Tobacco Research Institute, Cuba

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Introduction

The rates of growth and quantities of nutrients taken up vary considerably and depend on the type of tobacco, soil type and fertility, management and cultural practices (Layten and Nielsen,1999). Most of the information related to, adequate range of nutrient levels for tobacco is available for Virginia (Bergman, 1992), Burley and Flue-cured (Hallyday and Treukel, 1992), not for dark type. The deficiency ranges for black tobacco is not defined in the literature, as far as to our knowledge. Cuba, the land of the international famous “Habanos” cigars, produces mainly dark Tobacco. The aim of this project was to establish nutritional patterns of three commercial varieties of tobacco that guarantee the basis for an efficient fertilization management.

The project was also aimed to establish nitrogen requirements for three cuban commercial varieties, in the main productive regions, with the crop technology, that distinguish each one and set up their importance for cuban dark tobacco production.

Results

Greenhouse experiments. Institute of Plant Nutrition, University of Hohenheim, Germany. (Year 2005).

Nutrient concentration ranges that determine the appearance of deficiencies visual symptoms.

Experimental Design: Six treatments of nutrient deficiencies (N, P, K, Mg, Zn, B) and a control. Full nutrient solution according to Walch-Liu et al. 2001

Variety: *Nicotiana tabacum* L. var. Criollo 98

Nitrogen

First visual symptoms were observed in the treatment with no nitrogen (-N solution), four days from the beginning of the trial. Light green to yellowish colors were almost homogeneous. Adult leaves rapidly passed from yellow color to brownish and subsequently died (figure 1). At the time of symptoms appearance it was not observed any growth reduction in length (Fig. 1 a). Nevertheless, three days after height of deficient plants was reduced (Figure 1b), which agree to reports by Bergmann (1992) and Mengel & Kirkby (2000). Stem elongation inhibition as result of nitrogen source scarcity has been described as response to cytokinin transport from roots to the stem. (Wagner, 1993; Walch-Liu *et al.*, 2001). Adult leaves chlorosis is the characteristical symptom for nitrogen deficiency (Mengel & Kirkby, 2000).

Figure 1. Nitrogen availability effect on stem´s longitudinal growth.

Nitrogen percentage in control plants tissues was 3 y 6.5% on dry matter (DM). Nitrogen content was higher in roots than in stem. Old leaves from control plants had about 3% N, but deficient plants were below 1,5%. It seems that in experimental conditions critical N level is bellow 1,5% for old leaves and around 3% for young ones. This critical N level for adult leaves has been reported for tobacco plants by Chouteau (1969) and Choteau & Fauconnier, (1993). Normal N values reported for mature leaves of Virginia tobacco range from 25 to 2,5% DM. (Bergmann, 1992). Comparatively values from our work are quite high, but this difference may be explained by the higher requirements of dark tobacco if compared to Virginia cultivars (Layten & Nielsen, 1999).

Phosphorous

Phosphorous deficient plants rested small, with narrow spear/like leaves. Intensive dark green color, reported for P deficient leaves was not observed probably due to the normal dark green color of dark tobacco leaves. From the appearance of first symptoms, damage gets severe very rapidly. As untypical symptom with deepening of deficient condition small brown spots were observed on leaf lamina. The color of the injury may also be bronzed and of a bigger size at the edge of the leaves. (Figure 3). Red and/or violet spots have been reported by Chishaki & Horuguchi (1997) as a result of anthocyanin accumulation in P deficient tissues.

For control plants values from 0.14- 0.55% P DM in leaves were observed. For Virginia cultivars 45 days of age have been reported sufficiency values from 0.2 – 0.45% DM (Bergmann, 1992). Nevertheless, it is not possible to compare the values due to the difference in age and tobacco type. It may be considered that values are acceptable, because P absorption remains constant during all the stages of growth and development of tobacco plant. (Layten & Nielsen, 1999). Therefore, differences may be assigned to the tobacco type. For P deficient plant P values were much lower tan reported by Mengel & Kirkby (2000).

Figure 3. Dark tobacco plants with P deficiency symptoms.

