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Nutrient dynamic in cocoa leaves under different nitrogen sources: a reference tool for foliar analysis

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Abstract: Cocoa is a crop in increasing demand and cultivated worldwide. However, basic information concerning the movement of nutrients in leaves over time is still unknown, and methods to find an optimal time to collect a sample are still ambiguous. The present work focused on describing the movement of foliar nutrients (N, P, K, Ca and Mg) in productive 5-year-old cocoa clone CCN51 plants at the same dose of 114.8 kg ha^{-1} under different sources of nitrogen fertilization (Urea, calcium nitrate, ammonium sulfate and a control without application). Samples were taken from the time the leaf reached 70% of its total expansion until 10 months of age. The results indicated that the contents of N, Ca and Mg increased as the leaf grew, remained stable between 116 and 158 days of shoot emergence (DSE) and then decreased at the beginning of the leaf senescence period. While the K and P contents decrease from the beginning of the trial until 158 DSE where they are stable until the final stage of leaf life. Around 110 to 120 DSE, the leaves of cocoa CCN51 show a more stable nutritional content, a period in which samples can be collected for leaf analysis.

Index terms: nitrogen fertilization, nutrient absorption, sampling, cocoa leaves, nutrition.

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Dinâmica nutricional em folhas de cacau sob diferentes fontes de nitrogênio: um instrumento de referência para a análise foliar

Resumo: O cacau é uma cultura em demanda crescente que é cultivada em todo o mundo. No entanto, informações sobre os mecanismos de transporte de nutrientes nas folhas, em função do tempo, ainda continuam desconhecidas, e os métodos para encontrar um período ótimo para a amostragem permanecem ambíguos. O presente trabalho teve como objetivo descrever o transporte de nutrientes foliares (N, P, K, Ca e Mg) em plantas produtivas de 5 anos de cacau (clone CCN51), na mesma dose de 114,8 kg ha^{-1}, sob diferentes fontes de fertilização nitrogenada (Ureia, Nitrato de Cálcio, Sulfato de Amônio e um controle negativo sem tratamento). A amostragem foi efetuada quando a folha alcançou 70% de sua expansão total até aos 10 meses de idade. Os resultados revelaram que os conteúdos de N, Ca e Mg aumentaram à medida que a folha crescia, mantendo-se estável entre 116 e 158 DSE e diminuindo no início do período de senescência da folha. Entretanto, os conteúdos de K e P foram diminuindo desde o início do ensaio até 158 DSE, onde se mantiveram estáveis até à fase final da vida da folha. Nos valores entre 110 e 120 DSEs, as folhas de cacau CCN51 mostraram um conteúdo nutricional mais estável, período em que as amostras podem ser recolhidas para análise foliar.

Termos para indexação: fertilização com nitrogênio, absorção de nutrientes, amostragem, folhas de cacau, nutrição.

Introduction

Cocoa (*Theobroma cacao* L.) is a major cash crop in many tropical countries (LÓPEZ et al., 2019). In Ecuador, cocoa is one of the main export products, with a planted area of 601,954 ha, annual production of 283,680 t and a yield of 0.63 t ha^{-1} (MÁRQUEZ; CUICHÁN, 2021), currently being the first producer in Latin America and the fifth in the world ranking of cocoa producing countries, contributing 5% of production (FAOSTAT, 2021; HERRERA et al., 2022).

Nutrients are necessary for the plant in different quantities, so their deficit or excess can affect the physiological functioning of the plant. Nutrients are generally taken from the soil, but also by the leaves and other organs (SORIA, 2008). The content of a mineral element in the leaf is a measure of the actual nutrient uptake of the plant, and can show a strong correlation with yields (FOSTER; PRABOWO, 2002).

Due to the influence of environmental conditions and aging, some nutrient concentrations increase while others decrease under similar conditions, depending on their mobility in the plant and the effects of dilution; limiting the use of foliar analysis for diagnostic purposes (WALWORTH; SUMNER, 1987). Therefore, leaf nutrient content depends on leaf age, new leaf development, fruiting, light intensity and seasonal effects. Standardized sampling techniques for each crop and experienced personnel are required, since the leaves sampled must be of the same age to derive nutrient standards (VAN VLIET; GILLER, 2017).

