Effect of fertilization on the contents of macronutrients and chlorine in tobacco leaves cv. flue-cured (*Nicotiana tabacum* L.) in two municipalities in Huila, Colombia

Efecto de fertilización sobre el contenido de macronutrientes y cloro en hojas de tabaco (*Nicotiana tabacum* L.) cv. Virginia en dos municipios del Huila, Colombia

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ABSTRACT

In Colombia, there is little information on tobacco crops and the effect of fertilization on the nutrient content of leaves. The aim of this study was to determine the contents of mineral nutrients in tobacco (*Nicotiana tabacum* L., cv. flue-cured) plants subjected to different fertilizer rates and sources in two municipalities in the Huila Province (Colombia). Six foliar analyses, with three measurements from vegetative growth to flowering and three measurements during harvest, were carried out. Seven treatments, including six fertilization alternatives (three doses and two alternative sources of application) and a commercial control, were evaluated. The contents of the macronutrients N, P, K, Ca, Mg, S, and chlorine in the leaves were evaluated. The leaf analyses performed from vegetative growth to flowering showed differences in the foliar contents of nitrogen, calcium, magnesium, and chlorine between the two municipalities and over time, with contents that increased. During this stage, the elements with higher accumulations in the plants included N, K, and Ca. At harvest, the contents of potassium, magnesium, and calcium decreased over time. For nitrogen, sulfur and chlorine, differences were found in the time-municipality interaction.

Key words: nitrogen, phosphorus, potassium, leaf analysis, Solanaceae.

RESUMEN

En Colombia existe poca información del cultivo de tabaco y del efecto de la fertilización en el contenido de nutrientes minerales en hoja. El objetivo del presente trabajo fue determinar el contenido de nutrientes del tabaco cv. Virginia (*Nicotiana tabacum* L.) durante el ciclo de cultivo bajo diferentes dosis y fuentes de fertilización, en dos municipios del departamento Huila (Colombia). Se evaluaron siete tratamientos correspondientes a seis alternativas de fertilización (tres dosis y dos alternativas de fuentes de aplicación) y un testigo comercial, para conocer el contenido de nutrientes en hojas, se realizaron seis análisis foliares, tres durante crecimiento vegetativo hasta floración y tres durante la cosecha. Los macronutrientes evaluados en hojas fueron N, P, K, Ca, Mg, S y cloro. De acuerdo a los resultados de los análisis foliares realizados en durante crecimiento vegetativo hasta floración, se encuentran diferencias en el contenido de nitrógeno, calcio, magnesio y cloro en los dos municipios y a través del tiempo, presentándose aumento en los contenidos. Durante esta etapa los elementos que presentan mayor acumulación en las plantas fueron N, K y Ca. Durante la cosecha el contenido de potasio, magnesio y calcio disminuyó a través del tiempo. Para nitrógeno, azufre y cloro se presentaron diferencias en la interacción tiempo-municipio.

Palabras clave: nitrógeno, fósforo, potasio, análisis foliar, Solanaceae.

Introduction

Around the world, cultivated tobacco occupies about 3.9 million ha, of which 60% is 'flue-cured' tobacco, 13% is Burley tobacco, and 12% is Oriental (Rojo, 2008). The mineral nutrient requirements of different tobacco types vary according to their specific characteristics, resulting in the need to formulate different fertilization programs in order to maximize the yield, quality, and profitability.

Nitrogen is the element that has the highest effect on the growth and quality of 'flue-cured' tobacco (Parker, 2009; Smith, 2009). Nitrogen determines the performance of leaf blade, the qualities and taste of aroma, and the smoke taste (Marambe and Sangakkara, 1988; Lu *et al.*, 2005; Marchetti *et al.*, 2006; Smith, 2009). Excessive or improper applications of N may affect the yield and quality of 'flue-cured' tobacco (Marchetti *et al.*, 2006; Reed *et al.*, 2011). Excess nitrogen produces strong and spicy flavors, which are not associated with high nicotine contents. For the cultivation of tobacco, recommendations for this element vary according to different authors, between 90 and 100 kg ha^{-1} (Rojo, 2008), 67 and 78 kg ha^{-1} (Reed *et al.*, 2011), 56 and 90 kg

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ha^{-1} (Smith, 2009), or 181 kg ha^{-1} (Ballari, 2005) for a yield of 3,200 kg ha^{-1}.

Phosphorus is essential in the early stages of plant development and the requirements in 'flue-cured' tobacco are generally lower than those for nitrogen and potassium. This mineral element influences root development, plant growth, and the quality of the first leaves and improves the color and quality of leaves; moderate applications accelerate growth and maturity. An excess of phosphorus reduces quality, causes drying and results in rough, irregular leaves, producing black ash instead of white ash. The P content in leaves is positively related to the sugar content, a quality parameter (Ballari; 2005; Smith, 2009). The recommendations for phosphorus (P_2O_5) in tobacco vary according to the authors: between 60-70 kg ha^{-1} (Rojo, 2008), 44 kg ha^{-1} (Reed *et al*., 2011), 17 kg ha^{-1} (Smith, 2009), or 23 kg ha^{-1} (Ballari, 2005) for a yield of 3,200 kg ha^{-1}.

Potassium is a major mineral element necessary for the growth and quality of tobacco leaves (color, texture, sugar content, nicotine, and combustibility) (Ballari, 2005; Gurumurthy and Vageesh, 2007; Yang *et al*., 2007). High concentrations of K (>25 g kg^{-1}) in cured leaves improve the quality of leaves by increasing the burning rate and heat retention capacity (Miner and Tucker, 1990) and low concentrations of K (<2%) in leaves reduce the quality of the tobacco (Zhao *et al*., 2010). Concentrations of K higher than 2% dry weight are usually essential to producing upper leaves (Zhao *et al*., 2010). Recommendations for potassium (K_2O) for cultivation vary according to the authors: 160-180 kg ha^{-1} Rojo (2008), 110-195 kg ha^{-1} (Reed *et al*., 2011), 100 kg ha^{-1} (Smith, 2009) or 250 kg ha^{-1} (Ballari, 2005) for a yield of 3,200 kg ha^{-1}.