Potassium

Visual symptoms of potassium deficiency were observed after 15 days from the transfer of plants to the deficient solution. Injuries were clearly observed in medium to low leaves with the well reported marginal and intercostals chlorosis beginning from the leaf apex. Necrotic stains appeared with the development of the condition, which grew up to form patch - like injuries. After that leaves bend backward and downward (Figure 4), as result of impeded water control in the plant.

Figure 4. Black tobacco plants with visual symptoms of potassium deficiency.

Potassium deficiency did not affect drastically length growth in an early stage, length reduction is much slower and internodes' shortening is stronger in the upper part of the plant. Potassium content of healthy plants was in the range 1.5-3.8% DM, values from control adult leaves were higher than from the younger ones. When visual deficiency symptoms were observed K values were around 0.3%. Critical K value reported by Layten &

Nielsen (1999) for adult tobacco plants was <1%, no reports on critical K values were found for tobacco seedlings.

Range proposed by El Bergmann (1992) for healthy Virginia plants is 2.5 - 4.5%. Surely values bellow 2,5% DM obtained in our experiments are not critical, due to the potassium accumulation by adult leaves. This effect is described by Bergmann (1992) in plants with facilitated K absorption, due to sufficiently applied potassic fertilizers or in hydroponical conditions.

Magnesium

Magnesium deficiency symptoms were clearly showed by lower central leaves. Intervenal chlorosis of fully expanded leaves was the typical visual symptom, while central vena rested green. Color gradient was from light green to yellow. Leaf edges turned wavy (Figure 5). Apparently leaves from middle stem were the most affected, as reported by Pinkerton (1970) for Flue cured tobacco.

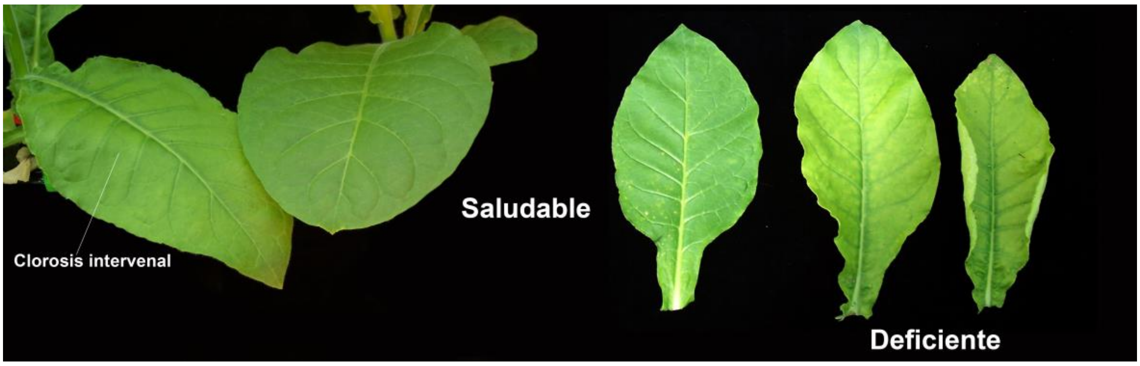


Figure 5. Intervenal chlorosis in Mg deficient leaves of black tobacco plants.

In control plant tissues magnesium percentage ranged 0.9- 1.4% for adult leaves and 0.35-0.4% for young ones. On the other side, in deficient plants, observed values (<0.5%) were higher than reported by Chouteau (1969), who described deficiency symptoms where Mg content in leaf DM was 0.4%.

Zinc

In deficient plants leaf expansion was reduced, leaf edges turned wavy and internodes shortened, in agreement with Römheld & Marschner (1991) report for dicotyledonous. Medium mature leaves showed chlorosis with some necrotic spots (Figure 6); in bibliography this is described as a secondary effect due to boron and phosphorous toxicity or due to photoxidative stress (Marschner, 1995).