In addition, Wessel (1971) suggested standards for determining N and P content of cocoa leaves at different ages and times of the year. The young leaves sampled were the second and third fully green leaves of the last shoot below the apex of the fan-shaped branches; the older leaves were those directly adjacent to these.

N is an essential nutrient for all plants and is a key participant in plant metabolism, and a prime constituent of amino acids, proteins, enzymes, nucleic acids, chlorophylls and hormones. For this reason, nitrogen deficiency rapidly inhibits plant growth (TAIZ et al., 2016). When N fertilizers are applied to the soil can follow different ways like, rapidly taken by the cacao trees or else leached and/or volatilized (DOGBATSE et al., 2021). In addition, the chemical form of N taken up, whether inorganic (such as nitrate) or organic (such as amino acids), may significantly influence plant shoot and root growth, and N use efficiency (FRANKLIN et al., 2017). N 'drives' uptake of P, K, S and possibly other nutrients; for this reason, the reliable interpretation of sufficiency levels of these nutrients in the leaf is possible only where N concentrations are non-limiting (MILES, 2010).

A main problem with using cocoa leaf analysis is that cocoa leaf nutrient content depends on many factors, like: leaf age, the development of new leaves, fruit bearing, light intensity, and seasonal effects (VAN VLIET; GILLER, 2017). Consequently, knowledge about the movement and evolution of nutrients content during leaf development using different N sources is essential to understand processes and changes at the physiological level on the nutritional status of source tissues to improve fertilizer use, management and cocoa production. The aim of this study was to evaluate the nutritional dynamics at the leaf level of cocoa (*Theobroma cacao* L.) clone CCN 51 under the effect of different nitrogen sources in fertilization.

Material and Methods

The study was carried out in Piuntza sector, Zamora Chinchipe province in the southeast of Ecuador, at a latitude of 3º52`27.89`` S and a longitude of 78º53`8.87`` W. One of the main cocoa-producing areas and considered the possible center of origin of cocoa worldwide (MOTAMAYOR et al., 2002). The climatic classification of the study site is Af (equatorial or humid tropical climate) according to Köppen-Geiger (RUBEL; KOTTEK, 2010), at an altitude of 849 meters above sea level, with an average temperature of 22.4 ºC and annual rainfall of 1918 mm. The experiments were carried out in a 5-year-old cocoa crop, clone CCN51 in production. Forty trees were selected, homogenized in age, number of main branches, plant height, phenological stage and canopy cover. A completely randomized design (CRD) was used, with 4 treatments and 10 replications, with the following treatments: T1 Control without Nitrogen, T2: Urea as Ammoniacal source, T3: Calcium nitrate as Nitric source and T4: Ammonium sulfate as Ammoniacal source.

To avoid limitations or masking of the effect of the N sources, an initial soil analysis was performed (Table 1), in which fertility was evaluated and a base fertilization of the elements P, K, Ca, and B was applied, with the exception of N and S (no sulfur deficiency), prior to the application of treatments. In addition, 1 kg plant^{-1} of lime in the form of Ca $(OH)_2$ was applied to raise the pH to 6.99 and promote the dynamic equilibrium of the elements in the soil. For treatments, using cocoa nutrient extraction information (FURCAL-BERIGUETE, 2017) and soil nutrients based on soil analysis (Table 1), a dose of 114.8 kg ha^{-1} of N was established, divided monthly during nine months (November 2018 to August 2019).

During nine months, leaf samples were taken at 60, 88, 116, 158, 199, and 275 days after shoot emergence for nutrient content analysis. For this purpose, 10 or 15 leaves per repetition were labeled and collected from non-productive shoots of the last season, from the

middle third of the canopy following a standard protocol for foliar analysis as suggested by De Mello and Rozane (2020). The minerals analyzed were: N, P, K, Ca, and Mg, where N was analyzed by the Kjeldahl method, P by colorimetry, and the elements K, Ca, and Mg by atomic absorption were carried out according to the methodology of the Association of Official Analytical Chemistry – AC (2016). Analyses carried out at the soil and water management laboratory of the INIAP Santa Catalina Experimental Station.