Magnesium is an essential component of chlorophyll and plays an important role in plant development (Pinkerton, 1972; Smith, 2009). In tobacco, an increased magnesium content in a leaf (up to 2%) enhances the combustibility and appearance of ash (color and texture), resulting in porous, loose, and light-colored ash. A magnesium deficiency reduces leaf quality, produces dark, cured leaves with uneven coloration and reduces the sugar content along with increases in the amount of ash (Pinkerton, 1972; Rojo, 2008; Smith, 2009). The K:Mg ratio in soil is a useful indicator of the magnesium supply for plants and, for tobacco, its value ranges from 4:1 to 7:1 (Pinkerton, 1972; Smith, 2009). The requirement for MgO in tobacco, according to various authors, is 10-12 kg ha^{-1} (Rojo, 2008), 33 kg ha^{-1} (Reed *et al*., 2011), 16-20 kg ha^{-1} (Smith, 2009), or 31 kg ha^{-1} (Ballari, 2005) for a yield of 3,200 kg ha^{-1}.

Calcium is a mineral element with a much higher demand by tobacco plants than potassium and its content in a cured leaf can be between 1.5 and 2.0% (López-Lefebre *et al*., 2001). The requirement for CaO is 20-24 kg ha^{-1} (Rojo, 2008), 44-56 kg ha^{-1} (Smith, 2009; Reed *et al*., 2011), or 141 kg ha^{-1} (Ballari, 2005) to obtain a yield of 3,200 kg ha^{-1}.

Sulfur deficiency is characterized by stunted growth, yellowing and rolling of young leaves. Excess sulfur affects combustibility and decreases the alkalinity of ash (Smith, 2009; Rojo, 2008) and S over 1% in leaves can affect aroma, a fundamental characteristic in 'flue-cured' tobacco that is used as flavoring in cigarettes mixtures (Rojo, 2008). The recommendations for S applications for tobacco growth vary as follows: 35-45 kg ha^{-1} (Rojo, 2008), 20-30 kg ha^{-1} (Smith, 2009), or 31 kg ha^{-1} (Ballari, 2005) to obtain a yield of 3,200 kg ha^{-1}.

A determining factor in the quality of tobacco is the detrimental effect of chlorine, which reduces flammability and increases hygroscopicity, causing problems during leaf drying and fermentation (Elliot and Back, 1963). High chlorine concentrations (> 10 g kg^{-1} or more than 1%) in cured leaves reduce their quality by reducing the burning rate and heat retention capacity and increasing the moisture contents (Miner and Tucker, 1990; Rojo, 2008). Recommendations for chlorine in tobacco cultivation are 5-7 kg ha^{-1} (Ishizaki and Akiya, 1978).

In Colombia, there is little information on tobacco crops and the effect of fertilization on nutrient content in leaves. Given the importance of soil fertilization and the development of adequate nutrition strategies for growing tobacco plants under local conditions, the aim of this research was to determine the nutrient content in tobacco leaves during the crop cycle under different doses and fertilizer sources in Huila, Colombia.

Materials and methods

Location and plant management

The study was done in 2010 on two tobacco farms located in the province of Huila (Colombia) in the municipalities of Campoalegre (525 m a.s.l., 28°C average temperature, 68% relative humidity, and 32 MJ m^{-2} average solar radiation) and Garzon (790 m a.s.l., 25°C average temperature, 74% relative humidity, 28 MJ m^{-2} average solar radiation). These locations have sandy loam soils of slightly and moderately acidic pHs, respectively, contents of P, K, Mg, S and Cl that are unsuitable for the cultivation of tobacco, and high N contents (Tab. 1). Tobacco (*Nicotiana tabacum* L.) plants, NC297 'flue-cured' variety, were used. Seedlings were produced and, when the plants had 5 true leaves, were transplanted to the field with a density of 20,833 plants ha^{-1} in rows that were 1.2 m wide with 0.4 m between the plants. The crop management in the field was performed in accordance to the production practices in each study area.

Experimental design

The experiment were set up under a completely randomized design (DCA) with a 3x2 factorial arrangement of seven treatments and three replicates per treatment, which corresponded to six fertilization alternatives and a commercial control. Each experimental unit consisted of four crop rows and 30 plants per row. The control consisted of incurrent management practices recommended by the Protabaco company (Plaza *et al*., 2011) and used by the growers. The treatments corresponded to three combinations of fertilizer doses and two groups of fertilizer sources (Tab. 2), keeping the nitrogen forms constant as 50:50 NO_3:NH_4. The fertilizer doses were considered according to the soil analysis (Tab. 1) and following the methodology of ICA (1992) and Gómez (2005), which corresponded to 100, 50, or 150% of the recommended dose. To achieve the recommended fertilizer dose, the extraction levels for mineral elements reported by Ballari (2005) and an expected production of 3,200 kg ha^{-1} were employed.