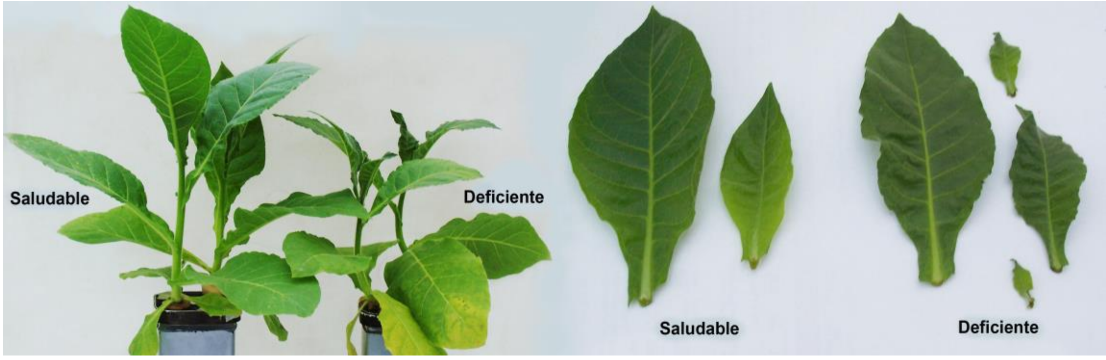


Figure 6. Visual symptoms of Zn deficiency

In control plants Zn content was in the range 25 - 82 µg Zn g^{-1} DM. Adult leaves showed higher values than young ones. Time - concentration curve showed a drastically drop after 28 days of deficient treatment.

Zinc concentration in deficient plants ranged 20-30 µg Zn g-^{1} DM. Young leaves from deficient plants showed zinc content almost half as much as control plants. Very low values were reached after 31 days in the deficient nutrient solution. Control plants behave as reported by Bergmann (1992) for Virginia tobacco, with blues in the range 25-70µg Zn g^{-1} DM. Comparatively with critical values proposed by Marschner (1995) (15-20µg Zn g^{-1} DM) our values are quite high, nevertheless deficiency symptom were severe.

Boron

No boron treatment was the second to show visual deficiency symptoms, seven days after the transfer of the plants to the boron deficient nutrient solution, probably due to the fact that plants were in a very active vegetative growing stage. First deficiency symptoms appeared on young leaves, with an intense green color and necrosis of the base. The other leaves expanded more and finally the (Figure.8). Observed symptoms match to the ones reported by Umesh (1993) for tobacco, based on the immobility of this nutrient. The only missing symptom was the intervenal necrosis reported by this author.

Figure 8. Boron deficiency inducted length growth inhibition. A) Death of the terminal bud.

Boron concentration in control plants tissue ranged 25-36µg B g^{-1}DM for adult leaves and around 16µg B g^{-1}DM for the young ones. In deficiency conditions boron content dropped drastically to 19 - 15 µg B g $^{-1}$DM. In young leaves values were around 6 µg B g^{-1}DM and in the extreme deficiency after 6 days of boron omission dropped to 2µg B g^{-1}DM. Bergmann (1992) reports normal values ranging 30-80ppm for mature leaves of bright tobacco and in a general chart points up to 25 µg B g^{-1}DM.

Conclusions

- According to our experiments results of dark tobacco visual deficiency symptoms appear with contents of nitrogen <1.5%, phosphorous <0.1%, potassium < 0.4%, magnesium <0.5%, zinc < 30µg Zn g^{-1}DM and boron < 20 µg B g^{-1}DM.

Nitrogen nutrition of dark tobacco grown in pots. Normal ranges of nutrients by leaf levels.

Variety *Nicotiana tabacum* L. var Sancti Spiritus 96

Pot experiment

Treatments: Interaction between nitrogen dosages and its percentage distribution in each application.

	1.	**60 kg N/ha**		**60%-40%-0%**
Nitrogen:	**2.**	**100 kg N/ha**	**Distributions**	**40%-60%-0%**
	3.	**150 kg N/ha**		**20%-75%-5%**

The rest of the nutrients (P, K, Mg) were applied according to the current recommendations

Results

Stem height and mass.

Statistical comparison of plant height at 45 days from planting did not show significant differences (p=0.2167). Final plant height was not significant either (p=0.0743). Taking into account that as an agronomical measure plants are topped and the terminal bud is removed to allow the growth of a number of leave pairs, you can't wait for significant differences and agronomical meaningness of this parameter, even if some differences arise among plants.

