Scientific Paper

Agronomic performance of three alfalfa (*Medicago sativa* L.) varieties with different phosphate fertilization doses

Wilson Oñate Viteri[1] and Enrique Flores Mariazza[2]

[1] *Facultad de Ciencias Pecuarias, Escuela Superior Politécnica de Chimborazo EC060155, Riobamba, Ecuador*

[2] *Laboratorio de Ecología y Utilización de Pastizales. Universidad Nacional Agraria, Lima, Perú*

E-mail: wilsonovec@yahoo.es

ORCID: https://orcid.org/0000-0001-7533-3481

Abstract

The objective of this study was to evaluate the agronomic performance of three *Medicago sativa* L. varieties with three doses of phosphate fertilization, during establishment, in the Ecuadorian mountains. The trial was conducted at the Tunshi Research Station, located in the Riobamba canton, Chimborazo province –Ecuador–. The treatments were: the alfalfa varieties as factor A (abunda verde, cuf-101 and sw-8210) and the fertilization doses (0, 50, 100 and 150 kg P/ha) as factor B, compared with the ecotype flor morada (control). The experimental plots were distributed in a completely randomized block design, with split-plot arrangement and three repetitions; variance analysis and mean comparison according to Tukey were carried out. Significant differences ($p < 0,05$) were recorded for the A x B interaction. The emergence percentage when 100 kg P/ha were applied was 95,67 % for the variety sw-8210, which differed significantly ($p < 0,05$) from the ecotype flor morada without fertilization (90,33 %). This ecotype reached 99,59 % of aerial cover and 34,09 % of basal cover in the first cut (110 days), and differed significantly ($p < 0,01$) from the introduced varieties. The ecotype flor morada without fertilization produced 17,23 and 4,52 t/ha per cut of green and dry forage, respectively; and surpassed ($p < 0,01$) the variety abunda verde (7,46 and 2,79 t/ha, respectively). It is concluded that the ecotype flor morada showed higher green and dry forage yield, and better cover and plant height without phosphate fertilization; likewise, the harvest was performed at 110 days after sowing, when the plants showed 10 % of flowering.

Keywords: ecotypes, emergence, plant establishment, yield

Introduction

Alfalfa (*Medicago sativa* L.) is a perennial forage legume cultivated in all the regions of the world, in subtropical, temperate and dry climates (Liu *et al.* 2015). Its importance lies on its production potential, its nutritional value (Rogers *et al.*, 2014) and its utilization as green forage, hay, silage, pellets, and others (Milic *et al.*, 2014; Rojas *et al.*, 2017). It is more effective than annual crops to reduce runoff and soil erosion (Fan *et al.*, 2014); however, the yield and nutrient content can be affected mainly on saline soils (Ensiye *et al.*, 2018). At present the alfalfa breeding programs have been focused on the forage yield and quality, resistance to biotic and abiotic stressing factors and the lack of water (Hawkins and Long, 2018), which is a limiting factor for crop production in many parts of the world (Raza *et al.*, 2014).

The survey of agricultural surface and production carried out by the National Institute of Statistics and Surveys (INEC, 2016) showed that in the Chimborazo province –Ecuador–, from the 523 340 ha of soil use, 79 951 ha (15,22 %) are used for cultivated pastures and from them 5 250 ha are aimed at the cultivation of *M. sativa*, which represents 6,57 %. In Ecuador cattle farmers are dedicated only to the production of green forage using imported seed from the United States (Alaska, 2015). Between 2000 and 2013 more than 1 700 metric tons of *M. sativa* seeds were imported, from which 93 % were from that country (Banco Central del Ecuador, 2014).