The fertilizers used were: 17-9-18-3, Nutrimon (Monomeros Colombo-Venezolanos, Barranquilla, Colombia), which contained N:P:K:Mg:S equal to 17:9:18:3:6 (N-NH_4:N-NO_3 ratio = 60:40, potassium sulfate source of K); SolunK®, Nutrimon (Monomeros Colombo-Venezolanos, Barranquilla, Colombia), which contained N: P: K equal to 13:3:43 (N-NH_4:N-NO_3 ratio = 5:95); Nitromag® (Yara, Barranquilla, Colombia), which contained N:Ca:Mg equal to 21.0:11.0:7.5 (N-NH_4:N-NO_3 ratio = 50:50); SAM®, Nutrimon (Monomeros Colombo-Venezolanos, Barranquilla, Colombia), which contained N:S equal to 21:24 (N-NH_4:N-NO_3 ratio = 100:0); Sulfate K®, Nutrimon (Monomeros Colombo-Venezolanos, Barranquilla, Colombia), which contained K:S equal to 50:17; and DAP®, Nutrimon (Monomeros Colombo-Venezolanos, Barranquilla, Colombia), which contained N:P equal to 18:46 (N-NH_4:N-NO_3 ratio = 100:0).

TABLE 1. Chemical properties of the soil before the transplanting in the municipalities of Campoalegre and Garzon, Colombia.

Campoalegre												
pH	**N-NO_3**	**N-NH_4** (mg kg^{-1})	**N-total**	**Ca**	**K**	**Mg** (cmol kg^{-1})	**Na**	**Al**	**CEC**	**P** (mg kg^{-1})	**S**	**EC** (dS m^{-1})
6.3	3.49	4.51	8	5.52	0.19	1.54	0.08	0	7.33	44.1	5.52	0.74
Garzon												
pH	**N-NO_3**	**N-NH_4** (mg kg^{-1})	**N-total**	**Ca**	**K**	**Mg** (cmol kg^{-1})	**Na**	**Al**	**CEC**	**P** (mg kg^{-1})	**S**	**EC** (dS m^{-1})
5.6	7.86	6.55	14.41	10.3	0.44	3.24	0.08	0	14.1	53.5	9.94	0.34

TABLE 2. Fertilizer treatments in the municipalities of Campoalegre and Garzon, Colombia.

		Fertilizers (kg ha^{-1})					
Treatment	**Dose**	**17-9-18-3**	**SolunK**	**Nitromag**	**SAM**	**Sulfato K**	**DAP**
a50	50	275	288	175	50		
a100	100	550	575	350	100		
a150	150	825	863	525	150		
b50	50	550	163	75			
b100	100	1100	325	150			
b150	150	1650	488	225			
Control	Con	750	50		50	100	50
a50	50	250	288	63	25		
a100	100	500	575	125	50		
a150	150	750	863	188	75		
b50	50	475	125	150			
b100	100	950	250	300			
b150	150	1425	375	450			
Control	Con	790	105		155	155	

a: with SAM; b: without SAM; Con: commercial control.

Fertilizer applications were carried out according to the absorption curves of mineral elements by the plants (Rojo, 2008), which were split into two applications in two municipalities: 8 d after transplanting (DAT) and 35 DAT, times that coincided with the application of fertilizer in the control treatment.

Mineral nutrient content in the leaves

Six foliar analyses were carried out during the growth cycle with three measurements during the vegetative growth and flowering and three measurements during harvest. The first foliar analysis was performed during the slow vegetative growth (24 DAT, BBCH scale code 1105), the second one during the rapid vegetative growth (44 DAT, BBCH scale code 1110) and the third one at flowering (74 DAT, BBCH scale code 59) (Coresta, 2009). For all of the leaf samples, 300 g of dry matter were taken per experimental unit. For the samplings, 2-4 leaves without petioles (first sampling) and with plant leaves with the base and apex removed (second sampling) were taken per plant, according to the methodology proposed by Miner and Tucker (1990). For the third sampling, the central leaf (middle-third of the plant) was used because this leaf represents the nutrient status of the plants, with the midrib, base and apex of the leaf removed (Miner and Tucker, 1990). The last three samples corresponded to the optimal harvest time for 'flue-cured' tobacco leaves. The fourth analysis was performed at harvest (100 DAT); the sample unit was taken as the lower-third of the plant (lower leaves are defined as the lower four or five leaves). The fifth analysis was taken at 114 DAT (mid-harvest) on a sample taken from the middle-third of the plant. The sixth analysis was performed with the final harvest, 127 DAT, with the sample unit taken from the upper-third of the plant (the top leaves are defined as the top eight or ten leaves); the samples corresponding to approximately 20 plants per experimental unit were taken equally, removing the midrib, the base and the apex of the leaves. The leaf samples were taken in the morning and dried at 60°C for 24 h, and the total concentration of the mineral elements in the plant samples was determined according to Calderón and Pavlova (2001). The nutrient concentrations in the leaves of the tobacco plants were multiplied with the corresponding dry matter yields to obtain the nutrient uptake of the leaves of the tobacco plants.

Statistical analysis

Analysis of variance (ANOVA) with the SAS statistical software v. 8.1e (SAS Institute, Cary, NC) was performed and, when a significant difference appeared, LSD means comparison was performed with a reliability of 95%.

Results

Mineral contents in the leaves from vegetative growth to flowering

Foliar analyses at this stage of plant growth revealed significant differences in the contents of nitrogen, calcium, magnesium, and chlorine in the tobacco leaves; the time-municipality interactions were evaluated ($P \leq 0.001$). For these elements, their leaf contents increased progressively over time, presenting the highest levels at the stage of full bloom (76 DAT). The municipality with the highest values of N, Ca, Mg, and Cl in the leaves was Garzon (2.49, 1.40, 0.67, and 0.12 g, respectively) due to the highest dry matter accumulation in the leaves (Fig. 1).

For the time-dose interaction, significant differences were seen in the contents of nitrogen, phosphorus, calcium, and magnesium ($P \leq 0.01$). Similarly, a progressive increase in the contents of these elements was observed over time. The doses of 100 and 150% of fertilizer resulted in the highest values of the mineral element extraction (g), which were above 2.25, 2.25, 1.23, and 0.53 g for N, P, Ca, and Mg, respectively (Fig. 2).