For stem dry matter the ANOVA showed extremely high significant differences (P=0.0002***). Highest values were observed for treatments 3.II and 4.II, without significant differences among them, regardless the 50 kg/ha difference in total nitrogen supplied. Distributions I y III were not different among them, but the lesser values always related to early applications (Distribution I).

Total leaf biomass production

Differences in the total biomass production (g) as showed by ANOVA was extremely significant (p<0,0001***). Foliar development in the 60 kg/ha nitrogen treatment was minimal, these plants also showed characteristical visual symptoms of nitrogen deficiency. Plant growth was generally affected, as observed for all morpho-agronomical parameters.

We consider that this value represents a nitrogen deficient zone for experimental conditions. For N starvation conditions the best plant response appeared with late nitrogen applications, but without statistical differences. It is very interesting to point out that there were no statistical differences among treatments with distribution I, regardless to the fact that nitrogen applied at planting time may be even threefold (36 – 60 – 96 kg/ha) and only the treatment with 60 kg/ha total nitrogen showed visual deficiency symptoms. It is known that young plants at planting time has a limited capability to absorb nutrients, some authors consider that this situation lasts all along the first week after planting. (Diaz & Gonzalez, 1979). More than that, nutrient salts applications at planting time rises osmotic concentration of the soil solution and probably generates subtle pH variations conditioning additional environmental stress. Experimental results show that regardless to the amount of nitrogen applied (relatively feeble or relatively plentiful), tobacco plant is not able to respond positively in terms of biomass production to early nitrogen applications. Late nitrogen applications promoted a positive response of N deficient plants, even in treatment 2.It seems that response depends on the nutritional state of the plant which is able to absorb nutrients more efficiently when it needs are not fulfilled.

This differential behavior depending on nitrogen distribution along the live cycle of tobacco plant is more evident when composition of each separate foliar level is analyzed. It seems that the foliar level that reflects the best nitrogen source variations is "Centro Fino 1" (Central foliar level), where every change due to the treatments enforced changes in the growth pattern of this foliar pair, the second one in magnitude of response was "Centro Fino 2". (Centro Fino is named the 4th to 5-6th leaf pair of tobacco plant)

SPAD measures

Chlorophyll levels in the first measure were around 30-50 SPAD units. At this time the characteristical tobacco pattern, where nitrogen contents rise from the plant bottom to the top, was not observed, as reported by Layten & Nielsen, (1999) and Tso (1990). AT 50 days of age, the pattern is clearly established. At this time measures ranged 35-57 SPAD units. This agrees with Izquierdo *et al.,* 2007 (unpublished results) for 'Criollo 98' cultivar sun grown. Chlorophyll variations may be explained, following Gastal & Lemaire (2002), due to the fact that the leaves the most exposed to the light accumulate higher nitrogen contents. When leaves rise toward the top of the plant self shading is less important and therefore chlorophyll contents are higher.

For treatment 4 values for distributions II and III are lower than for the early application. It is possible that the plant was not yet able to assimilate the late applied nitrogen. Similar values for treatments 3.III and 4.I may mean that the plant was only able to assimilate the same amount of nitrogen, regardless to the different availability. We should remember the negative impact and growth limitations in plants subjected to early nitrogen applications, as analyzed earlier on the subject of total biomass production.

Foliar composition

Nitrogen

Nitrogen content ranged 1,4%- 4,5%. (Chart 10). In the named range are present deficient values, as confirmed by morphological parameters from treatment 1, where deficiency visual symptoms appeared after 40 days. Normal values reported by Bergmann (1992) in mature Virginia tobacco leaves are 2 y 2.5% DM. Our values were considerably higher, which may be explained by the difference in tobacco type. Dark tobacco cultivars require more nitrogen than Virginia ones (Layten and Nielsen, 1999). Normal nitrogen values for dark tobacco proposed by Choteau (1969) in France, are wider (2 - 5%$^{-}$) and agree with our results. Plants from treatment 1, in the central foliar level (Centro fino) showed a 1,4% N DM of deficient nitrogen content of while the top foliar level (Corona) showed a high value around 2,3 %N DM. (Corona are named the top pair of leaf of the tobacco plant).

