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Chipilín (*C. longirostrata* Hook. and Arn.) Capacity for Regrowth and Leaf Area Production in Response to Nitrogen and Phosphorus Fertilizer Application

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ABSTRACT

Background: Fertilizer application combined with successive foliage cuttings can positively affect plant growth, yield and quality. The objective of this study was to evaluate the response of chipilín to successive foliage cuttings and nitrogen and phosphate fertilizer application under greenhouse conditions.

Methods: During 2018, an experiment was carried out in a greenhouse under a completely randomized design, with six replications of each treatment. The treatments were as follows: control, 100 kg ha^{-1} N as urea and 60 kg ha^{-1} P_2O_5 as triple superphosphate. The fertilizers were applied one month after planting (MAP). Two MAPs, uniform cuttings were performed, followed by three successive cuttings, with one every 30 days. At each cutting, the traits evaluated included the number of new shoots; the length, diameter, leaf area and dry biomass of the new shoots by component (leaves and stems); the relative growth rate (RGR) of the biomass of the shoot leaves, the chlorophyll (a, b and total) and carotenoid content and NDVI.

Result: Cutting foliage every 30 days increased ($p<0.05$) the number of new shoots, leaf biomass, total biomass and leaf area, but reduced the length and diameter of the shoots. There was no effect ($p> 0.05$) of cutting on root biomass or nodulation, but there was an effect of fertilizer application, with both variables decreasing with nitrogen addition.

Key words: Fertilizer application, Leaf biomass, Native food legume, Photosynthetic pigments, Regrowth.

INTRODUCTION

Chipilín (*Crotalaria longirostrata* Hook. and Arn.) is a common species native to southeastern Mexico where its tender leaves and stems are eaten as a vegetable (Bautista-Cruz *et al*. 2011; Castro-Lara *et al*. 2014). From a nutritional viewpoint, it is considered one of the 16 most important vegetable species. Its edible leaves have high contents of calcium, iron, thiamine, riboflavin, lysine, niacin, ascorbic acid, polyphenol and flavonoid content and antioxidant activity (Jiménez-Aguilar and Grusak, 2015). This species has many other uses: it is used medicinally as a sleep inducer, in paper (Morton, 1994; Arias *et al*. 2003) and as a protein supplement in vegan bread and whole grain cookies (Palacios *et al*. 2017; Ek-Chulim *et al.* 2018). In Mexico there are few studies on the chipilín chemical and nutrient composition although this species is grown in family gardens on a small scale for home use as food (Mariaca, 2012) and there are no studies about its agronomic management. In southern Mexico, mainly in the state of Tabasco, chipilín is part of the gastronomic culture; its leaves and young sprouts are consumed in several regional dishes (Pérez *et al.* 2005; Jiménez-Aguilar y Grusak, 2015).

Since it is mainly the leaves that are consumed, agronomic improvement of chipilín should focus on increasing and improving quality of its leaf biomass production. Several studies have indicated that the shoot biomass yield of plant species can be improved by successive cuttings of the foliage to promote regrowth by eliminate apical dominance (Salemaa and Sievänen, 2002; Dun *et al*. 2006). In this respect, in studies on leaf growth in gramineous and leguminous plants, it has been observed that foliage cutting and the frequency at which it is performed affect the velocity of regrowth and its biomass yield, which decrease as the regrowth advances in age (Kabi and Bareeba, 2007; Mendoza *et al*. 2010; Giambalvo *et al*. 2011).

Among the agronomic management practices that positively affect plant growth, yield and quality is fertilizer application (Näsholm *et al*. 2009). The Fabaceae family is

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characterized by its ability to fix N_2 biologically; this ability is affected by the application of nitrogen fertilizer (Xie *et al*. 2015). Nitrogen addition reduces nodulation, the size and weight of the nodules and nitrogenase activity (Heggo and Barakah, 2004; Xie *et al*. 2015). However, in the production of commercial legumes, fertilizer application is necessary to maintain adequate yield. In beans (*Phaseolus vulgaris* L.), the application of low to moderate doses of N and P stimulates N_2 fixation and increases yield (Mitova and Stancheva, 2013). As in other legumes, fertilizer application could increase foliage biomass in chipilín.

The objective of this study was to evaluate the regrowth capacity of chipilín after successive foliage cuttings, with and without nitrogen and phosphate application.