Only the potassium contents had statistical differences for the maximum interaction, time-municipality-sources-dose ($P \leq 0.01$). In Campoalegre, the increased potassium content in the leaves was achieved with the treatment (b150) without ammonium sulfate at 150% of the dose (1.48 g). In Garzon, the highest K content in the leaves was obtained with the same source of fertilization, but at 100% of the dose (4.73) (Fig. 3). This could be explained by the increased solubility of the K sourced fertilizer (Solun K), which allowed for increased availability of this element in the soil and its rapid absorption by the plants.

During the cycle of plant development before flowering, the highest contents in the tobacco plants were observed for nitrogen, potassium, and calcium (Fig. 4). For Campoalegre (Fig. 4), the element with the highest accumulation was nitrogen and, in Garzon, it was potassium, both measured at 76 DAT (flowering) (Fig. 4).

At 76 DAT (full bloom), the plants of the control treatment in Campoalegre had the highest concentration of nitrogen and calcium, an intermediate concentration of potassium, a low concentration of chlorine, and an optimal concentration of N and Ca. The plants in Garzon had the lowest percentages of nitrogen, potassium and calcium and the highest percentage of chlorine (Tab. 3). At the same time, the treatments without SAM at 100 and 150% of the dose (b100 and b150) resulted in the optimal concentrations of foliar K at this phenological stage for Garzon.

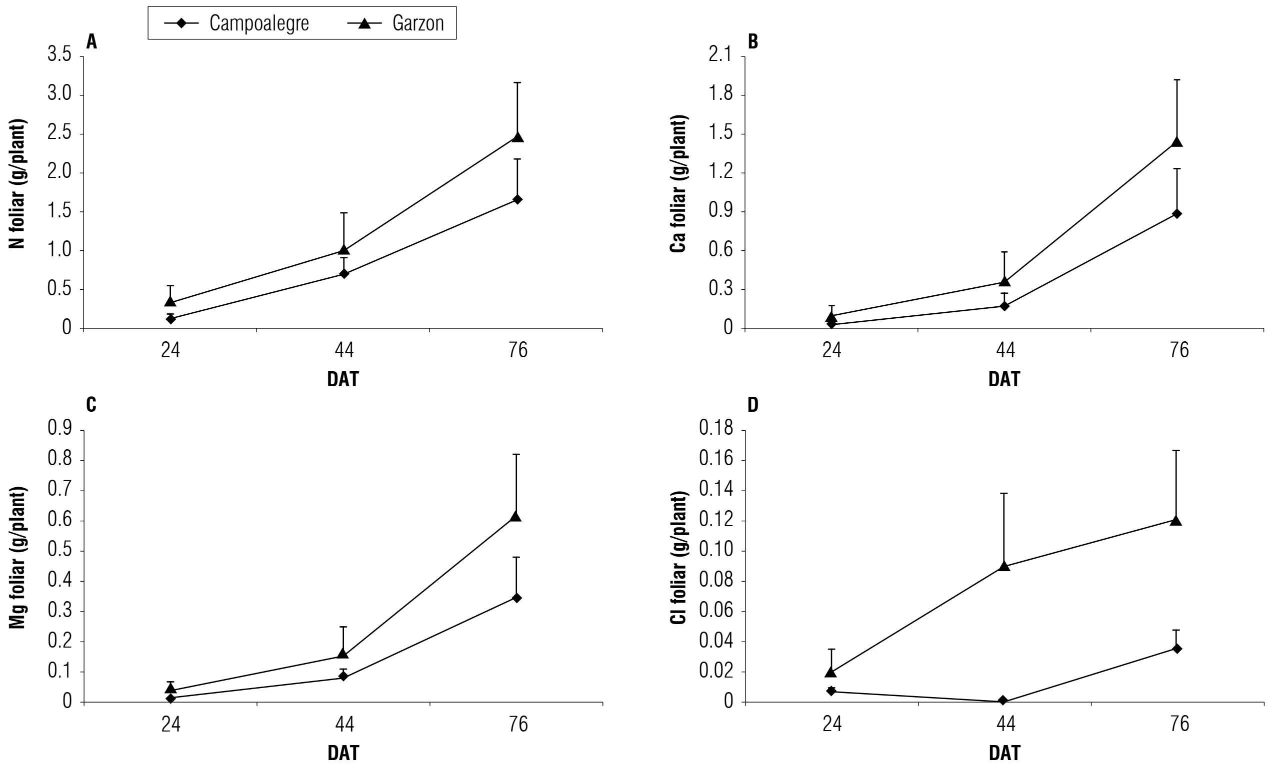

FIGURE 1. Contents of nitrogen, calcium, magnesium, and chlorine in the tobacco leaves during vegetative growth before flowering in the municipalities of Campoalegre and Garzon (Colombia). A, nitrogen; B, calcium; C, magnesium; D, chlorine. Error bars indicate standard deviation.

