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Yield of dry matter and other components in Leucaena leucocephala cv. Cunningham using Liplant

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ABSTRACT

In the Agricultural University of Havana biostimulators are produced which have been successfully used in the agricultural production of different plant species. Among them is Liplant –a product derived from earthworm humus containing hormonal fractions-, with which satisfactory results have been obtained in the *in vitro* propagation of several crops, as well as in *ex vitro* applications of others. The objective of this work was to determine the influence of the doses of this product on the bromatological composition and growth, as well as the yield of DM and some of its components. A factorial design was used under semi-controlled conditions and three treatments: 0, 1/20 and /40 (v/v), with two studied factors: without fertilization and with fertilization, on a lixiviated Ferralitic Red soil. Significant difference ($P<0,05$) for plant growth and number of shoots with the application of Liplant in the dilution1/20 was found; while no differences were found in the dry matter yield when applying the product. The analysis allowed to conclude that the dilution 1/20 favored the evaluated indicators, except the DM yield, althoughr it is necessary to test these results under field conditions.

Key words: *Leucaena leucocephala* cv. Cunningham, plant growth substances, yields

INTRODUCTION

At present the use of ecologically innocuous products that produce benefits to crops is booming, such as brassinosteroid analogs (Izquierdo *et al*., 2004), Biostán and Liplant, from earthworm humus (Huelva *et al*., 2004), which have been obtained and synthesized in Cuba. Seedling growth stimulation through the use of these compounds is a way to obtain optimum-quality seedlings; it is performed in two ways: imbibing the seed or spraying them over the seedlings.

Liplant is a plant growth biostimulator, obtained by the Agricultural University of Havana "Fructuoso Rodrìguez" from vermicompost. This natural product is in balance with the environment, for which it is a viable solution in sustainable agriculture (Garcés, 2000), because it facilitates nutrient and energy recycling to the agricultural environment.

In its composition the presence of plant hormones, free aminoacids, polysaccharides, carbohydrates, inorganic elements and humified substances has been reported; each of these fractions may exert, individually, a stimulation effect on the plants, and as a whole they reinforce their activity, which allows a marked response in crop growth and development (Garcés *et al*., 2004).

Leucaena, especially *Leucaena leucocephala*, has been the object of many studies and it is the most planted species in agroforestry systems, in addition to being one of the forage legumes with better characteristics for livestock production. There are many reasons for which it has been widely used, especially its high biomass production (even in the dry season), its acceptability by different animal species and its regrowth capacity after cutting and/or browsing. The combination of the characteristics found in this tree is likely to be unique, besides having a high nutritional value, for which in different parts of the world the interest in the study of its agronomic management and the forms of utilization in animal production systems has increased. However, it has some limitations, such as: poor adaptation to acid soils, particularly when they are associated to highly exchangeable aluminum; slow establishment and susceptibility to competition by young plants.

Likewise, other important factors are considered to affect the growth of young seedlings are: seed inoculation with *Rhizobium* spp. and the existence of vesicular-arbuscular mycorrhizal symbiosis (Ruiz and Febles, 2006). Young seedlings require the fast infection with mycorrhizae for the supply of adequate phosphorus quantities. The levels of vesicular-arbuscular mycorrhizae in the soil depend on a group of factors, such as the history of the planting area, natural vegetation and waterlogging.

According to Tang (1994) there are few results regarding the use of *Rhizobium* in leucaena plants, in addition to the fact that the strains accepted by this genus are very specific. In this sense, Murgueitio (2003) reported that leucaena may be multiplied through seeds in nurseries or by direct seeding (inoculated –or not– with *Rhizobium*, nitrifying bacteria or mycorrhizae). On the other hand, Corbea and Blanco (2005) stated that inoculation is a treatment previous to seeding which is necessary in the case of some tree legumes, although the leucaena species were not considered as there were several results in that regard, and due to their specificity with preselected *Rhizobium* strains; for which it is necessary to study alternatives to attenuate this problem. For such reasons, the objective of this work was to determine the influence of Liplant doses on the yield of DM and some of its components.

