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Effect of a Fungi Complex in Nine Ecotypes of *Cenchrus purpureus* (Schumach) Morrone

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ABSTRACT

Objective: The objective of this study was to evaluate the response of different concentrations of a fungal consortium on the growth and yield of *Cenchrus purpureus* ecotypes under storm conditions.

Design/Methodology/Approach: An experiment was established under a completely randomized design with a 9×2×4 factorial arrangement. The factors were 9 ecotypes of *Cenchrus purpureus*, two seasons of the year (Summer-Fall and Winter-Spring), and four levels of mycorrhizal consortium. The variables evaluated were: number of buds, number of leaves, height of the bud, leaf length, total biomass, and leaf-stalk ratio.

Results: The response of the ecotypes had a differential effect on the variables NB, LLL, TB and L/SR, while the season affected the variables LN, PH, LLL, MLL, SLL, TB and L/SR. Low inoculation levels increased the variables NB, PH, LLL, MLL and TB.

Study Limitations/Implications: The evaluated factors indicate that the level of inoculant and the season determine the growth and yield of *Cenchrus purpureus*.

Findings/Conclusions: The mycorrhizal consortium dose and season of the year mainly determined the growth and yield of *C. purpureus*. Ecotypes respond differently to changes in the season and in mycorrhizal consortium dose. The evaluated factors indicate that the mycorrhiza dose and the season of the year determine the growth and yield of *C. purpureus*. Two growth strategies of the ecotypes are visualized: 1) many buds, with few small leaves and 2) few buds with many large leaves.

Keywords: Growth, Forage, Mycorrhiza, Elephant grass, Yield.

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INTRODUCTION

The distribution of forage yield throughout the year in conditions of natural and induced pastures, and established pastures, show a decrease in the dry matter yield in the dry season, affecting importantly the bodily condition and yield of animals (Amamou *et al*., 2018). A strategy for this problem is the establishment of perennial fodders, with capacity for adaptation to the deficiency in water resources, as long as there are favorable conditions in the soil (Paredes, 2018). Among the species introduced there are grasses of the *Cenchrus* genus, which are characterized by adapting to various adverse situations, and therefore have a broad distribution. Their resistance to conditions of water stress and the disposition of their germplasm stand out, due to their presence in different genomic banks (Pattanashetti *et al*., 2016). In addition, they provide ecosystem services since they prevent erosion (Hendrickson and Sanderson, 2017), and their roots have the capacity to increase the presence of beneficial microorganisms in the soil (Crotty *et al*., 2015).

When the levels of fertility in the soil are not adequate for the development and survival of these fodder species, mycorrhizal fungi have been used that strengthen the capacity to withstand environmental stress, when improving the radicular development and the exchange of phytoregulators, as well as minerals that allow a better plant performance (Lenoir *et al*., 2016). Mycorrhizal fungi participate to a large extent in environmental conservation and they can be used in the regeneration of soils and in reforestation processes (Rocha *et al*., 2015). The mycorrhizae have been isolated and used as inoculants, although their use in agriculture is limited and is slightly developed (Goss *et al*., 2017). It has been shown that some fungi such as *Funneliformis mosseae* have effects on the growth of plants by improving phosphorus absorption, and increases up to 60% the appearance of sprouts (Jiang *et al*., 2016). Likewise, it gives resistance to water stress (Bernardo *et al*., 2019) and tolerance to contaminated soils with pesticides (Rivera-Becerril *et al*., 2016). When there is excess water it also helps to retain nutrients in the soil avoiding its lixiviation (Köhl and Van der Heijden, 2016). *Diversispora ebúrnea* creates an environment of competition of growth between inoculated plants (Shi *et al*., 2016). Hernández-Zamudio (2017) reported a high survival and resistance of this mycorrhiza in arid and semiarid ecosystems. On the other hand, *Rhizophagus fasciculatus* increases the density in roots and aerial part of the plant in soils of low fertility (Channabasava *et al*., 2015). Rożek *et al*. (2019) report this species in temperate forests. Tarraf *et al*. (2017) report that *Septoglomus viscosum* increases significantly the biomass of plants, and also gives excellent quality, which is why Pellegrino and Bedini (2014) recommend the inoculation to increase the absorption of nutrients in the soil, which allows improving the yields in the harvests. However, it is important to consider different variables, such as the biochemical conditions of the soil and the climatic variation, which influence their degree of effectiveness (Garzón, 2016). Therefore, the objective of this research was to evaluate the effect of a mycorrhizal consortium made up of four species in the growth and yield of ecotypes of *C. purpureus* under conditions of two seasons in a year.