MATERIALS AND METHODS

This experiment was conducted under greenhouse conditions in the Colegio de Postgraduados, Campus Tabasco, Tabasco, Mexico. Seeds were donated by the Intituto de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) bank of forage species, in Tabasco, Mexico. Before planting, the seeds were disinfected with 1% sodium hypochlorite for 1 min and treated with hot water to break dormancy. Planting was performed on May 14, 2018 in 5-kg containers with a soil:substrate mixture (vermiculite, 75:25) and two plants per container. The soil used in the substrate had a loam texture, pH of 7.1, 5.2% organic matter, 0.25% N, 5.9 µg mg^{-1} P and 0.25 meq 100 g^{-1} K. Three treatments were evaluated with six replications each: a control, 100 kg ha^{-1} N as urea (1.29 g per container) and 60 kg ha^{-1} P_2O_5 as triple superphosphate (0.78 g per container), which were applied one month after planting (MAP). A completely random design was applied to treatments in the greenhouse. Irrigation was performed manually to maintain hydration at field capacity. The treatments were subjected to a uniform cutting two months after planting (July 13) at the third node of the main stem. After the uniform cutting, three successive cuttings were performed, one every 30 d (August 12, September 11, October 11). At each cutting, the number of new shoots was counted (only shoots longer than 4 cm were counted) and their lengths and diameters were measured. The biomass of the new shoots was separated by component (*i.e.*, leaves and stems). Leaf area was measured with an integrator of leaf area LI-3100. The dry matter of each component was obtained after drying at 50°C for 48 h in a forced-air oven. The relative growth rate (RGR) of the biomass of the shoot leaves indicates the accumulation of leaf biomass per day. This was calculated with the biomass data of each component with the formula:

$$RGR = \frac{\ln P2 - \ln P1}{t2 - t1}$$

Where

P = leaf dry weight of the plant at any time interval.

t = time in days.

To determine the concentrations of total chlorophyll, chlorophyll a and b and carotenoids, 1 cm^2 leaf samples were taken from each treatment in triplicate prior to each cutting and placed in test tubes with 5 mL N, N-dimethylformamide. The samples were read at 470, 647 and 664 nm in a UV-VIS spectrophotometer (Thermo Scientific model *Multiskan Go*). The concentration of each photosynthetic pigment was calculated following Wellburn (1994). The normalized difference vegetation index (NDVI) was measured every 30 days (before each cutting) and in each treatment with a Green Seeker portable sensor. Parallel to the evaluations of the shoots, three intact plants per treatment were collected to count the number of nodules on the roots and to determine the total root biomass after drying in a forced-air oven. The data obtained were subjected to an analysis of variance under a factorial array, considering the effect of cutting (1, 2 and 3) and of the treatment (control and N and P fertilizer application). The means were calculated and compared with a Tukey test by InfoStat software.

RESULTS AND DISCUSSION

Regrowth production

The successive cuttings of chipilín foliage every 30 days increased the number of shoots and shoot and leaf biomass, but they reduced the length and diameter of the new shoots, indicating a significant effect of cutting at this time interval. There was no effect of fertilizer application or of the interaction between cutting and fertilizer application on the plant traits evaluated, except for shoot diameter (Table 1). The highest average number of new shoots (7.5 per plant) was observed in the third cutting; this regrowth increased the average total biomass of the new shoots (3.16 g per plant). Although the new shoots were shorter and had smaller diameters, the leaf area was larger (1224.6 cm^2 per plant). The effect of nitrogen application on shoot diameter (Fig 1) is reflected in a decrease of this trait. The ability to promote chipilín regrowth after a cutting of growing shoots seems to be the result of interrupting apical dominance, which stimulates the growth of the lateral meristems of the stem and forces the plant to complete its life cycle by generating multiple sites for seed production (Dun *et al*. 2006). In chipilín, forcing a plant to develop lateral meristems through successive cuttings seems to be very costly and the plant must modulate the growth and size of growing vegetative structures; this was the case in our study, in which the length and diameter of the regrowth decreased even though the plant received 100 kg N ha^{-1}.

The daily rate of leaf biomass accumulation of the new shoots per plant was affected by the cuttings (Fig 2). Between cuttings 1 and 2 (August to September) in the control treatment, there was no increase in leaf biomass, but the application of phosphorus and nitrogen maintained the growth of this trait. The lack of leaf biomass accumulation in the control treatment may have been due to an attack by *Trialeurodes vaporariorum*, which affected the leaves of the plants in this treatment. Between cuttings 2 and 3 (September to October) the effect of nitrogen was observed.

Table 1: Number of new shoots, leaf area and shoot biomass per chipilín plant subjected to successive foliage cuttings.