MATERIALS AND METHODS

The trial was conducted under semi-controlled conditions at the Experimental Station of Pastures and Forages “Indio Hatuey”, which is located at 22º 48’ and 7" latitude North and 79º 32’ and 2" longitude West, at an altitude of 19,9 masl, in the Perico municipality, Matanzas province, Cuba (Academia de Ciencias de Cuba, 1989).

The soil was classified by Hernández *et al*. (2003) as lixiviated Ferralitic Red, hydrated ferruginous nodular humic, of rapid desiccation, clayey and deep on limestone. This type is equivalent to the group of Ferrosols, in the FAO-UNESCO classification system (Alonso, 2003). The average depth to the limestone is 150 cm.

Used plant material. For this study *L. leucocephala* cv. Cunningham, which is preserved in the germplasm bank of the institution, was used.

Treatments and design. The treatments were: 0, 1/40, 1/20 (v/v) of Liplant, with two studied factors: without fertilization and with fertilization, in a factorial design.

Procedure. The soil was taken at a depth of 0-20 cm, it was air-dried and later sieved with a 0,6-cm mesh; 6 kg were introduced in polyethylene bags. Before seeding, irrigation was performed daily with tap water during three days, until completing 85 % of the field capacity.

The seeds were submerged in dissolution of 1/40 of Liplant during 24 hours and they were planted afterwards (three per bag). For each treatment 20 bags were used, 50 % of them were fertilized with 400, 100 and 150 kg of N, P and K/ha, respectively. Fertilization was applied in liquid form on the surface of 50 % of the bags, two weeks after being planted, and over the others in the next two weeks; and for the calculation the weight of one hectare per row was taken as basis.

Liplant (0, 1/40, 1/20 v/v) was prepared in the indicated concentrations and applied to the leaves four weeks after planting the seeds. Irrigation was performed daily, with tap water. The cutting was made when the plants were nine weeks old.

Measurements. Before cutting the following indicators were measured: plant height, number of shoots per plant, DM yield (g/bag) and bromatological composition (N, P, K, Ca, Mg) expressed in percentage.

For the data processing a factorial analysis was made and the option GLM (General Linear Model) was used, corresponding to the statistical pack SPSS version 11.5 for Microsoft Windows ® (Visuata, 1998). The multiple comparison test was performed through the SNK (Student-Newman Keuls) and the means were compared according to P<0,05.

RESULTS AND DISCUSSION

Regarding height, there was no interaction between the fertilizer and the biostimulator. It was observed that with fertilization (fig. 1) plant height increased significantly (P<0,05). There was higher growth with the application of the product and the highest values were obtained with the dilution 1/20 (fig. 2).

In the reviewed literature no reference is made to the use of this type of products to stimulate the

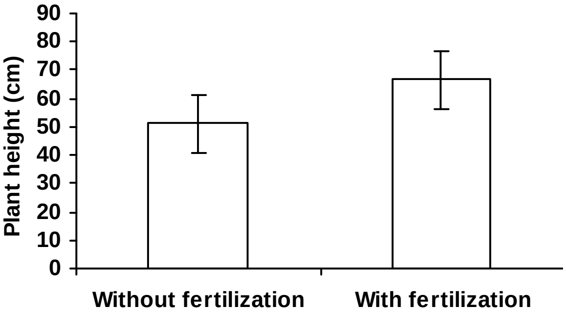
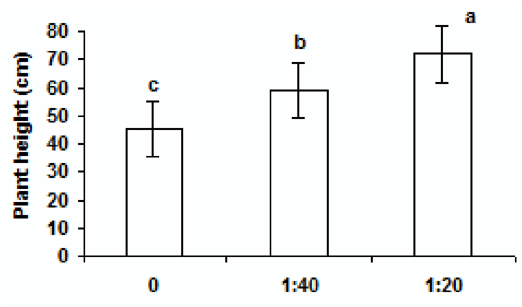


Fig. 1. Effect of fertilization on height before the cutting of *L. leucocephala* cv. Cunningham plants.