MATERIALS AND METHODS

The experiment was established in June, 2017, and ended in June, 2018, in the Technological Institute of Valle de Oaxaca (ITVO) located in the municipality of Santa Cruz Xoxocotlán, Oaxaca. The coordinates are 17° 01′ 16′′ N and 96° 45′ 51′′ W, with predominant Vertisol soils (INEGI 2010). According to the National Meteorological Service, the closest station to the experiment is No. 20354 – Zaachila, which is located 8 km away in a straight line from the experimental place. With average temperature of 20.6 °C, maximum of 23.1 °C in the month of May, minimum of 17.5 °C in the months of January and December. The average annual precipitation is 709 mm; the month of June is the most rainy month with 146 mm, and January and December are the driest months with 1.5 and 3.2 mm, respectively (CONAGUA, 2015).

Nine ecotypes of the Cenchrus purpureus species were used, which were: Elephant, Maralfalfa, CT-115, Roxo, Vruckwona, Taiwan, Merkeron, Mott and King Grass. These materials were donated by the Experimental Agricultural Field "La Posta" of the INIFAP, Veracruz Unit.

A compound mycorrhizal consortium of four mycorrhizae species was used: *Diversispora ebúrnea*, *Funneliformis mosseae*, *Rhizophagus fasciculatus* and *Septoglomus viscosum* provided by the Sierra Juárez University (UNSIJ), obtained from an agroecosystem of granadilla (*Passiflora ligularis*) from the community of San Antonino el Alto, Zimatlán, Oaxaca, Mexico, through isolation and its consequent reproduction. Four levels of inoculation were used: 0, 5, 7.5 and 10 g per plant, which were applied at the time of establishment, directly with the stake in the ground. The experiment was carried out in a period of 12 months, and it was divided into two seasons, the rainy season, during the months of July to December, 2017 (Summer-Fall, S-F) and January to June, 2018 (Winter-Spring, W-S). In each season the plants were registered six times every 30 days. Soil preparation was carried out with farming tasks, through plowing and trawling with a tractor. The sowing method was using stakes at 40° distributed in squares using the nine ecotypes of *C. purpureus* where two cuts are performed.

Variables

The following response variables were evaluated. Number of buds per plant (NB), considering the buds on the main axes of the plant. Number of leaves per bud (NL); the total number of leaves on each bud was counted. Plan height (PH), measured level on the ground up to the top plant tissue, for this variable a metric tape of 1 m was used. Leaf length, large leaf (LLL), medium (MLL), small (SLL), for each bud the largest, medium and small leaf were selected, which were measured with the metric tape from the ligule to the apex. Total biomass (TB), which was determined through dry matter six months after establishment of the crop, and for this the method of the square (1 m^2) was used, cutting the plant five cm from the ground level and kept in paper bags; they were put in the Riossa brand Model H-33 drying stove, at 55 °C for 96 h, and finally the weighing of each bag was carried out to obtain the total biomass. Leaf/stalk ratio (L/SR), obtained through the separation of the leaf and the stalk from the samples of total biomass, which were weighed separately to later divide the value of the leaf by that of the stalk.

The experiment was established in a completely randomized design (CRD), with factorial arrangement 9×2×4, where: A is the factor that corresponds to the nine ecotypes; B is the factor that corresponds to the two seasons of the year; and C is the factor that corresponds to the four levels of inoculation. In total there were 72 treatments with four repetitions. The data were analyzed to estimate the effect of the inoculant on the ecotypes of the Elephant grass, the means were compared with the Tukey test ($p \leq 0.05$) and SAS for Windows version 9.3 was used (SAS Institute, 2011).