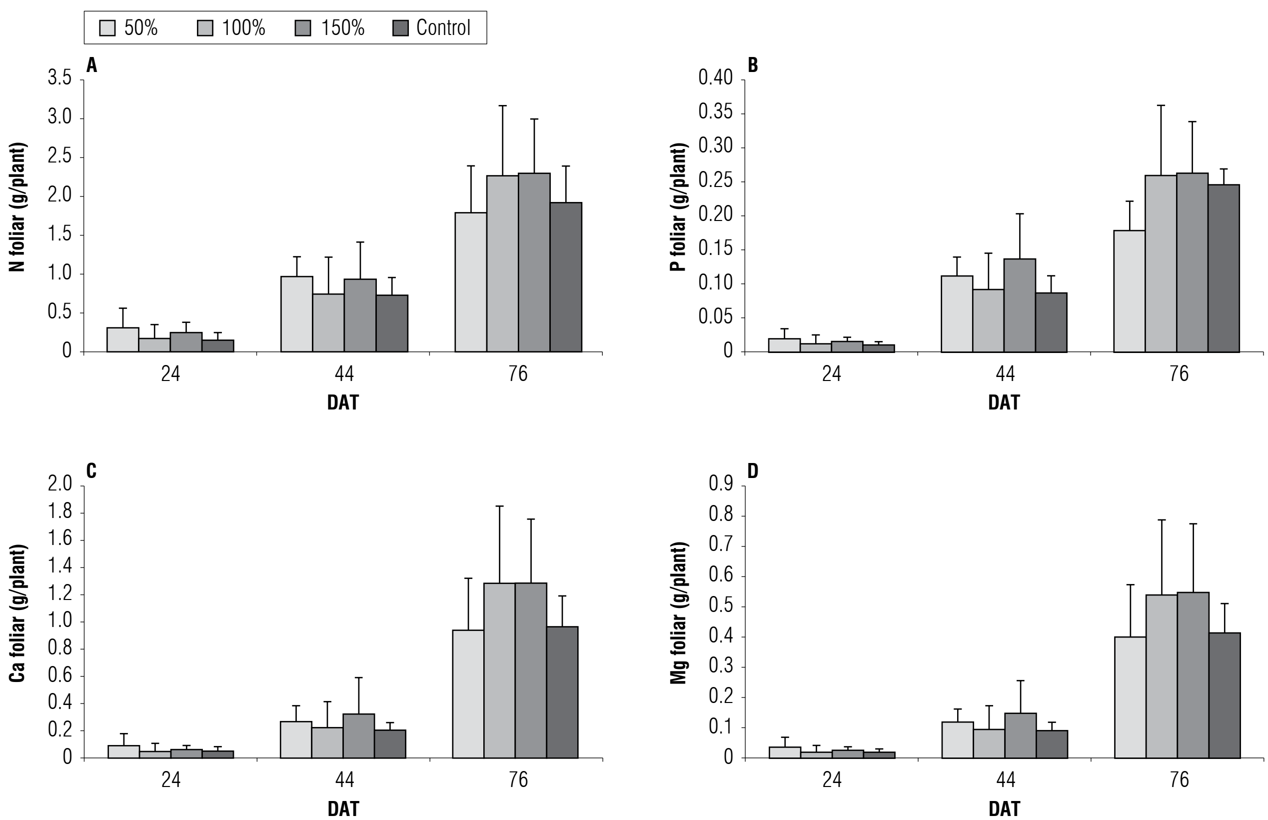

FIGURE 2. Contents of nitrogen, phosphorus, calcium, and magnesium in the tobacco leaves during vegetative growth before flowering in the municipalities of Campoalegre and Garzon (Colombia). A, nitrogen; B, phosphorus; C, calcium; D, magnesium. Error bars indicate standard deviation.

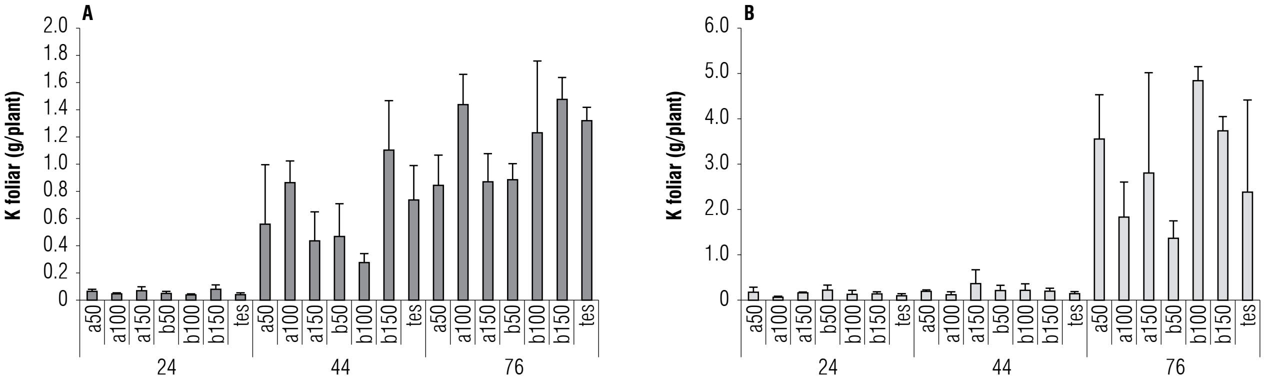

FIGURE 3. Potassium content in the tobacco leaves during vegetative growth before flowering in the municipalities (Colombia): A, Campoalegre; B, Garzon. Error bars indicate standard deviation. Abbreviations see Tab. 2.