Pastos y Forrajes, Vol. 36, No. 1, January-March, 50-55, 2013



a, b, c: values with different letters differ statistically at P<0,05.

Fig. 2. Effect of Liplant on the height of *L. leucocephala* cv. Cunningham plants.

germination of leucaena seeds, for which the experiments where they are used are novel in the cultivation of this species, which is particularly interesting for research. No reports have been found either of the use of growth stimulating products to increase height in these plants, but there is reference to them in other crops of economic interest (Baños *et al*., 2009). Nevertheless, leucaena is known to be a plant species capable of expressing its high productive potential with an adequate supply of nutrients to the soil. In that sense, many studies have been conducted proving it, as long as the soil and climate factors are favorable.

This increase regarding the height of *L. leucocephala* is logical, considering the report by Huelva *et al.* (2004) regarding that the fraction of humic and fulvic acids present in this biostimulator has the property of being assimilable by plants through their root system or their leaf tissue, by participating in metabolism and activating the photosynthetic process, for which a favorable response should be expected. The properties of this substance, which also has plant hormones, free aminoacids, polysaccharides and inorganic elements, allow a marked response in crop growth and development. This could have caused the performance of each of the yield components of *L. leucocephala* cv. Cunningham, such as plant height and other indicators, which were favored with the application of the stimulant derived from vermicompost. Huelva *et al.* (2004) obtained higher leaf mass and an increase of photosynthesis in soybean, allowing it to capture more radiant energy and higher accumulation of photosynthates.

In this sense, Figueroa *et al.* (2001) reported that the application of humic acids to the soil and foliage increases fertility and improves their physical properties, in addition to the better utilization of some higher nutrients, aspects which have an important repercussion on crop yields. Likewise, when spraying dissolutions of this product in different growth stages in corn cultivation, Caro (2004) found that leaf width increased remarkably as compared with the untreated ones.

Another yield component is number of leaf shoots, in which significant differences were observed using the dose 1/20 of Liplant and fertilization, with regards to the treatment without fertilization (figs. 3 and 4). It was also observed that the concentration 1/20 of Liplant almost doubled the effect.

If the results obtained in the number of leaf shoots are related, it is possible to suggest an action of Liplant on leaf expansion, which may be ascribed to the auxin-type effect of the humic substances present in this type of liquid humus or to the existence of phytohormonal substances in this product. Thus, Huelva *et al*. (2004) found favorable modifications in the specific leaf area when applying liquid humus to the leaves in soybean cultivation.

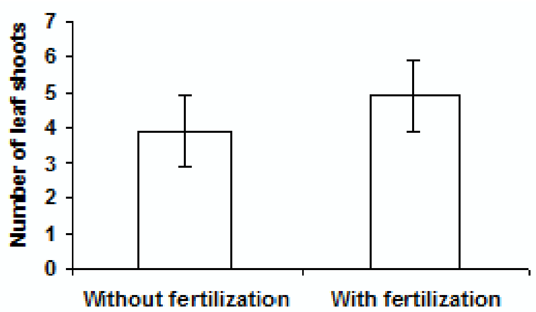
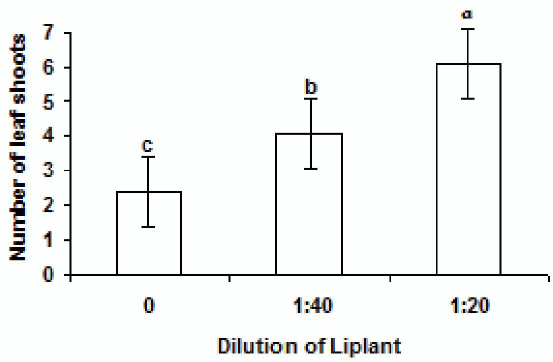


Fig. 3. Effect of fertilization on the number of leaf growths of the plants.



a, b, c: values with different letters differ statistically at P<0,05.

Fig. 4. Effect of the Liplant dilution on the number of leaf growths.

