Scientific Paper

Influence of EcoMic® and Pectimorf® on the establishment of *Leucaena leucocephala* (Lam.) de Wit. cv. Cunningham

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Abstract

The objective of the study was to evaluate the influence of the biofertilizer EcoMic®, the bioactive product Pectimorf® and their combination, on some morphobotanical variables during the establishment of *Leucaena leucocephala* (Lam.) cv. Cunningham. For such purpose a randomized block design was used with three replicas, and the following treatments: T1: thermal scarification (control), T2: inoculation with EcoMic®, T3: imbibition in Pectimorf®, T4: imbibition in Pectimorf® + inoculation with EcoMic®. The variables height, number of branches and stem diameter were measured until the plants were 12 months old. The best values of each variable were obtained when, after thermal scarification (T1), the seeds were inoculated with EcoMic® (T2) or imbibed in Pectimorf® (T3), without significant differences between them; while the control showed the lowest values. The height increased in T2 and T3 by 12 %, with regards to T1. It is concluded that the imbibition in Pectimorf® or the inoculation with EcoMic® of the seeds from *L. leucocephala* cv. Cunningham, after thermal scarification, improved the biological response of the plants during the establishment regarding their height, number of branches and stem diameter. Hence they can be used indistinctly, in order to reduce such period in the animal husbandry systems where it is used.

Keywords: height, scarification, inoculation, seeds

Introduction

Legumes perform functions of high agroecological relevance in silvopastoral systems (SPS), because they improve the quality of the animal diet and contribute nitrogen to the system, which implies an advantage from the biological and economic point of view; hence the interest in ensuring the establishment of these species in the systems.

Among the most widely used legumes in SPS is *Leucaena leucocephala* (Lam.) de Wit., due to the crude protein content of its foliage (Petit-Aldana *et al*., 2010), to its capacity to regrow after pruning or browsing and to be rapidly re-established after biotic or abiotic stress, among other aspects; nevertheless, this species shows difficulties for its establishment due to the dormancy of its seeds.

In this sense, the establishment constitutes one of the most vulnerable stages in the life cycle of plants. In the case of the species with exclusively sexual reproduction, persistence depends on the establishment of new seedlings and on the longevity of adult plants (Ramos-Font *et al*., 2015).

At present, the use of biofertilizers and bioactive products constitutes a common practice in agriculture to reduce the establishment period of crops and, in turn, improve productivity; among the most widely used for this purpose are Azofert®, EcoMic® and Pectimorf® (João *et al*., 2016).

The application of *Glomus mosseae* significantly increased growth indicators (fresh leaf biomass, fresh root biomass and height) in *L. leucocephala*, with regards to the addition of native mycorrhiza; and, also, mycorrhizal infection exceeded 90 % of the roots (Cuesta *et al*., 2006). The bioactive product Pectimorf® has been validated in sugarcane, rice, tomato and citrus fruits (Terrero, 2010) and also in sorghum (Pentón *et al*., 2011), but in *L. leucocephala* it has been tested only under nursery conditions (Bover-Felices *et al*., 2017).

That is why the objective of this research was to evaluate the influence of the bioproducts EcoMic® and Pectimorf® on some morphobotanical variables, during the establishment of *L. leucocephala* cv. Cunningham.

Materials and Methods

Locality and geographical location. The trial was conducted in 1,6 ha belonging to the animal production areas of the Pastures and Forages

Research Station Indio Hatuey, which is located at 22º 48' N, 79º 32' W and 19,9 m.a.s.l.

Soil and climate characteristics. According to the classification proposed by Hernández-Jiménez *et al.* (2015) the soil of the experimental area corresponds to a Ferralitic Red one, with good internal drainage and pH between 6,0 and 6,2. During the study (June, 2015-June, 2016) the mean annual temperature was 24,7 ºC, in the rainy season it was 26,5 ºC while in the dry season, 22,3 ºC; and mean annual rainfall, 1 637 mm (Meteorological Station Indio Hatuey).

Treatments and experimental design. A randomized block design was used with three replicas. The experimental plots measured 1 200 m^2. The treatments were: T1: thermal scarification (control), T2: inoculation with EcoMic®, T3: imbibition in Pectimorf®, T4: imbibition in Pectimorf® plus inoculation with EcoMic®.

Experimental procedure. For the preparation and later application of the treatments, the seeds from *L. leucocephala* cv. Cunningham (seven months after being harvested) were scarified before seeding with hot water at 80 ºC during 2 minutes (thermal scarification, TS), according to the procedure described by González and Mendoza (2008).