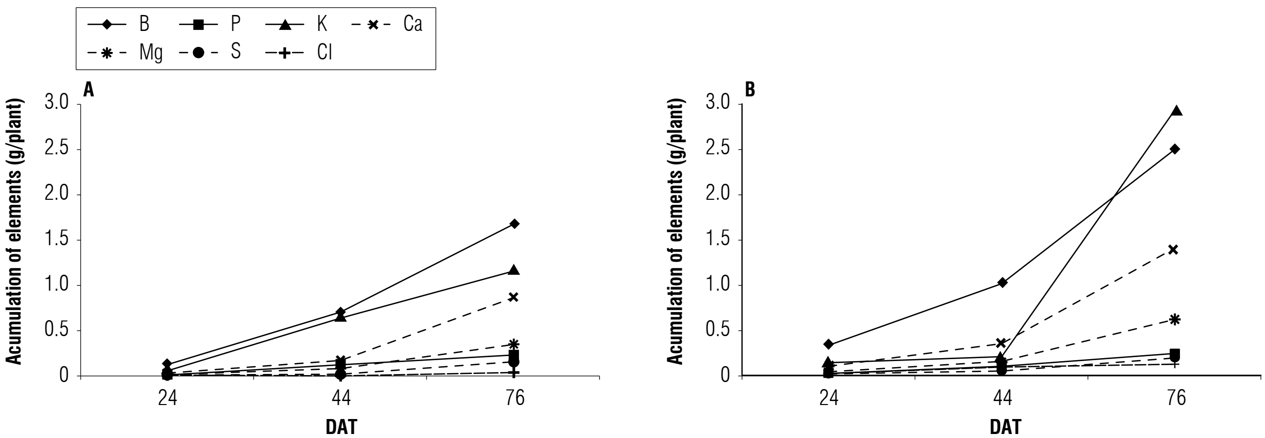

FIGURE 4. Accumulation of mineral elements in leaves of 'flue-cured' tobacco during vegetative growth before flowering in the municipalities (Colombia). A, Campoalegre; B, Garzon.

TABLE 3. Foliar concentrations (%) of the main mineral elements during flowering at 76 DAT in the 'flue-cured' tobacco in the municipalities of Campoalegre and Garzon (Colombia). The optimal ranges of mineral nutrients (%) in leaves are given according to Rojo (2008).

Municipality	Treatment	N	K	Ca	Cl
		%			
Optimal contents		**2.9 - 3.9**	**2.5 - 3.3**	**1.5 – 2.5**	**0.4 – 0.75**
Campoalegre	a50	2.650 ab	1.677 d	1.343 abc	0.063 bc
	a100	2.663 ab	1.997 dc	1.323 abc	0.050 c
	a150	2.693 ab	1.647 d	1.270 abc	0.060 bc
	b50	2.810 a	2.127 bcd	1.337 abc	0.070 bc
	b100	2.687 ab	1.800 d	1.393 ab	0.067 bc
	b150	2.693 ab	1.963 dc	1.447 ab	0.057 bc
	Control	2.857 a	2.087 bcd	1.520 a	0.060 bc
Garzon	a50	2.187 c	3.493 a	1.297 abc	0.103 ab
	a100	2.160 c	2.320 abcd	1.247 bc	0.093 abc
	a150	2.380 bc	2.057 bcd	1.313 abc	0.087 abc
	b50	2.317 c	1.307 d	1.100 dc	0.123 a
	b100	2.173 c	3.367 ab	1.333 abc	0.103 ab
	b150	2.313 c	3.193 abc	1.443 ab	0.103 ab
	Control	1.783 d	1.927 dc	0.840 d	0.130 a

*a: utilization of SAM, b: non-utilization of SAM. Abbreviations see Tab. 2.

Means with different letters in each column indicate significant differences according to the LSD test ($P \leq 0.05$).

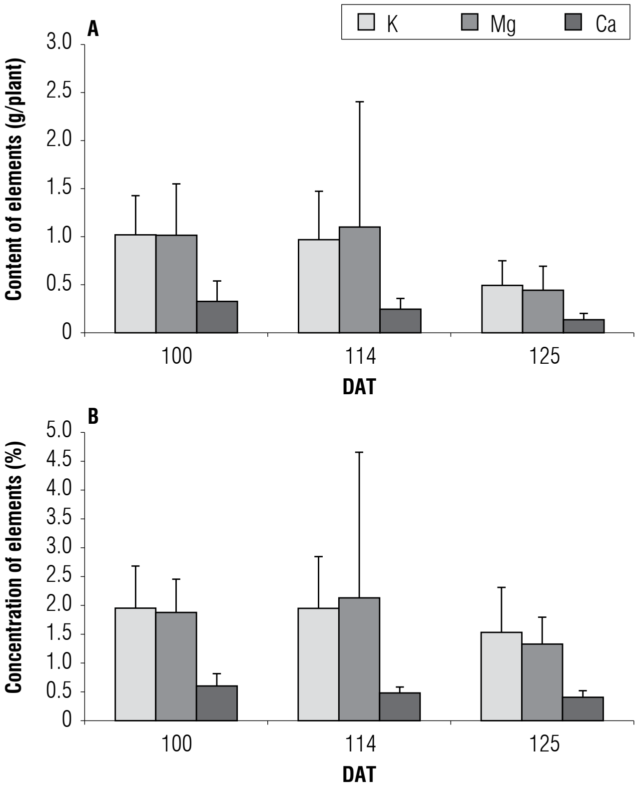

FIGURE 5. A, Contents (A) and concentrations (B) of potassium, calcium and magnesium in the tobacco during harvest in the municipalities of Campoalegre and Garzon (Colombia). Lower-third: 100 DAT; Middle-third: 114 DAT; Upper-third: 125 DAT. Error bars indicate standard deviation.

Mineral contents in the leaves at harvest

The foliar analysis conducted during the harvest showed significant differences in the contents of potassium, magnesium, and calcium over time ($P \leq 0.001$). For these elements, the leaf content decreased throughout the harvest time, presenting the lower contents in the upper-third of the plants at 125 DAT (Fig. 5).

For nitrogen, sulfur, and chlorine, statistical differences in the time-municipality interaction ($P \leq 0.05$) were seen. The plants in Campoalegre had the highest N and S contents during harvest because they had higher dry matter accumulation than the plants in Garzon (Fig. 6). The highest content of chlorine was in Garzon, because of the higher concentrations of Cl in the leaves (Fig. 6). For these elements, a decrease in their contents was observed at the last sampling date (125 DAT), corresponding to the upper-third of the plants.