Seemingly, the increases of these growth variables could be related to the composition of Liplant. It is necessary to remember that this is an extract obtained from vermicompost through physical and chemical procedures. The components of these products, according to De la Huerta *et al*. (2012), are mainly humic substances, from which their effects and their participation in the different physiological-biochemical processes of plants are known, with positive intervention in respiration and the rate of enzymatic reactions of the Krebs cycle (which propitiates a higher production of ATP); and also their selective effects on protein synthesis and the increase or inhibition of the activity of diverse enzymes (Arteaga *et al*. 2004).

Humic substances are not only biostimulators, but they can also have a nutritional effect due to their deposition of small amounts of substances on the leaves; in addition, when applied on the plants such effect may be higher, because higher availability is guaranteed for the incorporation of their components through the roots (Álvarez, 2005; Torres *et al*., 2005).

The possible participation of these components in the above-mentioned processes may favor the higher values obtained in these indicators. Likewise, auxin-type phytohormones are found in higher concentration in the composition of this liquid humus and they are known to influence cell elongation (De la Huerta *et al*., 2012).

This liquid humus contains minerals and aminoacids (Reinés *et al*., 2006), chemical fractions which alone –or combined with the above-mentioned ones- could cause nutritional and/or biostimulating effects on plant growth and development (Saracco-Álvarez, 2007).

Regarding DM yield (g/bag), there was no interaction among the principal factors. Figures 5 and 6 show the effects in this indicator, in which there were no highly significant differences ($P<0,05$). It was also observed that with the application of Liplant, in any of the concentrations, there were no significant differences for such indicator.

All these aspects favored the accumulation of DM in the leucena accessions. Similar results have been obtained in other crops of agricultural importance, such as: plantain, lettuce, pineapple, onion, corn, beans, garden beans, tomato (Garcés *et al*., 2004), rice (Izquierdo *et al*., 2004) and soybean (Huelva *et al*., 2004); although in tree or shrub species the results are very scarce.

The effect of Biostán and Liplant, concentrates of strong biological activity, soluble, stable, easy to

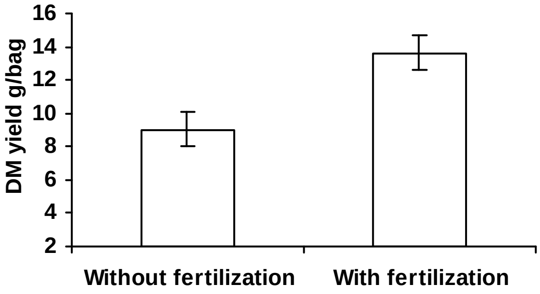


Fig. 5. Effect of fertilization on DM yield.

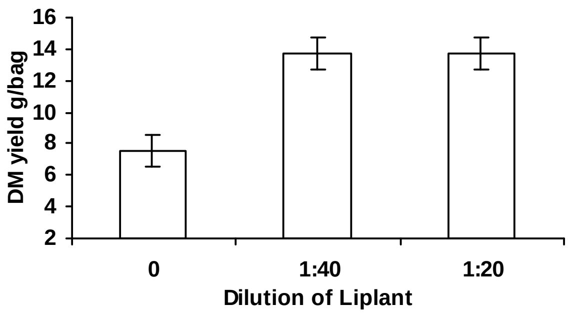


Fig. 6. Effect of Liplant dilution on DM yield.

handle and of low cost, was studied by Jefferson Díaz and García (2004) in onion cultivation, on such indicators as germination and on different physiological values of the plants, which grew under field conditions. An increase was observed, with regards to the control, in the bud mass per plant and the equatorial diameter in the cultivar Texas Grano, when the seeds were submerged (before planting) in dilutions of 1/20 v/v of Liplant and then the seedlings were sprayed after 21 days with equal dilution; while the treatment with Biostán offered a similar response.