RESULTS AND DISCUSSION

The results obtained from the factorial analysis show that there are highly significant differences ($p<0.05$) between ecotypes of *C. purpureus* in all the variables evaluated. Regarding the seasons, the variables with higher significance were number of leaves, height of the bud, and length of the small leaf. These results are related to the precipitations, since in most of the fodders there is an increase in the elongation of leaves during the rainy season (Cruz *et al*., 2017a). As consequence, a maximum growth is reached in a short time, as reported by Pérez *et al*. (2004), obtaining a maximum growth at four months in a period of six months. For the level of inoculation of mycorrhizae, the variables were highly significant except in the number of leaves and length of the small leaf. In the interaction ecotype-level, there were highly significant differences for the number of buds and height of bud, for the ecotype-season interaction all the variables were highly significant, in the season-level interaction there was no significance in any of the variables, in the ecotype-season-level interaction it was determined that in the variable of number of bud there were significant differences (Table 1). The effects that are observed between the interactions of the factors are positive, showing differences in the plant's organs; these results agree with Calzada-Marin *et al*. (2018) who observed that the morphological composition in these ecotypes varies between different ages.

Table 1. Variance analysis considering the levels or factors.

Variable	Factor a	Factor b	Factor c	Inter. a*c	Inter. a*b	Inter. b*c	Inter. a*b*c	Rep.	C. V.
NB	2856.55 **	0.35 ns	1402.36 **	216.87 **	764.06 **	119.61 ns	213.24 **	316.13 ns	65.82
NH	89.78 **	4830.47 **	42.52 ns	24.20 ns	77.93 **	43.93 ns	22.07 ns	39.61 ns	66.77
AB	9767.38 **	347864.7 **	5929.50 **	1232.25 **	2967.24 **	774.30 ns	559.86 ns	5101.73 **	50.47
LHG	5767.78 **	887.45 ns	3662.80 **	501.01 *	884.24 **	100.81 ns	295.61 ns	2639.23 **	43.21
LHM	1376.18 **	341.86 ns	874.20 **	130.45 ns	250.60 **	79.93 ns	60.44 ns	747.96 **	44.13
LHC	77.20 **	921.12 **	14.29 ns	17.49 ns	83.00 **	11.50 ns	17.89 ns	27.17 ns	92.74

**=Highly significant; *=significant; ns=no significant; Factor a=ecotypes of *C. purpureus*.; Factor b=Season; Factor c=level; Inter=Interaction; Rep=Repetition; C.V.=coefficient of variation; NB=number of regrowth; NH=number of leaves; AB=regrowth height; LHG=large blade lenght; LHM=medium blade lenght; LHCh=blade length small.

Ecotype factor

As indicated in Table 2, when the means comparison is made, the results show that for the NB, the ecotype Elephant was the one that showed the highest value (21.9), producing 82% more stalks compared to the ecotypes CT-115, Merkeron, Mott and King Grass, which were the ones that evidenced the lowest numbers of resprouts (p>0.05), and not different between one another (p>0.05), which produced in average 12 stalks. Meanwhile, in the variable number of leaves, with the exception of the ecotype Elephant, the rest of the ecotypes did not show differences, with the ecotype Maralfalfa showing the highest number of leaves per stalks (7.07). In plant height, the ecotype Merkeron was the one that showed the highest value (54 cm), exceeding in 70% the ecotypes Elefante, Maralfalfa, Roxo and King Grass (p>0.05), which were the lowest ecotypes, respectively.

This is similar to what was observed by Calzada-Marín *et al*. (2014) who explain that Maralfalfa is characterized by a constant increase in its growth. This makes it an alternative for zones with similar characteristics to the medium where it was established (Uvidia, 2013). The efficiency of Elephant in some studies reaches a maximum growth in a short time (Vivas-Quila *et al*., 2019), with an efficient production in quality and quantity of fodder (González *et al*., 2011). The results obtained are affected primarily by the capacity for adaptation that Elephant, Maralfalfa and King Grass have in comparison to the remaining ecotypes and to environmental conditions (Sterling and Guerra, 2010).

Season factor

As indicated in Table 3, the means comparison of the variables showed that the variable NB during the two seasons was the same, producing in average 13 buds. In the variables NL, PH, LLL, MLL and SLL, differences were observed (p<0.05) during the two seasons, with higher values in the S-F season. The lower development of Cenchrus purpureus in W-S is adjudicated to the absence of the water resource which was lower compared to the S-F season. Likewise, these fodders have adapted to environmental conditions of precipitation and temperature (Rojas *et al*., 2011), reaching a higher average per cut of 3.38 t ha^{-1} in rainy periods (Álvarez *et al*., 2013). On the other hand, Reyes-Castro *et al*. (2018) reported an increase in yield of the ecotype Moott in the rainy season compared to the dry season. For their part, Cruz *et al*. (2017b) reported a higher number of buds in the rainy season. Therefore, Murillo *et al*. (2014) recommend sowing Elephant grass in seasonal conditions, specifically in the rainy season, since a maximum development of

Table 2. Comparison of variables considering the ecotypes of *C. purpureus*.