This gradation in the leaves composition from different foliar levels defines the organoleptic characteristics for cigar making process. The higher foliar levels are characterized by the strength, due to the high nitrogen contents. Analysis showed that the nitrogen content for the central foliar level is below the critical content established by Choteau (1969) (<1,5% N) objectively corroborating the visual symptoms observed in treatment 1. Nevertheless, the top foliar level did not so low the nitrogen values. It is known that nitrogen is a very labile nutrient and in deficient conditions the plant mobilizes the reserves toward growing buds. That is the reason why the young part of the plant always shows higher nitrogen contents (Bergmann, 1992). Therefore, critical nitrogen content for adult plants should be different for every foliar level.

When 100 kg/ha nitrogen was applied nitrogen values logically were higher, bottom value was 2,09% N DM. For both treatments (1 y 2) from the three parts of the plant sampled, the lesser values always corresponded to the central leaf (Centro fino 1). Otherwise happened for the rest of the treatments where the steady rise of nitrogen content toward the top of the plant was clearly observed, according to Layten and Nielsen (1999); Tso (1990) Choteau & Fauconnier (1993). It seems that the plant, developing in a N deficient environment has the central foliar level as the most sensitive one. The same way, it was observed that nitrogen content of central foliar level at the 150 kg/ha N was higher than in the 200 kg/ha N treatment, what point to a sufficient level around the 150 kg/ha N mark and the response to the increment may be considered a toxicity sign in our experimental conditions. If we accept that treatment 3 is in the sufficient zone in our experimental conditions, it states that nitrogen content rises with foliar levels and values range 2,5% - 4,4% N DM.

Nitrate concentration in lamina sap was very low in treatments 1 and 2 for any one of nitrogen distributions. Accepting that nitrate accumulation happens when nitrogen demand is satisfied, according to (Marschner, 1995) it is once more corroborated that these treatments are in zone below of the sufficient zone. For the rest of the treatments higher nitrate sap values were observed, the highest was observed in the treatment 4, corroborating our hypothesis that the amount of applied nitrogen exceeds the plant needs. According to Layten and Nielsen (1999) report in Burley tobacco, nitrate accumulation began after total nitrogen content rise over 2,2% N DM^{-}.

Other nutrient behavior

Phosphorous

P contents in leaves ranged 0,1%-0,4% P DM ; this range is inside the Wolf *et al.*, 1990,proposed range, but the author´s range is wider, up to 1% P DM. Differences among foliar levels were as follow: lower level 0,1-0,13% P, medium level 0,14%- 0,22% P; top level 0,24-0,39% P. Statistical comparison of these results shows that significance grew in upward direction of the plant: lower and middle level were not significant and top level was highly significant. Results from the upper level are related to treatment 4.I which showed the highest P content. The rise of P contents in the plant upward direction agrees with reports given by Choteau & Fauconnier (1993) on tobacco plants.

Potassium

Potassium content ranged 2,6%- 4,5% K, research results on Virginia tobacco report as normal K range 2.5% -4.5% (Bergmann, 1992). It seems that there are not strong differences in potassium contents between these tobacco types. The highest K contents were found in the lower foliar level 3,3%-4,5% K, in the middle level 2,6%-3,6% K and in the top level 3%-3,6% K. It is possible that nitrogen deficiency may restrict the potassium uptake. Reduction of K content toward the top of the plant was observed by Choteau & Fauconnier (1993) for tobacco harvest by leaves. This result may be explained following Kafkafi (1998) when states that a rise of radiant energy promotes higher nitrogen demand and lower potassium demand.

Magnesium

Magnesium inside the green leaf ranged 0,37%-1,23% Mg. Lower leaf levels have higher Mg content than the top ones, which agrees with tobacco report by (Layten and Nielsen, 1999). Obtained Mg values are lower, but not enough to reach the deficient range (< 0,25% Mg) reported by Tso (1990). Nevertheless Choteau (1969) reports as critical Mg level for dark tobacco less than 0,4% Mg. In our experiments visual symptoms for Mg deficiency were not observed, so a critical value for this nutrient wasn´t possible to establish.