Sanz *et al.* (2017) indicated that nitrogen and phosphorus are the main plant macronutrients that limit growth. Although P is abundant in many soils, its availability for the plants is low. The alfalfa crop requires soils with neutral pH, medium to light texture, good drainage and depth and high phosphorus availability, where it can express all its productive potential. In that sense, phosphate deficiency is a nutritional factor that limits legume production, particularly on acid and calcareous soils (Martins *et al.*, 2017). For such reason, the chemical analysis of the topsoil is essential to know the particular situation and aim the fertilization programs. The benefits of initial phosphate fertilization of alfalfa are perceived, among others, in a higher formation of nodules, stimulation of initial growth, higher root development, better competition with weeds and advance of the first utilization. Taking such antecedents into consideration, the objective of this study was to evaluate the agronomic performance of three *Medicago sativa* L. varieties with three doses of phosphate fertilization, during establishment in the Ecuadorian mountains.

Materials and Methods

The study was conducted in a land lot of the Tunshi Research Station, belonging to the School of Animal Husbandry Sciences of the Higher Polytechnic School of Chimborazo (ESPOCH for its initials in Spanish), located on the Kilometer 12 road to Licto, Chimborazo province; at 79º 40′ West longitude and 01º 65′ South latitude, and an altitude of 2 754 m.a.s.l. The temperature is 10 to 20 ºC, with a mean of 13,6 ºC; annual rainfall varies between 550 and 800 mm, and relative humidity, between 54 and 96 %, with a mean of 82 % (INAMHI, 2017).

Soil characterization. The soil analysis of the Tunshi Research Station is shown in table 1. The soil of this site has neutral pH and low P content in the topsoil; its topography is flat (1,5 % slope) and the structure is loamy sandy, with good drainage.

Table 1. Soil characterization of the Tunshi Research Station.

Indicator	Value
pH	7,40
OM, %	2,53
N, %	0,33
P, ppm	7,80
K, cmol/kg	0,86
Ca, cmol/kg	9,52
Mg, cmol/kg	3,55
Fe, ppm	6,10
Mn, ppm	7,16
Cu, ppm	9,61

Source: Laboratory of soil and fertilizer analysis of Agroquality (2016).

Treatments and experimental design. The treatments were the alfalfa varieties as factor A (abunda verde, cuf-101 and sw-8210) and as factor B the fertilization doses (0, 50, 100 and 150 kg P/ha), against the ecotype alfalfa morada (control), with three repetitions. The design was completely randomized block design with split-plot arrangement, adjusted to the following linear additive model:

$$Yijk = \mu + Ri + Aj + E_A + Bk + AjBk + E_B + Ts + Eijk$$

Where:

Yijk: estimated value of the variable

μ: general mean

Ri: effect of the blocks (R)

Aj: effect of the alfalfa varieties (A)

E_A: effect of the randomization of the varieties within the blocks

Bk: effect of the doses of phosphate fertilization (B)

AjBk: effect of the interaction alfalfa varieties and phosphate fertilization (AB)

E_B: effect of the randomization of phosphate fertilization doses on the *M. sativa* varieties

Ts: effect of contrast (*M. sativa* ecotype morada compared with the interactions alfalfa varieties by fertilization doses)

Eijk: residual effect

Experimental procedure

Once the site was selected the land preparation started, which consisted in three steps: plowing, harrowing and land leveling; afterwards the plots were delimited, with an area of 5 x 4 m (20 m^2) which was the experimental unit, and a 1-m^2 separation between blocks. For the study there were three blocks made up by 13 plots (12 for the introduced varieties and one for the ecotype flor morada), with an area of 780 m^2 (39 plots).

A soil sample was taken to make the chemical and physical analysis before sowing. It was carried out in December, 2016, by broadcasting, with certified seed for the introduced varieties and artisanal seed for the ecotype flor morada, and a density of 22,73 kg/ha was used. The chemical fertilization (0, 50, 100 and 150 kg P/ha) was applied at the moment of sowing, with triple superphosphate (TSF) as phosphorus source.

In the case of the introduced varieties, before sowing each variety it was randomly raffled in each block (main plot), and in each variety of each block (subplot) the phosphorus doses were randomly raffled. However, in the control sowing was performed in the first plot of each block.

The cultural labors were homogeneous for all the experimental units, mainly weed control and irrigation, which was in correspondence with the environmental conditions of the Tunshi Research

Station. The evaluations of alfalfa establishment and growth were made every 30 days, until the first cut (110 days). The following indicators were evaluated: emergence percentage, number of stems per plant, number of plants per square meter, plant height, percentage of aerial and basal cover, regrowth number and height, botanical composition, leaf/stem ratio, green forage and dry matter production, and days to harvest.

