude protein, NDF: neutral detergent fiber, DOM: digestibility of organic matter. Averages with unusual letters in the same column differ significantly at $p<0.05$, according to Duncan's test

With growth stimulation produced by the increase in the N availability in the soil, there is an increase in the available carbohydrate use for the formation of cells, instead of increasing the cell wall thickness. In this way, fiber and lignin content are reduced, increasing the digestibility and nutritional value of the pasture [42].

When comprehensively analyzing the results obtained in this experiment, it was observed that the pasture response of to the AMF inoculation depended on the strain effectiveness. This fact agrees to that observed by other researchers when evaluating the introduction effect of AMF species in forage pastures [6] and that seems to be associated with the existence of different compatibility degrees between the host plant and the fungus.

In other studies carried out, it is argued that, although there is no evidence of a strict fungus-plant specificity, not all AMF species colonize the different plant species with the same intensity and efficiency, being demonstrated the existence of different compatibility degrees in the symbiosis, because of environmental influences on the genotypic expression of both symbionts [43].

The soil influence on the introduced strain effectiveness cannot be ruled out either, since AMF inoculation studies carried out in Cuba in different crops demonstrate the existence of a high relationship between the strain efficiency and the edaphic environment, in crop response to biofertilization with arbuscular mycorrhizal fungi [44].

The *G. cubense* effect, which was undoubtedly the most effective strain, in increasing pasture yield, is closely related to the improvement of its nutritional status. This was evident both by the higher N, P and K concentrations in the aerial biomass that were reached as a result of its inoculation, as well as by its greater participation in pasture nutrition. Such behavior seems to be closely related to an increase in soil nutrient use, from the amounts formation of mycorrhizal structures that facilitated the access of plants to such resources [45]. Thus, biofertilization with

G. cubense is as a promising alternative seen to improve the productivity and nutritional value of Yacaré pasture biomass, at least under similar conditions to those present in this study.

CONCLUSIONS

- Biofertilization with arbuscular mycorrhizal fungi improves biomass yield and nutritional value of Yacaré pasture (*Urochloa* hybrid cv. CIAT BR 02/1752) grown on leached Red Ferrallitic soil.

- *Glomus cubense* is the most effective strain for inclusion in biofertilization with arbuscular mycorrhizal fungi of Yacaré pasture grown in this soil type.

- The *Glomus cubense* effect on the yield and nutritional value of the biomass was to the nutritional status improvement of the pasture related.

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