Chart.1. Average distribution of analyzed nutrients by foliar levels and treatments.

Foliar Composition Macronutrientes (%)												
Treatment	**1**			**2**			**3**			**4**		
Distribution	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
Nitrogen												
Uno y Medio	1.71**c**	1.88**bc**	1.92**bc**	2.72**abc**	2.31**abc**	2.58**abc**	2.56**abc**	3.12**a**	2.97**a**	2.49**abc**	2.76**ab**	2.87**a**
Centro Fino	1.41**cd**	1.42**bcd**	1.47**bcd**	2.62**abcd**	2.12**abcd**	2.09**abcd**	3.08**ab**	3.32**a**	3.22**a**	2.95**abc**	2.88**abc**	3.07**ab**
Corona	2.38**b**	2.32**b**	2.41**b**	3.30**ab**	2.99**ab**	2.75**ab**	4.42**a**	3.61**ab**	3.84**a**	4.43**a**	3.68**ab**	3.63**ab**
Phosphorous												
Uno y Medio	0.13	0.13	0.11	0.13	0.12	0.12	0.11	0.12	0.11	0.13	0.11	0.10
Centro Fino	0.16	0.16	0.16	0.19	0.16	0.15	0.20	0.18	0.17	0.22	0.16	0.14
Corona	0.26**b**	0.25**b**	0.27**b**	0.25**b**	0.27**b**	0.24**b**	0.32**ab**	0.27**b**	0.28**b**	0.39**a**	0.31**ab**	0.29**b**
Potassium												
Uno y Medio	4.54	3.39	3.37	3.93	3.49	3.97	4.13	4.54	4.01	3.77	4.25	4.21
Centro Fino	2.87	2.68	2.64	2.87	3.09	3.58	3.44	3.41	3.58	3.23	3.39	3.39
Corona	3.25	3.15	3.25	3.01	3.33	3.52	3.22	3.35	3.34	3.18	3.57	3.25
Magnesium												
Uno y Medio	1.00	0.81	0.85	0.92	1.01	1.23	1.05	1.12	1.11	0.92	0.99	1.02
Centro Fino	0.46**b**	0.37**b**	0.37**b**	0.52**ab**	0.58**ab**	0.60**ab**	0.72**ab**	0.87**a**	0.85**a**	0.71**ab**	0.75**ab**	0.84**a**
Corona	0.51	0.54	0.51	0.55	0.56	0.58	0.67	0.70	0.69	0.63	0.65	0.77

***Different letters show the statistical differences**

Zinc

Zinc concentrations ranged 16 μg/g DM - 65μg/g DM. (Chart 2). This range agrees with Wolf *et al*. , (1990) and Choteau (1969). The higher contents were found in the lower foliar level. (40 μg/g DM - 65 μg/g DM). Middle and top level contents do not follow a definitive pattern, but values from middle level tend to be higher than in the top foliar level, and may be related to a certain nutritional unbalance.

Manganese

Manganese contents in tobacco leaves range 26ppm-398ppm, (Wolf *et al*. , 1990) and even higher (140ppm- 700ppm) following Tso (1990). In our experiment Mn values ranged 63 μg/g DM- 169 μg/g DM. Lower foliar level shows the maximal values of Mn content.

Copper

From all nutrient, analyzed in our experiment, copper was the only one that did not meet establish ranges for tobacco. Obtained values (0,86 μg/g DM – 6,8μg/g DM) were far below published nutritional standards for Cu (10ppm- 34ppm Wolf *et al*., 1990) and (30ppm-60ppm Choteau 1969). Absence of locally obtained Cu values limits our judgment on this matter.

The behavior of nutrient contents of tobacco leaves leads to the conclusion that their uptake is strongly related to nitrogen nutrition. Potassium and magnesium were the closest related to nitrogen application levels. Scarcity of micronutrients results does not allow us to reach any general conclusion.

Conclusions

- In a nitrogen sufficient state and for our experimental conditions the following ranges may be considered as normal: for phosphorous 0.1 - 0.3%, for potassium 3 - 4% and for magnesium 0.6 – 1.15%.
- Normal nutritional ranges for dark tobacco differ from the ones published for bright tobacco.