Table 1. Soil chemical parameters prior to base fertilization and treatment application.

Parameter	Quantity	Parameter	Quantity
MO (%)	4.00	Cu (µg/ml)	6.50
pH	4.70	Fe (µg/ml)	711.00
N-NH4 (µg/ml)	47.00	Mn (µg/ml)	34.00
P (µg/ml)	22.00	B (µg/ml)	0.50
K (µg/ml)	145.00	CIC (meq/100g)	112.00
Ca (µg/ml)	494.00	Σ Bases	4.45
Mg (µg/ml)	195.00	Ca/Mg	1.54
S (µg/ml)	16.00	Mg/K	4.32
Zn (µg/ml)	4.10	(Ca+Mg)/K	10.96

Results and Discussion

Leaf nutritional dynamics (Figure 1) shows an increase in N content ($\bar{x}$=2.19%) at 116 days of shoot emergence (DSE) that then gradually decreases until the end of the observation as mentioned by Santana and Igue (1979), in mature leaves total N levels decrease during new shoot development. Significant statistical differences and higher N contents are presented: at 88 DSE with the control and at 116 DSE with the application of N treatments. The curves of the ammonium sulfate and urea treatments showed stable concentrations during the evaluation period, while the curves with calcium nitrate and control showed greater variations.

Leaf nitrogen content between 88 to 199 DSE present values higher than 2 % that are considered a normal concentration in cocoa leaves according to Van Vliet and Giller (2017), an indicating an increase of N as leaf grew and photoassimilate production increased.

N is a structural element of chlorophyll, where 75% of leaf N is directed to the chloroplast, consequently affecting leaf greenness and chlorophyll accumulation (Yamashita et al., 2020). So, during leaf growth the process of cell division and expansion is continuous, requiring an accumulation of N in the chloroplasts, resulting in a constant increase in leaf N content until the leaf stops growing and the senescence stage begins.

It is important to remember that, when an adequate supply of N is provided, vigorous vegetative growth and intense green color are observed; conversely, excessive amounts can prolong the growing season and delay maturity (TISDALE; NELSON, 1970). Then, if inadequate soil N supply, N from the leaves is mobilized to younger plant organs (BALTA et al., 2015). In the case of rice, N deficiency hinders the synthesis of chlorophyll and proteins, thus reducing photosynthesis and affecting dry matter production (WANG et al., 2020).