In the seeds that were treated with EcoMic® [from the National Institute of Agricultural Sciences (INCA, for its initials in Spanish) of Cuba, solid inoculant that contains the arbuscular mycorrhizal fungus (AMF) *Glomus cubense* Y. Rodr. & Dalpé], the covering technique was used, in a proportion of 10 % of their weight (with a mixture formed by 600 mL of H_2O for every kilogram of this bioproduct); and when they were covered by a uniform film they were dried under shade, according to the recommendations in the EcoMic® technical handbook (INCA, 2003).

In the case of the bioactive product Pectimorf®, from the plant physiology and biochemistry laboratory of INCA, the seeds were imbibed in it at a concentration of 10 mg L^{-1} of H_2O, after TS, by the immersion method during 16 h.

Soil preparation of the experimental area. In correspondence with the type of vegetation present in the field (natural pastures, mainly) a full preparation was carried out (plowing-harrowing-crossing-harrowing) and strips 2 m wide and 320 m long were formed.

Seeding. The seeding orientation was carried out according to the sun trajectory (from East to West), to prevent shade excess between the furrows; planting density was 1 kg of seeds ha^{-1}, which were covered with a soil layer of 1 cm. the distance between plants was 0,20 m, and a 5-m space was left between furrows. The area was considered established when the plants reached a height of 1,5- 2,0 m (Seguí *et al*., 2002).

Variables. The height and number of branches were measured since emergence started, and stem diameter since the plants were three months old until 12 months, with a monthly frequency and in 40 plants per replica, which represented 3 % of the total *L. leucocephala* plants.

Plant height was measured with a ruler graduated in centimeters, placed perpendicularly to the soil; the number of branches was counted *in situ* in the experimental field; and to measure the stem diameter a caliper was used, which was placed at 25 cm over the soil surface.

Statistical analysis. The statistical program SPSS® version 22.0 was used to verify whether the data fulfilled the normal distribution through the Shapiro-Wilk test, as well as the variance homogeneity through Levene's test, and once those assumptions were proven a variance analysis was carried out. The differences among means were determined through Duncan's test, for 5 % significance.

Results and Discussion

When analyzing the performance of the variables that were evaluated during the establishment (table 1), no significant differences were observed when treatments T2 and T3 were applied, which statistically differed from T4 and T1; in the case of height, the last two ones did not differ between them. This indicates that the two bioproducts (EcoMic® and Pectimorf®) can be an additional complement for the nutrition of this tree after breaking dormancy with the TS.

In this stage the highest values of each variable were obtained when, after the TS, the seeds were imbibed in Pectimorf® or inoculated with EcoMic®; and the lowest values, in the control. The height increased by 12 % with regards to T1, which is important if it is taken into consideration that *L. leucocephala* cv. Cunningham, for being a tree, is a slow-growth plant, and this can be a disadvantage for its utilization in SPS.

Duran-Alvernia (2017) stated that most tree legumes have slow growth in the first phase, which makes them vulnerable to competition with weeds and predators during the establishment. Hence this period constitutes one of the most difficult stages in the development of a system, because it is

Table 1. Effect of Pectimorf®, EcoMic® and their combination on some morphological characteristics.

Indicator	Treatment	n	Mean ± SE	Sig.
Height (cm)	T1	1 200	128,0 (± 1,81)[b]	***
	T2	1 200	143,2 (± 2,03)[a]	
	T3	1 200	143,0 (± 2,07)[a]	
	T4	1 200	126,9 (± 1,84)[b]	
Stem diameter (mm)	T1	840	8,1 (± 0,13)[c]	***
	T2	840	9,8 (± 0,15)[a]	
	T3	840	10,0 (± 0,17)[a]	
	T4	840	8,9 (± 0,13)[b]	
Number of branches	T1	1 200	7,1 (± 0,18)[c]	***
	T2	1 200	8,6 (± 0,18)[a]	
	T3	1 200	8,7 (± 0,19)[a]	
	T4	1 200	7,8 (± 0,16)[b]	

Means with different superscripts in the same column significantly differ at $p \leq 0{,}001$.

T1: thermal scarification (control), T2: inoculation with EcoMic®, T3: imbibition in Pectimorf®, T4: imbibition in Pectimorf® plus inoculation with EcoMic®.

necessary to combine the conditions inherent to the soil, climate and characteristics of the variety.