Ecotypes	NB	NH	AB (cm)	LHG (cm)	LHM (cm)	LHCh (cm)
Elefante	21.89 a	4.84 c	34.42 c	32.42 cd	16.86 cd	4.03 c
Maralfalfa	7.3 d	7.07 a	33.95 c	25.68 cd	14.04 ef	5.59 a
CT-115	12.57 c	6.29 ab	48.65 ab	41.56 a	21.23 a	5.02 abc
Roxo	16.68 b	5.8 abc	34.83 c	29.36 de	14.98 de	4.04 bc
Vruckwona	13.03 c	6.75 ab	43.89 b	36.01 bc	18.28 bc	4.61 abc
Taiwan	13.95 bc	6.58 ab	46.47 b	36.73 abc	18.35 bc	4.31 abc
Merkeron	12.26 c	6.69 ab	54.46 a	38 ab	19.81 ab	5.35 ab
Mott	11.46 c	6.8 ab	44.63 b	33.94 bcd	17.59 bc	4.13 bc
King Grass	11.97 c	5.45 bc	28.59 c	22.79 f	11.83 f	3.72 c

Values with different letters in the same column are significantly different according to the test of Tukey (P<0.05); NB=number of regrowth; NH=number of leaves; AB=regrowth height; LHG=large blade lenght; LHM=medium blade lenght; LHCh=blade length small.

Table 3. Means comparison taking into consideration the season.

Station of the year	NB	NH	AB (cm)	LHG (cm)	LHM (cm)	LHCh (cm)
Summer - Autumn (rains)	13.6 a	7.94 a	56.01 a	33.87 a	17.54 a	3.77 b
Winter- Spring (dry)	13.52 a	4.36 b	24.74 b	32.13 b	16.62 b	5.4 a

Values with different letters in the same column are significantly different according to the test of Tukey (P<0.05); NB=number of regrowth; NH= number of leaves; AB=regrowth height; LHG= large blade lenght; LHM= medium blade lenght; LHCh= blade length small.

the plant is shown (Pilco and Pérez, 2017). Likewise, there is an increase in the appearance of leaves (Ramírez *et al.*, 2010).

Level of inoculant factor

This factor did not affect (P>0.05) the variables NL and SLL; the variables NB, PH, LLL and MLL were higher (P<0.05) with the doses of 0 and 5 g of the consortium than with the others (Table 4).

Ojeda *et al.* (2018) reported that arbuscular mycorrhizae increased the yield of biomass, raw protein, and mineral extractions of the soil, with mycorrhizal efficiency of 100% in *Rhizoglomus intraradices*, which is why it is an option for fertilization. For that purpose, the use of mycorrhizae is important, allowing the plant to capture, translocate and transfer nutrients, in addition to adopting a lower dependency to chemical fertilizers (Beltrán and Fiallos, 2016). When comparing with chemical fertilization, these ecotypes have high rates of production and fodder yield (Vivas-Carmona *et al.*, 2019). Fodders under conditions of good fertility and moisture represent forage potential (Ramos-Trejo *et al.*, 2012).

Total Biomass and Leaf/Stalk Ratio

The variance analysis of total biomass (TB) showed significant differences (p<0.05) between the three factors (Table 5). The ecotype, the season and the level of inoculant had a significant effect on the increase of total biomass. In this sense, Karti *et al.* (2018) showed similar results when inoculating with mycorrhiza, they observed an increase of 30% in the production of dry weight in *P. purpureum*. Likewise, Rao *et al.* (1985), when inoculating arbuscular mycorrhizae in *Pennisetum* reporte dan increase of 41.7 % of dry matter. Meanwhile, for the leaf/stalk ratio (L/SR) there was no significance for the factor of level of mycorrhiza inoculated. For the interactions that were performed solely in ecotype-season, there were highly significant differences only for the variable leaf/stalk ratio. These performances can be caused by environmental conditions of precipitation and temperature that were present during the experiment.

In the TB variable, there are differences (p<0.05) between the means of the ecotypes studied, where Merkeron, Taiwan, Ct-115, Vruckwona and Elephant predominate with a higher yield (1.73, 1.40, 1.38, 1.23 and 1.18 t MS ha^{-1}, respectively) (Table 6). This agrees with what was reported by Goyes-Vera *et al.* (2018), that the ecotype that better adapts to the absence of chemical fertilizers is Elephant, showing higher amounts of biomass.