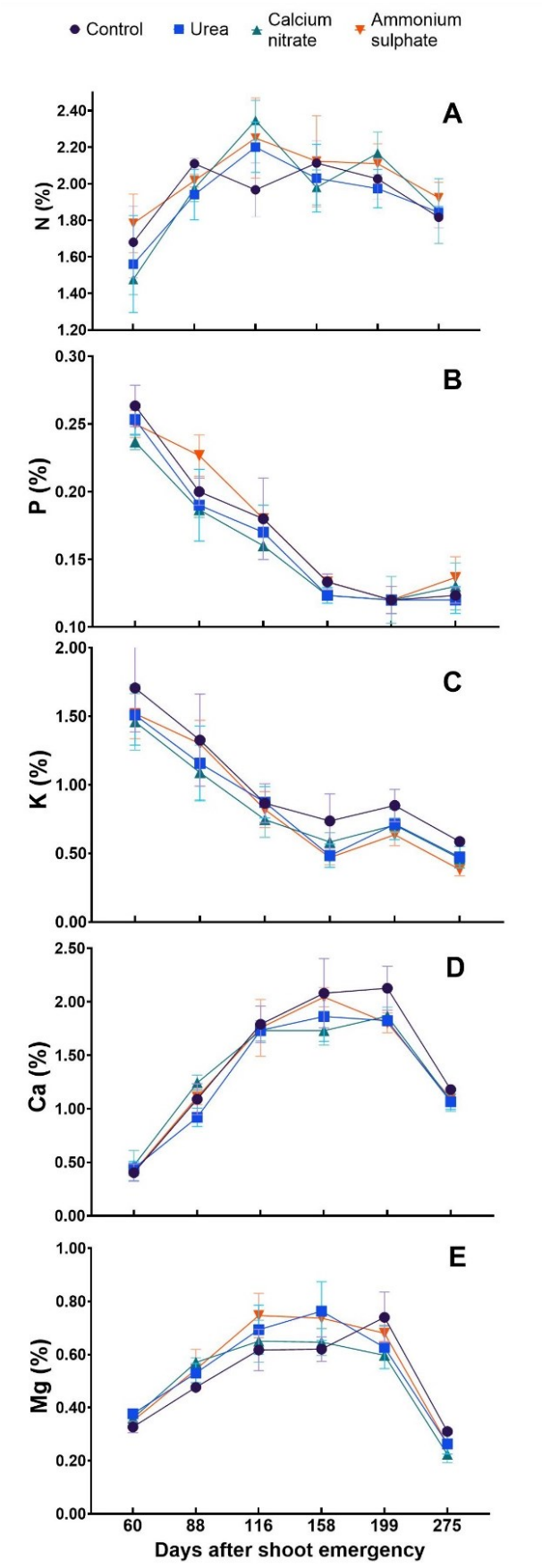

Figure 1. Macronutrients at leaf level in cocoa CCN51 under application of different N sources. A) Nitrogen; B) Phosphorus; C) Potassium; D) Calcium, and E) Magnesium. Error bars are ± S.E.

When analyzing the concentration of P and K, a decrease was observed as the leaves aged. This is probably due to the fact that the developing fruits serve as sinks for K and P, which mobilize these nutrients from the leaves (KANT; KAFKAFI, 2002). Significant statistical differences were detected at 88 DSE and a higher P content with the use of ammonium sulfate. Meanwhile, the presence of urea at 116 DSE and the absence of N sources (control) at 275 DSE revealed higher K concentrations (Figure 1). The values reported by Hosseini et al. (2017) for foliar P concentrations range from 0.10 to 0.15 %, similar to those found from 116 to 275 DSE. In the case of K concentrations reported by Hosseini et al. (2017) between 1.64 and 1.96 %, are higher than those presented in the present investigation.

P is a major component of nucleic acids, phospholipid membranes and phosphorylated compounds, 22-26% of total leaf P is required for membranes and other cellular structures, suggesting a basic P cost for leaf "infrastructure". Another percentage of leaf P is used in metabolism, where it is rapidly cycled (CROUS; ELLSWORTH, 2020). Therefore, as it is easily cycled the amount of P remaining in the leaf is low, as shown by leaf analysis, in addition to the decrease in P content as the leaf grows.

Low P content severely limits leaf growth, due to reduction of compounds such as adenosine triphosphate (ATP) that photosynthetic limitations for carbohydrate production (TIMLIN et al., 2017).

K is important in plant metabolism because it favors the synthesis of lipids, carbohydrates and proteins; it also regulates stomatal aperture, reduces water loss and optimizes plant photosynthesis processes (DE

MELO et al., 2021). K is necessary for assimilate translocation from leaves to fruits and other sinks (PATRICK et al., 2001). As the leaf grows its photosynthetic activity decreases, therefore the amount of K required to transport photoassimilates will be less, reflecting a low foliar K content. Due to K is required for sucrose loading in the phloem (ARIAS et al., 2018). A minimum concentration of tissue K is therefore essential to support metabolic processes, and uptake and transport mechanisms, and to maintain cellular turgidity (SINGH; REDDY, 2017)

In the case of Ca and Mg, their concentrations increased until 158 DSE, and then began to decrease until the end of the observation (Figure 2). Significant statistical differences were observed at 88 DSE when the presence of urea revealed the lowest Ca concentrations and at 199 DSE the absence of N sources (control) showed a higher Ca content. In the case of Mg, the presence of urea at 60 DSE and the control at 275 DSE reveals an increase in its content (Figure 1).