Measurements

Soil analysis. The soil samples were analyzed in the Agroquality Laboratory of the Ministry of Agriculture (Agrocalidad, 2016).

Emergence percentage. The percentage of plants that took root and/or emerged 21 days after sowing.

Number of plants. In each subplot (varieties), the number of plants in a 0,5-m^2 quadrant was counted; it was expressed in plants per square meter.

Number of stems per plant. It was determined by the manual count of each one of the stems from 10 plants at random; this indicator was measured for each treatment and the averages were calculated.

Basal cover (%). The basal cover was evaluated with the Parker's permanent transept method.

Floristic composition. The floristic composition was calculated based on the plant cover, in percentage.

Leaf/stem ratio. Subsamples of the samples were used for forage yield. They were separated into morphological components (leaves and stems), dried and weighed, to estimate later the leaf and stem percentage in the sample. The leaf/stem ratio was determined by dividing the leaf yield by the stem yield.

Plant height (cm). The plant height was taken as the mean distance comprised between the basal part of the stem to the leaf apex (canopy height).

Flowering percentage. This measurement was made only in the ecotype flor morada, because the introduced varieties did not flower; the pre-flowering status was considered when the crop reached 10 % flowering.

Number and height of new regrowths. The measurement was based on counting the new regrowths of the crown, after they reached 5-7 cm of height (Harris, 1978).

Dry matter production (t/ha). The green forage production of each variety was expressed in tons per cut; for such purpose the green sample weight in one square meter was taken, and this value was taken to its equivalence in dry matter production per hectare.

Nodulation. Digging was done carefully around the roots, the plant was uncovered and the live nodules were counted.

Days to harvest. In the introduced varieties the harvest was made when the plants reached four to five new regrowths with more than 5 cm height, because there was no flowering induction; and in the case of flor morada, when the alfalfa plants had 10 % flowering.

Statistical analysis. The results were tabulated in an electronic sheet Excel from Office 2016; and they were later subject to variance analysis, after testing the variance and normality homogeneity assumptions. The mean comparisons were made according to Tukey ($p < 0,05$) for the AB interaction. For the statistical processing the Infostat package was used (Di Rienzo *et al*., 2017).

Results and Discussion

Tables 2a and 2b show the performance of the evaluated indicators

Emergence. The emergence percentages of the alfalfa varieties abunda verde, cuf-101 and sw-8210 when applying phosphate varied between 92,67 and 95,67 %, and differed significantly ($p < 0,05$) of the ecotype flor morada (90,33 %). This coincides with the report by Alaska (2015) in those same varieties: between 94 and 95 % emergence. This may be due to the stimulus of phosphorus on root development, which propitiates the more intensive and long term initial vegetative growth, when good nutritional and soil humidity balance is guaranteed (Barber, 1980).

Aerial cover. The ecotype flor morada and the variety sw-8210 with 100 and 150 kg/P/ha showed the highest values in aerial cover, which were significantly higher ($p < 0,01$) than those of the varieties cuf-101 with 0 and 50 kg/P/ha and sw-8210 without fertilization. In the case of flor morada, this was due to the fact that it is adapted to limey-sandy soil and its nitrogen fixing capacity through interaction with the present rhizobia (Ohyama, 2017), which allows to increase the leaf area. In addition, the roots of the ecotype flor morada are deeper than the ones of the introduced varieties; thus, they extract better the soil nutrients for plant growth and development, the leaf area is increased and, consequently, the aerial cover increases. However, in the variety sw-8210 with 100 and 150 kg/P/ha there was an increase in cover, which proves that phosphorus is necessary to achieve successful establishment and good root development, as well as better water efficiency and competition with weeds (Sanz *et al*., 2017); therefore, there was better utilization of the available nutrients in the soil, and this favored the increase of cover.