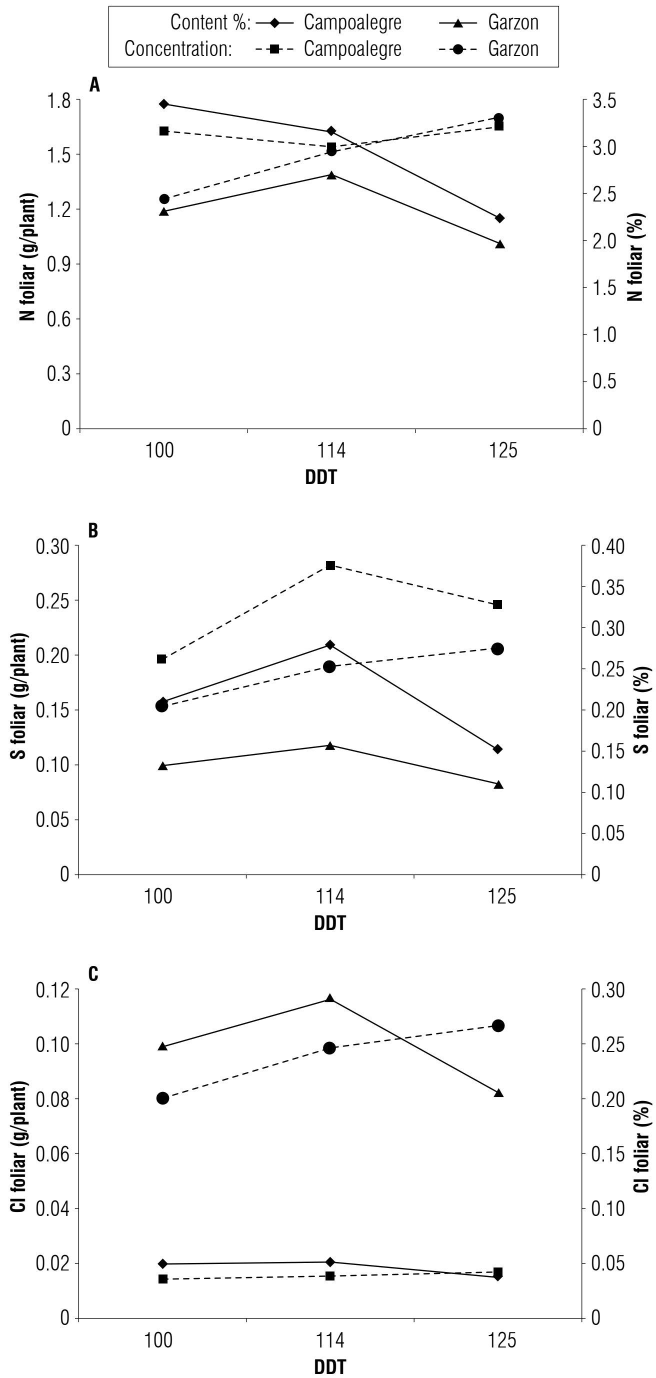

FIGURE 6. Contents and concentrations of nitrogen, sulfur, and chlorine in the tobacco leaves during harvest in the municipalities of Campoalegre and Garzon (Colombia). Lower-third: 100 DAT; Middle-third: 114 DAT; Upper-third: 125 DAT. A, nitrogen; B, sulfur; C, chlorine.

Discussion

The results in terms of element accumulation in the leaves over time were consistent with previous studies. Chavarria (2007) reported the largest accumulation of nutrients at the harvest stage in tobacco (48-78 DAT) due to the high rate of dry matter accumulation and, in ‘flue-cured’, this dry matter accumulation coincides with the rapid growth stage and leaf expansion (41 and 75 DAT) (Miner and Tucker, 1990; Moustakas and Ntzanis, 2005). For nitrogen and potassium, the rapid changes in absorption were reported between 41 and 68 DAT, when the entire plant absorbed 85% of the total nitrogen and 81% of the total potassium, with a maximum absorption at 55 DAT for N and 61 DAT for K (Moustakas and Ntzanis, 2005). Miller *et al*. (1967) reported that nitrogen uptake in tobacco plants is minimal in the last month of growth. In addition, phosphorus, calcium, and magnesium exhibited rapid changes in absorption between 41 and 75 DAT, when the whole plant absorbed 96% of the total phosphorus, 90% of the calcium, and 89% of the magnesium, with maximum absorption at 55 DAT for P and 61 DAT for Ca and Mg (Moustakas and Ntzanis, 2005). At the time of flowering, over 90% of the N and K and from 70 to 80% of the Ca, Mg, and P had been accumulated (Miner and Tucker, 1990).

Nitrogen, calcium, and magnesium increased their foliar concentrations over time, and the municipality of Garzon showed the highest values. Foliar analysis serves as an indicator of soil fertility and nutrient concentration in plants and reflects the influence of growth, soil, climate, management, and the availability of nutrients in the soil (Marschner, 1995). The soil of Garzon had higher contents of available (NH_4^+ and NO_3^-) and total N, organic carbon, calcium and magnesium, as compared to the soil of Campoalegre (Tab. 1). Furthermore, Garzon had less solar radiation, a lower average temperature and a higher relative humidity and precipitation.

The present study showed that the N, P, K, Ca and Mg concentrations in the tobacco were the highest at the flowering stage with the highest doses of fertilization (100 and 150%). Similar results were found in common beech (*Fagus sylvatica*), when increased fertilization resulted in higher levels of N, P and K in the leaves (Minotta and Pinzauti, 1996). However, in other species, such as rosemary (*Rosmarinus officinalis*), the highest concentration of elements in leaves was reported with balanced fertilization (Sardans *et al*., 2005). In *Lablab purpureus*, higher doses of phosphorus increased the foliar contents of nitrogen, phosphorus, potassium, and calcium (Naeem *et al*., 2010). In tobacco, phosphorus deficiencies delayed maturation and caused a reduction in foliar nitrogen and magnesium (Rojo, 2008). Increasing concentrations of other nutrients with increasing phosphorus levels can probably be attributed to the beneficial effect of phosphorus on the overall growth of the plants due to balanced plant nutrition (Naeem *et al*., 2010).