According to the report by Bonareri-Oruru and Mugendi-Njeru (2016), the positive response of plants (higher growth) to the application of EcoMic® is due to the fact that it is a biofertilizer composed by arbuscular mycorrhizal fungi (AMF), which provide or improve nutrient availability. They also participate in the absorption of phosphorus from the soil (Bagyaraj *et al.*, 2015; Guisande-Collazo *et al.*, 2016).

Phosphorus is one of the most limiting nutrients, and is essential for crop growth. It also has other beneficial effects, such as control of root pathogens; it is integral part of the cell activity, and establishes synergies with other soil beneficial microorganisms (Bagyaraj *et al.,* 2015).

Dantas *et al.* (2015) reported that AMF increase the root volume of the plant, which allows higher extension of the roots in the rhizosphere and, thus, higher quantity of spores that facilitate nutrient capture. Hence the positive response when using this bioproduct during the establishment of *L. leucocephala*.

Similar performance was found by João *et al.* (2016) with the application of AMF (*Funneliformis mosseae*) in the cassava crop, which originated significant increases ($p < 0{,}001$) in all the evaluated variables. More vigorous plants were achieved, with higher growth and a 21 % increase in height. Likewise, the yields of edible roots increased with regards to the control, and the mycorrhizal spores increased eight times; this indicated the effectiveness of the mycorrhizae under these edaphic conditions.

In the case of Pectimorf®, a positive effect was found on the variables under study, with emphasis on seedling and adult plant height. This product influences the activation of cell division and the elongation of the cell walls, according to Izquierdo *et al.* (2014). In addition, its biostimulator effect on the plant height of the herbaceous legume *Vigna unguiculata* (L.) Walp. var. Lina has been observed, according to the report by Nápoles-Vinent *et al.* (2016).

It is valid to emphasize that Pectimorf® is a mixture of pectic oligosaccharides, obtained from raw materials produced in Cuba by the citrus fruit agroindustry –specifically pectic acid– (Cevallos, 2000). It has as active component a mixture of oligogalacturonides (OGs) –functional structures of the cell wall belonging to the group of pectins (Mederos-Torres and Hormaza-Montenegro, 2008).

It is considered that OGs trigger physiological processes of cell wall regeneration and division, by stimulating the synthesis of substances that act in those processes (Cabrera, 2000). All this originates modifications in root architecture, as proven by Hernández *et al.* (2007) in *Arabidopsis thaliana*.

On the other hand, when using Pectimorf® in the *L. leucocephala* seeds under nursery conditions, Bover-Felices *et al*. (2017) obtained a favorable result, specifically in the percentage of emerged seedlings (71,43 %).

No significant effect was found when the bioproducts were combined, which differs from the report by Pentón *et al*. (2011) in an evaluation of the response of the variables effective height, stem basis diameter, number of opened leaves and leaf length, in *Sorghum bicolor* (L.) Moench.

In turn, Ayala-Boza *et al.* (2013), when evaluating the effect of the biofertilizers Azofert® and EcoMic® and of the biostimulator Pectimorf® on height and yield and its components in the soybean varieties Conquista and INCA soy-27, no significant differences were found between the use of bioproducts independently or in their combinations.

In this sense, Corbera and Nápoles (2013) stated that the OGs present in Pectimorf® have shown synergic interactions with other bioproducts (Azofert®, EcoMic®); at laboratory level, other authors proved that it can also have antagonist effect, mainly the mixture of OGs with auxins, which decreases the expression of genes and enzymes associated with the auxin activity (Kollárová *et al*., 2012; Savatin *et al*., 2013).

Vázquez and Torres (2006) reported that the excess of substances with rooting effect inhibits callus formation and, thus, the rooting onset of the cutting. This process is led by hormones, and it is possible that the mixture or combination causes hormonal disorganization inside the cells which hinders the occurrence of the cell elongation, differentiation and division processes (Doll *et al.,* 2013). In this regard, Ramos-Hernández (2014) found antagonist effects among the combinations of bioproducts in different crops.

It is concluded that the imbibition in Pectimorf® or the inoculation with EcoMic® of the seeds from *L. leucocephala* cv. Cunningham, after thermal scarification, improved the biological response of the plants during the establishment regarding their height, number of branches and stem diameter. Hence they can be used indistinctly, in order to reduce such period in this species in the animal husbandry systems where it is used. Likewise, to conduct other evaluations with the combination of these two bioproducts is recommended.

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