Basal cover. The basal cover of the ecotype flor morada, the varieties sw-8210 and cuf-101 with 150 kg/P/ha and abunda verde with 50, 100 and 150 kg/P/ha did not differ among them; yet, the ecotype flor morada showed the highest cover, which differed significantly ($p < 0,01$) from the varieties abunda verde without fertilization, cuf-101 and sw-8210 with 0, 50 and 100 kg/P/ha. This better cover for flor morada without fertilization was due to the fact that it is adapted and naturalized in the mountains of Ecuador; while in the introduced varieties phosphorus is essential for their cultivation. According to Honghua *et al.* (2017), the phosphorus supply in the soils affects the accumulation of other mineral nutrients by plants. If there is a high supply of P, the capture of some cations also increases; and a high concentration of P in the growths of some plants is accompanied by high concentrations of potassium, calcium, magnesium and manganese (Aziz *et al*., 2015).

Leaf/stem ratio. The leaf/stem ratio for the introduced alfalfa varieties varied between 1,11 and 1,30, with significant differences ($p < 0,05$) with regards to the ecotype flor morada (0,96), which indicates that the latter has lower quality. The alfalfa leaves are the plant components that show higher nutritional value and high consumption potential at the moment of cutting; thus, the quality of this forage can be improved with cultivars that have higher leaf quantity.

Lamb and Hans (2014) observed a higher leaf/stem ratio in June (early summer), and related it to the early regrowth age; in addition, the leaf/stem ratio was lower in the late flowering stages.

Plant height. Plant height showed highly significant differences ($p < 0,01$) between flor morada (77,97 cm of average height) and the introduced varieties with phosphate fertilization (between 50,1 and 56,27 cm). This is possible due to the fact that the ecotype flor morada is a naturalized species and has genes adapted to the climate and soil conditions of the Ecuadorian mountains. In addition, the successful establishment of alfalfa depends on the formation of effective nodules by the bacterium *Sinorhizobium meliloti*, and many nodules per plant can remain occupied by naturalized rhizobium strains present in the soil (Wigley *et al*., 2015).

Bayas (2003), when using *bokashi*, manure tea and biosol as biofertilizers, obtained heights at the pre-flowering stage of 40,60; 43,14 and 34,71 cm, respectively, lower values than the ones in this study; while Chacón (2011), when applying foliar fertilizer, recorded height values between 63,35 and 79,63 cm. Similar performance was reported by Avci *et al.* (2013) in seven alfalfa varieties, in two years of production and two localities, which reached an average height of 66 cm.

Green forage production. The green forage production of the ecotype flor morada and that of cuf-101 with 50, 100 and 150 kg/P/ha and sw-8210 with 50 and 150 kg/P/ha were significantly higher ($p < 0,01$), compared with the varieties abunda verde with 0, 50, 100 and 150 kg/P/ha, cuf-101 without fertilization and sw-8210 with 0 and 100 kg/P/ha. These results can be due to the inclusion of phosphorus in the introduced varieties, which contributes to improve the chemical characteristics of the soil and favor plant growth and development.

In a study conducted in Colombia, the green biomass production and better nutritional quality appeared in the stage of flower bud, 210 days after sowing; no significant differences were found among the alfalfa varieties for the environmental conditions of the Pamplona municipality, for which all of them (cuf-101, moapa-69, sw-8210 and sw-8718) could be introduced in this agroecosystem (Capacho *et al*., 2018).

Such results do not coincide with the ones in this study, because in the ecotype flor morada without fertilization and in the varieties sw-8210 and cuf-101 with 50, 100 and 150 kg/P/ha higher productions were recorded (between 10 and 17 t/ha per cut).

On the other hand, it should be stated that alfalfa has high phosphorus requirements; its critical level is from 18 to 20 ppm and, in turn, produces between 50 and 70 kg DM/kg P_2O_5 depending on the used quantity, nutrient content in the soil, and pasture status and age (Morón, 2000). If the sufficiency criterion is applied, which is based on the integration of the analytical result of P Bray with the required phosphorus quantity to achieve the response in dry matter production of alfalfa due to phosphorus addition, it would imply applying the minimum phosphorus quantity at sowing, so that it allows to maximize profitability (Marino and Echeverría, 2018).