In tobacco, increasing doses of nitrogen fertilization increased the concentration of total N (Kowalczyk-Juśko and Kościk, 2002). Similar reports have been found in maize (Vos *et al*., 2005) and rice (Wang *et al*., 2006). In tea plants, increased leaf P contents with increased application rates have been reported (Lin *et al*., 2012).

The treatment that resulted in the highest accumulation of potassium in the plants was the one without SAM at a 150% fertilization rate in the municipality of Campoalegre, contrary to what was found for N, P, Ca, and Mg. Similar results have been found in cotton, where the content of foliar K was affected by potassium fertilization levels, with foliar K concentrations that increased at a higher dose of K (Gerardeaux *et al*., 2010). In *Houttuynia cordata*, the maximum concentration was recorded in the treatment with an intermediate level of K, while increased supplies of potassium reduced its uptake, as compared with the intermediate levels (Xu *et al*., 2011).

In this research, the elements with a higher accumulation included nitrogen, potassium, and calcium; however, there were differences in the order of importance of the mineral elements for the growth of 'flue-cured' tobacco. Miner and Tucker (1990) mentioned that potassium is the most important element for tobacco, followed by calcium, nitrogen, magnesium, and phosphorus. Collins and Hawks (1994) differed from the above-mentioned authors, stating that nitrogen was more important for the cultivation of tobacco. According to Moustakas and Ntzanis (2005), calcium is the third element in order of importance for the cultivation of tobacco. Chavarria (2007) reported that K has the largest accumulation in tobacco leaves, followed by N and, to a lesser extent, Ca. However, it could be concluded that N, K, and Ca are the most important elements for the production and quality of 'flue-cured' tobacco, with implications for qualities such as flavor, color, texture, sugar and nicotine contents, flammability, and smoke flavor (Smith, 2009; Ballari, 2005; Lu *et al*., 2005; Marchetti *et al*., 2006; Gurumurthy and Vageesh, 2007; Yang, *et al*., 2007; Marambe and Sangakkara, 1988).

The mean contents of nitrogen and potassium for all of the treatments at the flowering stage were below the normal range (Campbell, 2000; Ballari, 2005), except in Garzon without using ammonium sulfate at 100 and 150% of the dose (b100 and b150, respectively) and when using SAM at 50% of the dose (a50). According to the ranges reported by Rojo (2008), the control was above the normal range of N in Campoalegre and below the normal range in Garzon. The content of foliar calcium presented normal contents (Campbell, 2000); however, for Ballari (2005) and Rojo (2008), all of the contents of Ca were below this range, except for the Campoalegre control treatment. All of the treatments had a chlorine content in the leaves that was below the recommended ranges (Ballari, 2005). The optimal concentration of K and N in tobacco leaves should range between 2.5-3.0% for proper plant growth and optimal chemical balance in leaves (Zou *et al.*, 2005).

At harvest, the contents of K, Mg, and Ca in the leaves decreased over time. This occurred because of a high accumulation of leaf dry matter that reduced the nutrient concentrations in the leaves due to a dilution effect (Miner and Tucker, 1990). Dilution or accumulation of nutrients in plants can occur because of differences between the rate of plant growth and the rate of nutrient absorption (Marschner, 1995). Similar results were reported by Jiang *et al.* (2001) and Zhao *et al.* (2010), who found that the concentration of K in nearly all organs of tobacco plants was reduced from the time when the shoot apex was removed until the end of harvest. The Mg content in the leaves decreased according to the ascendant position of the leaves; the lower leaves had a higher Mg content, while the upper leaves had a lesser content (Miner and Tucker, 1990).

In the production of tobacco, the apex of the plant is removed for more leaf yield and higher quality; for the present study, this practice was performed at 86 DAT. However, in physiological terms, this practice can cause changes in sink-source relations and modify the distribution and translocation of assimilates and mineral nutrients in plants (Jiang *et al.*, 2001). Zhao *et al.* (2010) discussed three possible reasons for this decrease in the K content of whole plants, particularly in leaves. Firstly, it could be due to a dilution of nutrient contents as dry matter is accumulated at a greater rate than the element is absorbed; similar results were reported by Moustakas and Ntzanis (2005). Secondly, when leaves mature, outputs of K from leaves exceeds its absorption, causing decreases in the K content of plants. And thirdly, re-translocation and redistribution of K occur as a result of cleavage of the apex and leaf senescence (Zhao *et al.*, 2010). The latter occurs because potassium is highly mobile in the phloem and, therefore, a high degree of its reuse is done with re-translocation via phloem (Marschner, 1995).

In the cultivation of the 'flue-cured' tobacco, different fertilizer rates and sources had an effect on the accumulation of the mineral nutrients in the leaves. The foliar element concentrations during the vegetative stage of development increased over time and the highest values were found at the flowering stage; however, during the harvest, the contents of K, Mg and Cl decreased over time. Nitrogen, P, K, Ca, and Mg presented the highest concentrations at flowering with the highest doses of fertilization (100 and 150%). For potassium, the treatment with the highest accumulation in the tobacco did not use SAM and had a 150% fertilization rate in the municipality of Campoalegre. For the 'flue-cured' tobacco in the municipalities of Campoalegre and Garzon, the elements with the higher accumulations included nitrogen, potassium, and calcium.

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