The ones with lower performance were Maralfalfa and King Grass (0.55 and 0.40 t DM ha^{-1}). In the seasons, there is difference (p<0.05), where Summer-Fall was higher in yield in 100% compared to the other season. In the levels of inclusion of the inoculant, they present significant differences, where the control and the level of inoculation of 5 % presented the highest yield of total biomass. Mujica and Molina (2017) found that mycorrhizae increase yield. In the variable Leaf/Stalk Ratio differences were observed (p<0.05). The ecotypes had similar performance, except for King Grass, Elephant and Mott with averages of 1.57, 1.41 and 1.18, respectively; compared to Cuba CT-115

Table 4. Means comparison considering the level of inoculants.

Inoculants (g)	NB	NH	AB (cm)	LHG (cm)	LHM (cm)	LHCh (cm)
0	14.36 ab	6.12 a	43.82 a	35.17 a	18.02 a	4.75 a
5	15.59 A	6.45 A	45.72 a	36.57 a	18.83 a	4.67 a
7.5	11.12 C	6.55 A	37.81 b	30.29 b	15.76 b	4.41 a
10	13.19 B	5.85 A	37.25 b	30.03 b	15.55 b	4.33 a

Values with different letters in the same column are significantly different according to the test of Tukey (P<0.05); NB=number of regrowth; NH=number of leaves; AB=regrowth height; LHG=large blade lenght; LHM=medium blade lenght; LHCh=blade length small.

Table 5. Variance analysis of biomass and leaf/stalk ratio.

Factor / Variable	Factor a	Factor b	Factor c	Inter. a*b	Inter. a*c	Inter. a*b*c	Inter. b*c	Rep.	C, V.
BT	48935.08 **	247089.60 **	86899.40 **	23615.14 ns	12767.21 ns	7607.73 ns	26140.61 ns	39675.59 **	79.99
RH/T	1.57 **	26.01 **	0.30 ns	2.57 **	0.41 ns	0.51 ns	0.49 ns	0.45 ns	34.98

**=Highly significant; *=significant; ns=no significant; Factor a=ecotypes of *C. purpureus*.; Factor b=Season; Factor c=level; Inter=Interaction; Rep=Repetition; C.V.=coefficient of variation; BT=total biomass; RH/T=leaf/stem ratio.

with 1.95, so they are different ($p<0.05$) (Table 6). Considering the season, there were differences in the means of both, being higher in the W-S season. It can be observed that the accumulation of fodder, the growth and development of *Cenchrus* will be conditioned to the age of resprout and the climatic conditions, which are determined by the time or season of the year (Calvano, 2011; Fortes *et al*., 2015). Taking into consideration the levels of inoculation, no significant differences were observed, with a similar performance in the four levels.

Table 6. Means comparison of total biomass and leaf/stalk ratio.

Factor	Variable	BT (t MS ha^{-1})	RH/T
Ecotype	Merkeron	1.73 a	1.75 ab
	Taiwan	1.40 ab	1.77 ab
	CT-115	1.38 ab	1.95 a
	Vruckwona	1.23 abc	1.7 ab
	Elefante	1.18 abc	1.41 bc
	Mott	0.82 bcd	1.18 c
	Roxo	0.78 bcd	1.73 ab
	Maralfalfa	0.55 cd	1.76 ab
	King grass	0.40 d	1.57 abc
Season	Summer - Autumn	1.38 a	1.32 b
	Winter- Spring	0.73 b	1.98 a
Inoculation levels (g)	0	1.28 a	1.6 a
	5	1.51 a	1.67 a
	7.5	0.74 b	1.58 a
	10	0.71 b	1.7 a

Values with different letters in the same column are significantly different according to the test of Tukey ($P<0.05$); BT=total biomass; RH/T=leaf/stem ratio.

CONCLUSIONS

The response of the ecotypes had a differential effect on the variables NB, LLL, TB and L/SR. The season affected the variables NL, PH, LLL, MLL, SLL, TB and L/SR. The low levels of inoculation increased the variables of NB, PH, LLL, MLL and TB. The factors evaluated indicate that the level of inoculant and the season determine the growth and the yield of *Cenchrus purpureus*.

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