Dry matter production. The dry matter production showed highly significant differences

Table 2a. Agronomic performance of three alfalfa varieties with three phosphate fertilization doses, compared with the ecotype flor morada.

Variable	Phosphorus doses, kg/ha												Flor morada	± SE	P-value
	abunda verde				cuf-101				sw-8210						
	0	50	100	150	0	50	100	150	0	50	100	150			
Emergence, %	94,00^{a}	94,33a	94,00^{a}	92,67^{a}	95,00^{a}	95,33^{a}	95,33a	95,00^{a}	95,33^{a}	93,67^{a}	95,67^{a}	93,33^{a}	90,33^{b}	0,94	0,03
Number plants/m^{2}	18,33	19,33	21,33	24,67	17,33	17,67	17,00	20,00	17,67	18,33	17,67	20,67	20,33	1,74	0,18
Number of stems	8,87	10,50	10,67	13,63	9,90	11,70	9,27	9,57	10,33	12,43	11,80	10,63	11,30	1,03	0,13
Aerial cover, %	79,62^{bcd}	78,06^{bcd}	80,46^{bcd}	83,63^{bcd}	76,37^{cd}	75,66^{cd}	77,86^{bcd}	78,86^{bd}	73,8^{d}	80,40^{bcd}	88,19^{abc}	90,38^{ab}	99,59^{a}	2,61	0,00
Basal cover, %	26,02^{b}	28,23^{ab}	28,33^{ab}	29,96^{ab}	26,59^{b}	27,18^{b}	26,82^{b}	28,96^{ab}	24,59^{b}	26,85^{b}	27,78^{b}	29,30^{ab}	34,09^{a}	1,14	0,00
Floristic composition, %	93,60	94,07	94,50	93,40	94,73	94,43	96,40	93,73	93,73	92,63	95,43	96,20	95,33	1,06	0,39

Different letters in the same row significantly differ, according to Tukey ($p < 0,05$).
SE: standard error.

Table 2b. Agronomic performance of three alfalfa varieties with three phosphate fertilization doses, compared with the ecotype flor morada.

Variable	Phosphorus dose, kg/ha												Flor morada	± SE	P-value
	abunda verde				cuf-101				sw-8210						
	0	50	100	150	0	50	100	150	0	50	100	150			
Leaf/stem ratio	1,16^{a}	1,20^{a}	1,26^{a}	1,30^{a}	1,11^{a}	1,22^{a}	1,17^{a}	1,29^{a}	1,05^{a}	1,11^{a}	1,13^{a}	1,20^{a}	0,96^{b}	0,003	0,04
Height, cm	54,80^{b}	50,17^{b}	53,77^{b}	55,63^{b}	51,53^{b}	56,27^{b}	48,80^{b}	55,63^{b}	55,63^{b}	56,13^{b}	54,27^{b}	55,63^{b}	77,97^{a}	2,24	0,00
Regrowth height, cm	6,67	6,67	5,67	6,33	6,33	6,67	8,33	6,67	5,33	7,00	7,33	8,00	5,33	0,70	0,14
GF production, t/ha/cut	7,46^{b}	8,52^{b}	7,87^{b}	9,92^{b}	8,30^{b}	10,32^{ab}	10,92^{ab}	10,32^{ab}	8,25^{b}	11,29^{ab}	9,60^{b}	10,51^{a}	17,23^{a}	1,34	0,00
DM production, t/ha/cut	2,07^{b}	2,12^{b}	1,95^{b}	2,47^{b}	2,08^{b}	2,48^{b}	2,48^{b}	2,66^{b}	1,90^{b}	2,79^{b}	2,41^{b}	2,61^{b}	4,52^{a}	0,32	0,00
Days to harvest	90,0^{b}	90,0^{b}	90,0^{b}	90,0^{b}	90,0^{b}	90,0^{b}	90,0^{b}	90,0^{b}	90,0^{b}	90,0^{b}	90,0^{b}	90,0^{b}	110,0^{a}	2,82	0,03
Number of nodules/plant	-	-	-	-	-	-	-	-	-	-	-	-	4,00	-	-

Different letters in the same row significantly differ, according to Tukey ($p < 0,05$).
SE: standard error.