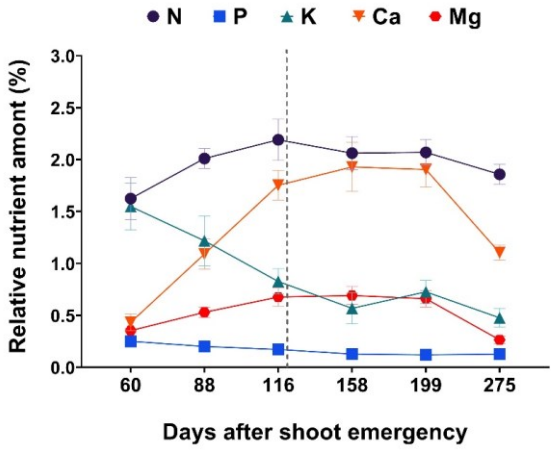

Figure 2. General movement of elements at the leaf level in CCN51 cocoa in days after shoot emergence. Dashed line indicates the ideal initial time to take samples for leaf analysis, approximately at the fourth month after shoot emergence. Error bars are ± S.E.

Ca exhibits a dual function, both as a structural component of cell walls and membranes and as intracellular second messenger. Thus, Ca concentration in the cytosol is found low level and increased to generate a signal when the plant required (THOR, 2019). This indicates that when the leaf arrives at the senescence stage, the leaf Ca content will be higher than at the beginning of leaf growth, due to its accumulation in the cell walls and a small portion in the cytosol.

The calcium content in the leaf increased with leaf growth from 116 to 199 DSE, showing values higher than those reported by Hosseini et al. (2017) between 1.70 and 1.43 %, and then the content decreased to 1.10 % until the end of the observation.

During the light-dependent reactions and the Calvin-cycle stages of photosynthesis, Mg is involved in three key biochemical processes: 1) in the light-dependent reaction, the chlorophyll molecule is composed of a central Mg ion surrounded by a group of atoms, 2) Mg can also promote the synthesis of adenosine triphosphate (ATP), 3) Mg is a cofactor and allosteric modulator for enzymes, and regulates the Calvin cycle by activating many enzymes (WANG et al., 2018). So, when the leaf begins to grow and increase its photosynthetic activity, the leaf Mg content increases as it is required in the photosynthetic process; when the senescence stage begins, the content of this element decreases.

In addition, the Mg content from 116 to 199 DSE was greater than 0.60 %; this these values are higher than those reported by Hosseini et al. (2017) where the concentrations were between 0.57 and 0.37 %.

It is important to take into account that the nutritional demand of the leaves changes

during their life cycle, having a close relationship with the rhythm and characteristics of vegetative growth and the phenological state. Because the longevity of the leaves is determined by the physiological state of the plants at the time of production (VINICIO, 2002). Not only the demand for nutrients determines the movement of elements in the plant, but also the absorption capacity of the plant (FAGERIA et al., 2009).

The movement of nutrients at leaf level expressed as a percentage of dry weight is equal to the mobility reported by Van Vliet and Giller (2017), where N and Ca increase with age, while P and K are more or less constant with decreasing concentration. Such movement may be due to retranslocation of nutrients to other sink organs as they are required, as well as the age of the leaf due to the fact that during leaf senescence there is a synthesis of proteolytic enzymes that hydrolyze nutrients, inducing the retranslocation of breakdown products, to storage tissues (RALHAN; SINGH, 1987; COVELO; GALLARDO, 2002). In the case of P it is potentially more subtle to analyses chemically than N, as it is commonly maintained at a concentration of about an order of magnitude lower in plants compared to leaf N (CROUS; ELLSWORTH, 2020).

It is important to remember that as mentioned by Wessel (1971), foliar analysis is mainly a useful tool to detect and identify pronounced nutrient deficiencies; it is difficult to use foliar analysis to establish a quantitative fertilizer recommendation without considering soil analysis.

Conclusions

Results indicate that leaf nutrient movement of N, P, K, Ca and Mg, did not show major differences when subjected to different sources of nitrogen nutrition or in the absence of fertilization over time, indicating that the plant regulates its own internal processes of nutrient uptake and mobilization in the leaf.

There is a great variation in nutritional content over time depending mainly on the age of the leaf regardless of the N fertilization supplied, so collecting samples for foliar analysis too early or too late may result in misinterpretations of results.

In this situation, it is possible to determine a period in which elements N, P, K, Ca and Mg, depending on their age, are more stable in their contents being an ideal moment to take a sample for foliar analysis, which is located around the fourth month after the sprout emission.

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