When evaluating the potentials of Liplant in the germination of pink radish, with different concentrations, similar results were found in seed germination, growth and development. Arteaga *et al*. (2004) observed that with 1/40 and 1/20, the highest percentage was obtained in the evaluated indicators, as compared with the other concentrations and the control. Likewise, Mesa *et al*. (2006) studied the effect of Liplant on the DM yield in *Morus alba* (L), with and without fertilizers, and concluded that the dilution 1:20 v/v favored most of the evaluated indicators.

These results could be influenced by the humic and fulvic acids or the aspartic acid, main aminoacids related to the formation of others through transamination, which influence the synthesis of proteins necessary for biomass production in the plant (Guerra, 2011). The results of this experiment confirmed that the application of Liplant caused biostimulation, which corroborated the effect reported in other crops of economic interest with dissolutions of liquid humus obtained from vermicompost in doses that oscillate between 1/30 and 1/100 v/v.

Figures 7 and 8 show that the fertilization applied to the soil and the concentration of Liplant influenced the mineral content, because there was no significant interaction between these two factors. Only the content of N and Ca increased significantly ($P<0,05$) with the application of fertilizer. On the other hand, the biostimulator significantly increased the N content, and there were no differences between the dilutions 1/40 and 1/20; something similar occurred with potassium. The other macronutrients were not affected by the application of this product.

Regarding the accumulation of minerals, the increase of the N and Ca content due to fertilization was significant, except for P, K and Mg. In studies conducted in different plants N has been proved to be one of the elements with higher influence on DM yield and, thus, on the accumulation of these elements, and also on the number of growths and leaf yield (Álvarez, 2005).

Another important aspect in fertilization, and which has a marked influence on yield as well as on chemical composition, is the increase in the rubisco/soluble protein ratio and in the chlorophyll content from the application of N and K_2O, which allows an increase in the CO_2 fixation rate and this may be inferred in the potential to increase the green biomass.

Although P was applied to the soil, seemingly this did not have repercussion on the accumulation of such elements in the plants, maybe due to its immobilization because of the pH it has. Several authors, such as Gallardo *et al*. (2001), have reported similar results.

The effect of Liplant was very significant for the N and Ca contents. Highly similar results were obtained by Huelva *et al*. (2004) in soybean, with an increase in protein content, and Mesa *et al*. (2006) in studies conducted in mulberry.

The bromatological composition of this biostimulator (Garcés *et al*., 2004) could have favored the accumulation of K, a very important aspect in the regulation of the water regime in forage species. Although it is not considered an important component in plant structure, it has the functions of intensifying nitrogen metabolism and protein synthesis, as well as influencing the exchange of carbohydrates and regulating the water regime of the plant.

The application of this diluted product (1/20) was concluded to favor plant height, number of leaf shoots and bromatological composition; although it is necessary to study these concentrations under field conditions, as well as the frequency of its application.

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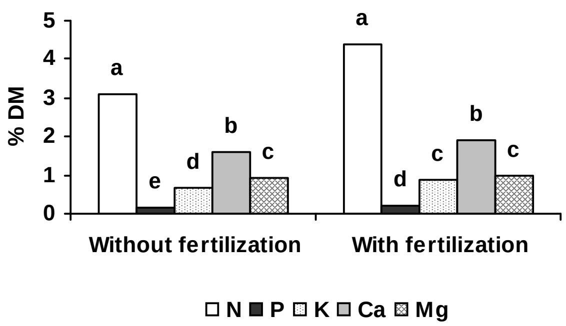

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Fig. 7. Effect of fertilization on mineral content.

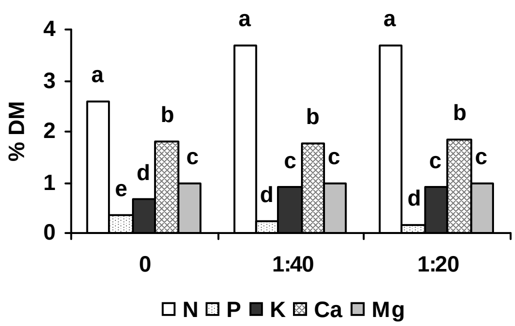

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Fig. 8. Effect of Liplant dilution on mineral content.

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