($p < 0{,}01$) between the mean of the control treatment and that of the three introduced varieties with the different doses of phosphate fertilization; 4,52 t/ha per cut were obtained for alfalfa flor morada and between 2,12 and 2,79 t/ha per cut for the introduced varieties. This shows that, in the establishment stage of the alfalfa plantations, the fertilization doses did not have effect on the introduced varieties, possibly due to the lack of nodule proliferation; because this provides energy through ATP and, consequently, affects the dry matter production. On the other hand, the fibrous roots of alfalfa proliferate in the first 20 cm of soil, and are the ones that have most of the nodules which favor plant growth and development (Barnes and Scheaffer, 1995).

Dammer (2004) evaluated four varieties of this legume regarding dry matter production per hectare in the second cut, in winter, and obtained 3 869 kg DM/ha in the variety moapa 69, followed by cuf-101 (3 319 kg DM/ha) and by sw-8210 (2 393 kg DM/ha), with lower values than the ones in this study.

On the other hand, Plevich *et al.* (2012) reported a dry matter production in the second cut of 2 820 kg DM/ha. Rojas *et al.* (2017) indicated that the forage yield was contrasting with the average number of plants in each season, and that the higher forage yields during the spring and summer are due to the favorable light and temperature conditions for alfalfa growth; in this regard, in five alfalfa varieties the growth rate, intercepted radiation, leaf area index and plant height were higher in spring-summer (Rojas *et al.*, 2017).

According to Rebuffo (2005), to achieve good establishment and production special attention must be paid to the quality of the soil where alfalfa is to be planted. The soil pH is an important factor that influences the growth of this species, which affects directly the symbiotic nitrogen fixation and availability of such essential elements as potassium, phosphorus, sulfur and boron –limiting nutrients in the alfalfa production–, although other deficiencies could occur in certain soils (Barnes and Scheaffer, 1995). On the other hand, several researchers report that the environment is a factor that influences forage production and quality, for which materials should be selected which are adapted to the management, soil and climate conditions of the region.

Days to harvest. The introduced alfalfa varieties were harvested at 90 days, which significantly differed ($p < 0{,}05$) from the ecotype flor morada which was harvested at the flowering stage (110 days). The harvest of the varieties abunda verde, cuf-101 and sw-8210 was performed when the plants showed new regrowths with height between 5 and 8 cm, because there was no induction of flowering. It is stated that timely cut allows to harvest all the energy produced by alfalfa; for each day of delay after flowering, its nutritional value decreases in 1 %. Thus, for the ecotype flor morada performing the cut when 10 % of the alfalfa plantation is flowering, is recommended, and for the introduced species, when the basal regrowths are 5 cm high. The frequent cuts, before the recommended time, decrease reserves in the roots, increase the plant susceptibility to high temperatures, and leave the plants more exposed to disease attack and, consequently, to a reduction in their yield and density. Yunga *et al.* (2015) reported that regrowth depends on the reserves, which are reduced when cuts are frequent.

The variables number of plants per square meter, number of stems per plant, botanical composition and regrowth height did not show significant differences in the varieties sw-8210, abunda verde and cuf-101 with phosphate fertilization, or with regards to the control; this was due to the fact that the climate and edahic conditions and the genetics of the alfalfa plantations behaved homogeneously (tables 2a and 2b).

Conclusions

The ecotype flor morada, without phosphate fertilization, showed higher green and dry forage yield and better cover and plant height; likewise, its harvest was carried out 110 days after sowing, when the plants showed 10 % flowering.

In the varieties abunda verde, cuf-101 and sw-8210 with phosphate fertilization, the emergence percentages were higher than those of flor morada and also of higher quality, with a better leaf/stem ratio; the harvest was carried out at 90 days, when the alfalfa plantations showed higher regrowths than 5 cm.