Number of cuttings	Number of shoots plant^{-1}	Shoot length, cm	Shoot diameter, mm	Dry matter of the regrowth's stem plant^{-1}, g	Dry matter of the regrowth's leaf plant^{-1}, g	Dry matter of total regrowth plant^{-1}, g	Leaf:stem ratio	Regrowth leaf area plant^{-1}, cm^2
1	2.72 b[†]	29.71 a	2.28 a	0.31 b	0.56 b	0.87 b	1.81 b	380.6 b
2	4.35 b	15.62 b	1.51 b	0.22 b	0.50 b	0.72 b	2.35 a	325.9 b
3	7.56 a	13.42 b	1.28 b	0.98 a	2.18 a	3.16 a	2.34 a	1224.6 a
HLSD	1.71	3.90	0.24	0.19	0.30	0.49	0.27	253.5
ANOVA Effect								
Cutting (C)	***	***	***	**	**	**	***	*
Fertilizer application (FA)	ns	ns	**	ns	ns	ns	ns	ns
C × FA	ns	ns	ns	ns	ns	ns	*	ns

[†]Different letters in a column indicate significant differences between cuttings (*P*≤*0.05*). HLSD: Honest least significant difference; ***, **, * and ns denote significance at 0.001, 0.01, 0.05 and not significant (P>0.05), respectively.

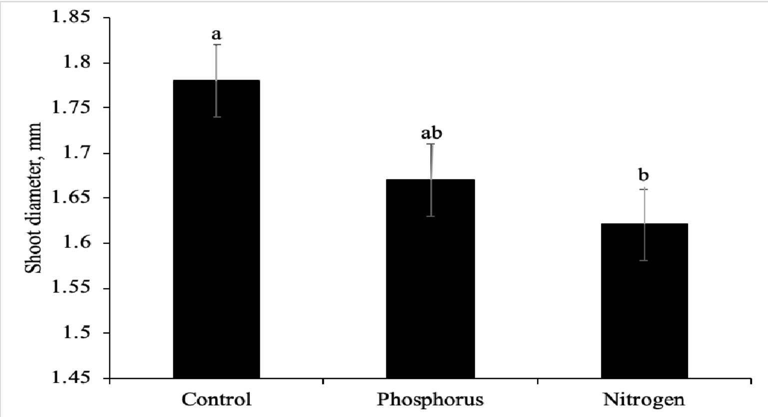

Fig 1: Average diameter of the regrowth's stem after application of successive cuttings to foliage of *C. longirostrata*, with and without fertilizer application. Different letters indicate significant differences between treatments (*P*≤*0.05*). Bars indicate the standard error.

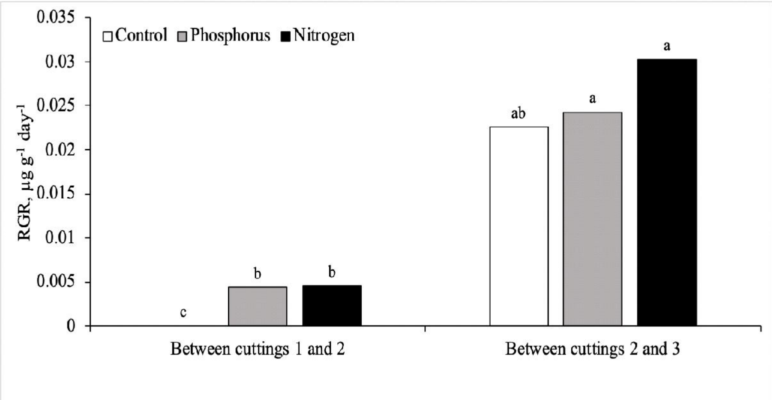

Fig 2: Relative growth rate (RGR) of leaf regrowth biomass by effect of fertilizer application in *C. longirostrata*. Different letters indicate significant differences between treatments (*P*≤*0.05*).

Nitrogen application promoted a higher rate of leaf biomass accumulation than that in the control; however, nitrogen application did not have a significant effect on the accumulation of total regrowth biomass per plant, as was observed in chickpea by Namvar *et al.* (2011).

Photosynthetic pigments and NDVI

Successive cuttings did not affect the concentrations of chlorophylls (Table 2), but they did affect NDVI and the concentrations of carotenoids, which are a source of provitamin A and have antioxidant activity and photoprotective characteristics (Jáuregui *et al*. 2011). Fertilizer application did not have a significant effect on the content of photosynthetic pigments, contrary to observations in other legumes such as *Vigna mungo* (Kulsum *et al.* 2007).