Acknowledgements

The authors thank the Higher Polytechnic School of Chimborazo, School of Animal Husbandry Sciences, Tunshi Research Station, for the facilities provided for this research.

Bibliographic references

Agrocalidad. *Análisis químico del suelo de Tunshi*. Ecuador: Ministerio de Agricultura y Ganadería del Ecuador, Laboratorio de Análisis de Suelos y Fertilizantes, 2016.

Alaska. *Pastos de clima frío*. Importadora de semillas http://www.imporalaska.com/26-semillas_de_pastos_de_frio.html, 2015.

Avci, M.; Ozkose, A. & Tamkoc, A. Determination of yield and quality characteristics of alfalfa (*Medicago sativa* L.) varieties grown in different locations. *J. Na. Vet. Adv*. 12 (4):487-490, 2013.

Aziz, T.; Lambers, H.; Nicol, D. & Ryan, M. Mechanisms for tolerance of very high tissue phosphorus concentrations in *Ptilotus polystachyus*. *Plant Cell Environ*. 38:790-799, 2015.

Banco Central del Ecuador. *Estadísticas de comercio exterior*. Quito. http://www.portal.bce.fin.ec/vto_bueno/comercioexterior.jsp, 2014.

Barber, S. Nutrient placement. In: *Soil nutrient bioavailability. A mechanistic approach*. John Wiley & Sons, Inc. p. 368-388, 1980.

Barnes, D. & Scheaffer, C. *Alfalfa. An introduction to grassland agriculture*. 5th ed. Vol. 1, cap. 16. Ames, USA: Iowa State University Press. p. 206-211, 1995.

Bayas, A. *Té de estiércol, biol, biosol como biofertilizantes en la producción de alfalfa*. Tesis de grado. Riobamba, Ecuador, 2003.

Capacho, A.; Flórez, D. & Delgado, J. Biomasa y calidad nutricional de cuatro variedades de alfalfa para introducir en Pamplona, Colombia. *Revista Ciencia y Agricultura*. 15:61-67, 2018. DOI: http://doi.org/10.19053/01228420.v15.n1.

Chacón, D. *Evaluación de diferentes niveles de abono foliar en la produccion de forraje de Medicago sativa*. Tesis de grado. Riobamba, Ecuador, 2011.

Dammer, M. *Adaptación de cuatro variedades de alfalfa (Medicago sativa) en la zona de Cananvalle*. Tabacundo. Cayambe, Ecuador, 2004.

Di Rienzo, J. A.; Casanoves, F.; Balzarini, Mónica G.; González, Laura A.; Tablada, M. & Robledo, C. W. *Infostat. Versión 1.6*. Córdoba, Argentina: Grupo InfoStat, FCA, Universidad Nacional de Córdoba. https://www.infostat.com.ar/index.php?mod=page&id=31, 2017.

Ensiye, A.; Jamshid, R. & Morteza, Z. Effect of salt stress on the growth and accumulation of alfalfa ions (*Medicago sativa* L.). *J. Plant Nut*. 41 (7):818-831, 2018. DOI: http://doi.org/10.1080/01904167.2018.1426017.

Fan, J.; Wang, Q.; Malhi, S. & Li, E. Effect of mulch on the storage of water in the soil and its depletion by alfalfa in the Loess Plateau in northwestern China. *Agric. J. Manage. Water*. 138:10, 2014.

Harris, W. *Defoliation as determinant of the growth, persistence and composition of pasture*. (Ed. J. R. Wilson): CSIRO. p. 67-85, 1978.

Hawkins, C. & Long, X. Recent progress in alfalfa (*Medicago sativa* L.) genomics and genomic selection. *The Crop Journal*. 6:565-575, 2018.

Honghua, H.; Peng, Q.; Xia, W.; Chenbin, F.; Jiayin, P.; Hans, L. *et al.* Growth, morphological and physiological responses of alfalfa (*Medicago sativa*) to phosphorus supply in two alkaline soils. *Plant Soil*. 416:565-584, 2017. DOI: http://doi.org10.1007/s11104-017-3242-9.

INAMHI. *Estación Meteorológica Facultad de Recursos Naturales*: ESPOCH, 2017.

INEC. *Encuesta de superficie y producción agropecuaria continua ESPAC 2106. Producción agropecuaria en la provincia de Chimborazo. Ministerio de Agricultura zona III*. Quito: Instituto Nacional de Estadística y Censos, 2016.

Lamb, J.; Hans, J. & Heathcliffe, R. Growth environment, harvest management and germplasm impacts on potential ethanol and crude protein yield in alfalfa. *Biomass and Bioenergy*. 63:114-125, 2014.

Liu, D.; Liu, G. & Yang, Z. The effect of sowing and harvesting factors on the yield of hay and the proportion of stem leaves of *Medicago sativa*. *Acta Prataculturae Sinica*. 24:48-57, 2015.

Marino, M. & Echeverría, H. Diagnóstico de requerimiento de fósforo para alfalfa (*Medicago sativa* L.) en argiudoles. *Jornal Agronomy and Crop Science*. 35 (1):11-24, 2018. DOI: http://dx.doi.org/10.31047/1668.298x.v1.n35.20449.

Martins, D.; Macovei, A.; Leonetti, P.; Balestrazzi, A. & Araújo, S. La influencia de fosfato, deficiencia en leguminosas. symbiotic N_2 fijación. En: S. Sulieman y L. S. Tran, eds., 2017.

Milic, D.; Karagic, S.; Vasiljevic, A.; Mikic, B.; Milo & Katic, S. Breeding and improvement of quality traits in alfalfa (*Medicago sativa* L.). *Genetic*. 46:11-18, 2014.

Morón, A. *Alfalfa. Fertilidad de suelos y estado nutricional en sistemas agropecuarios de Uruguay*: INPOFOS, 2000.

Ohyama, T. *The role of legume-rhizobium symbiosis in sustainable agriculture. Legume nitrogen fixation in soils with low phosphorus availability: Adaptation and regulatory implication*, 2017.

Plevich, J.; Delgado, A. & Saroff, C. El cultivo de alfalfa utilizando agua de perforación, agua residual urbana y precipitaciones. *Rev. bras. eng. agrícola ambiental*. 16 (12), 2012. DOI: http://doi.org/10.1590/S1415-43662012001200013.

Raza, A.; Moghaddam; Gollner, G. J. & Friedel, F. Evaluation of crop system for studying the effect of mulching with lucerne (*Medicago sativa* L.) in Austria. *J. Plant Interactions*. 9 (1):592-598, 2014. DOI: http://doi.org.10.1080/17429145.2013.877601.

Rebuffo, M. Alfalfa: Principios de manejo del pastoreo. *Revista INIA - No 5*. 43 (1):79-92, 2005.

Rogers, A.; Lawson, S. & Chandra, K. The limited application of irrigation water does not affect the nutritional characteristics of the Anim Pinchar alfalfa. *Sci*. 54 (10):1635-1640, 2014.

Rojas, G.; Torres, S.; Joaquín, C.; Hernández, G.; Maldonado, P. & Sánchez, P. Componentes del rendimiento en variedades de alfalfa. *Agrociencia*. 51:697-708, 2017.

Sanz, S.; Morales, F.; Arrese, I. & Aranjuelo, I. Phosphorus deficiency: An important limiting factor for rhizobial symbiosis. En: S. Sulieman, ed. *Tran LS*, 2017.

Wigley, K.; Liu, W.; Khumalo, Q.; Brown, D. & Ridgway, H. Effectiveness of three inoculation methods for lucerne (*Medicago sativa* L.) in two Canterbury soils. *New Zealand J. Agric. Res.* 58 (3):292-301, 2015. DOI: http://doi.org/10.1080/00288233.2015.1028652.

Yunga, A.; Ayora, B. & Llaguarima, P. *Manejo y cuidado del cultivo de alfalfa (Medicago sativa L.).* https://issuu.com/adrianchucuri/docs/cultivar_vivero, 2015.

Received: January 16, 2019

Accepted: July 04, 2019