Table 2: Concentration of photosynthetic pigments and reflectance index of the canopy (NDVI) in chipilín leaves subjected to successive cutting of foliage.

Number of cuttings	Chlorophyll a	Chlorophyll b	Chlorophyll total	Carotenoids	Ratio a/b	NDVI
	µg mL^{-1}					
1	4.02 a[†]	1.22 a	5.24 a	0.86 a	3.29 a	0.83 b
2	4.01 a	1.31 a	5.32 a	0.90 a	3.06 a	0.86 a
3	4.27 a	1.43 a	5.70 a	0.59 b	2.98 a	0.87 a
HLSD	1.14	0.42	1.29	0.24	1.86	0.02
ANOVA Effect						
Cutting (C)	ns	ns	ns	**	ns	**
Fertilizer application (FA)	ns	ns	ns	ns	ns	**
CxFA	ns	ns	ns	ns	ns	ns

[†]Different letters in a column indicate significant differences between cuttings (*P≤0.05*). HLSD: Honest least significant difference; ***, **, * and ns denote significance at 0.001, 0.01, 0.05 and not significant (P>0.05), respectively.

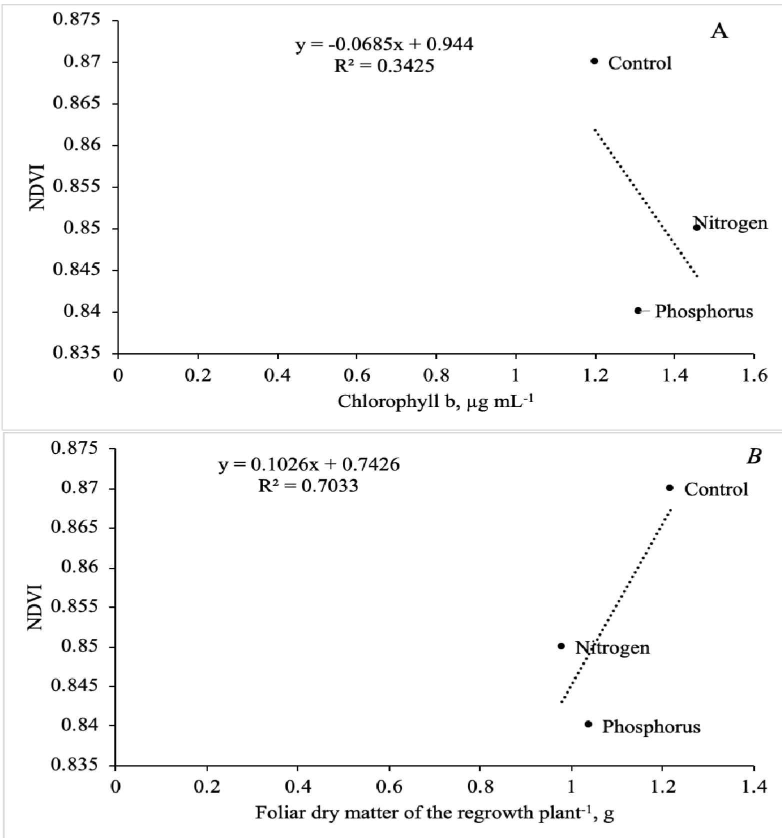

Fig 3: Relationship of NDVI, chlorophyll b **(A)** and foliar dry biomass of the regrowths per plant **(B)** in *C. longirostrata*.

NDVI is a widely used vegetation index to evaluate phenotypic and photosynthetic characteristics in crops (Walter *et al*. 2015). This index has been shown to correlate highly with canopy biomass, the leaf area index at specific phenological stages and the yield of legumes, such as beans (Monteiro *et al*. 2012). A strong relationship was also observed between NDVI and the regrowth biomass in the treatments applied (R^2=0.70). The relationship observed between NDVI and the photosynthetic pigments was weak (Fig 3), probably due to a lack of response to the applied N fertilizer treatment, since the content of chlorophyll a is determined by the availability of N and the reflectance of the leaves in the visible spectrum depends mainly on the concentrations of photosynthetic pigments (Gamon *et al*. 2015).

Root biomass and nodulation

The analysis of the data revealed an effect of fertilizer application on root biomass, while nodulation was affected by fertilizer application, cuttings and the interaction between

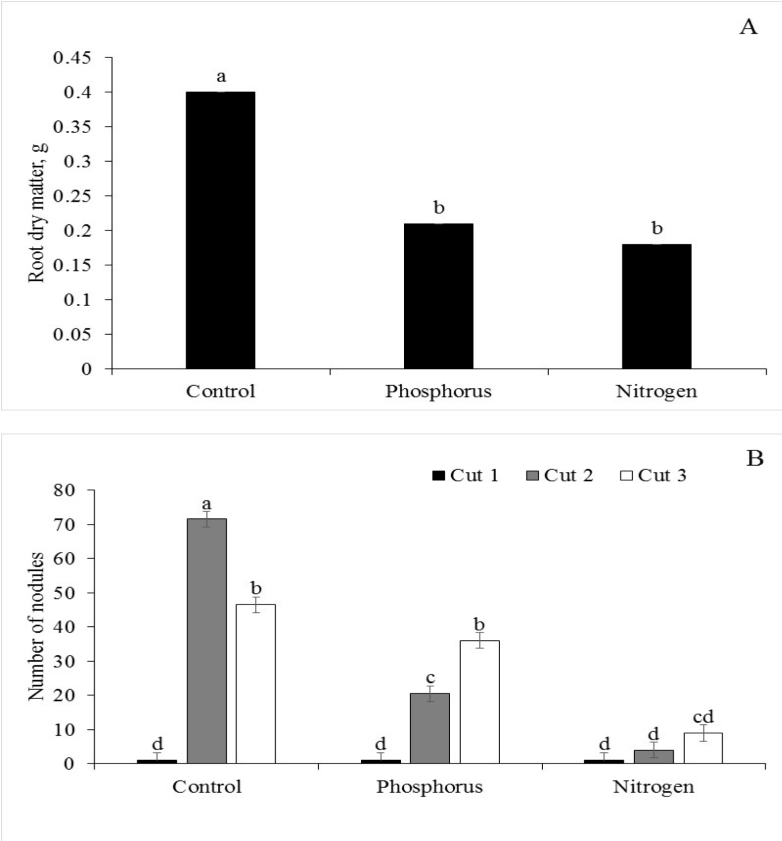

Fig 4: Effect of fertilizer application on root biomass **(A)** and of fertilizer application and cutting on number of nodules **(B)** of *C. longirostrata*. Bars indicate the standard error. Different letters indicate significant differences between treatments (*P≤0.05*).

fertilizer application and cuttings (Fig 4). Root biomass measured at the three cuttings, decreased with nitrogen application (0.18 g) relative to the control (0.40 g). Nodulation was affected in the same way as root biomass, with higher values in the control treatment (39.3 nodules per plant on average) than in the treatment with phosphate fertilizer application (18.3 nodules per plant on average) and a significant decreasing was observed in the treatment with nitrogen fertilizer (4.3 nodules per plant on average) as of the second cutting. Other authors (Heggo and Barakah, 2004; Xie *et al*. 2015) have also documented the inhibition of nodulation by nitrogen addition. However, in *Crotalaria juncea* and *Phaseolus vulgaris* the application of mineral N at low or moderate doses at the beginning of the crop cycle has a synergetic effect on N_2 fixation, which leads to better nodulation and dry matter accumulation (Mendonça and Schiavinato, 2005; Mitova and Stancheva, 2013). This effect was not observed in our study with chipilín at the applied dose; it is likely that the fertilizer added to the N content of the substrate, resulting in a high content of available mineral N that inhibited biological fixation of N_2 (Liu *et al.* 2011). Moreover, the decrease in root biomass, together with inhibition of nodulation with nitrogen application, such as that observed in alfalfa (Xie *et al*. 2015), could have resulted in a smaller root area for absorption in the treatments with fertilizer application and thus increases were not observed in either the photosynthetic pigments or leaf biomass yield.

CONCLUSION

The high plant biodiversity present in the southeastern region of Mexico has been used by the population to diversity and enrich their diet. Chipilín, a plant used widely in the regional gastronomic culture, is gathered from wild plants or growth in family gardens. However, changes in demographic patterns and climate that affect plant diversity in the region can lead to the loss of regional gastronomic heritage. An alternative to the a small-scale controlled production of this plant is its management through cuttings. The application of up to three successive cuttings of chipilín foliage increases regrowth, leaf biomass and leaf area, but it reduces the length and diameter of the regrowth and concentration of carotenoids in leaves. There were no significant differences between the fertilizer doses applied in the production of regrowth or in the concentration of photosynthetic pigments, but differences were found in the root biomass and nodulation, which decreased with nitrogen application. More studies on cutting frequency, fertilizer application and the concentrations of nutritional compounds and secondary metabolites are necessary to improve the production and quality of chipilín leaf biomass.

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