Field experiments in natural conditions. Tobacco Research Institute and Experimental Stations. (Cuba, 2006-2007).

Susceptibility of four tobacco varieties to nutritional deficiencies.

Hydroponics is an excellent method for every kind of research in Plant Physiology, Pathology and Nutrition. In nutritional studies it allows to design nutrient solutions without interferences from the growing media, creating deficient or toxic environments, resulting in an ideal technique for monitoring susceptibility of varieties to actually or potentially environmental conditions

Chart 2. Effect of treatments on micronutrient content by foliar levels.

Composición foliar Micronutrientes (µg/g)												
Tratamiento	**1**			**2**			**3**			**4**		
Distribución	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
Cinc												
Uno y Medio	59.25	45.46	53.16	48.08	42.59	53.33	65.6	43.19	53.14	40.67	43.77	39.23
Centro Fino	41.58	26.30	21.77	24.54	26.21	27.62	19.58	16.90	30.14	21.51	22.38	16.84
Corona	37.86	32.55	37.46	29.95	42.13	36.31	28.09	24.88	31.81	33.7	29.57	25.9
Manganeso												
Uno y Medio	138.6	127.4	96.88	113.2	148.9	131.5	161.4	156.8	168.2	139.7	157.6	159.7
Centro Fino	85.09	69.67	97.93	79.92	79.51	63.73	104.1	106.3	74.33	97.8	88.59	76.75
Corona	149.3	128.4	119.2	133.5	133.6	103.2	141.0	130.3	103.4	144.4	128	98.2
Cobre												
Uno y Medio	3.84	2.54	2.53	1.33	1.62	0,86	1.2	1.14	2.79	1.93	2.12	2.23
Centro Fino	1.24	3.67	3	4.06	3.05	3.19	6.4	4.36	5.15	5.15	5.79	6.78
Corona	5.21	3.38	2.75	3.13	3.67	1.33	4.65	1.51	4.28	6.42	4.15	6.29

happening in the soil, natural environment. Response to nutritional stress situations, evaluates genetic potential of the varieties to express its genetic productive potential.

Susceptibility of four Cuban dark tobacco varieties (Habana-2000, Criollo-98, Corojo-99 y Sancti Spiritus-96) were evaluated face to face several macro and micro nutrient deficiencies. Deficient treatments were designed to use modified University of Hohenheim’s basic nutrient solution. Plants were grown in 1 liter PE pots with one plant of each variety, with three replications randomly distributed. The deficiencies’ detection was carried by visual evaluation and leaves tissue analysis. Varietal differences to nutritional stress conditions were observed. Nevertheless, statistical analysis did not shown strong significant differences. Toxicity experiments were not conclusive, new solution design is needed for future studies.

2.2 Tobacco Research Station. San Juan y Martinez (Pinar del Rio, San Juan y Martinez)

Tobacco zone: Vuelta Abajo

Variety : *Nicotiana tabacum* L. Var Corojo 99

Technology: Shade grown tobacco Soil type: Kanhapludalf

Design Field experiment (Two Year)

Treatments: Interaction between nitrogen dosages and its percentage of distribution in each application.

Nitrogen:
0
70 kg/ha
140 kg/ha
280 kg/ha

Distributions
30% - 50% - 20%
0 - 40% - 60%
0 - 60% - 40%

The rest of the nutrients (P, K, Mg) were applied according to current production recommendations

Nitrogen Current Recommendation: 122 kg/ha; Distribution 20%- 40%-40%

Results

Morphological parameters

Plant height was not affected by treatments and nitrogen distribution, and the interaction between factors was not significant either. This is the result of plant topping. Mean values of leaf length at the harvesting time of each of the three foliar levels were significant for nitrogen doses, which explain the major percentage of total variation, without a response to nitrogen distribution. However, a solid trend to better leaf longitudinal growth as a result of late nitrogen applications was observed. Nitrogen doses positively affect leaf wideness at every leaf level, but early fertilizer application gives the better results. Carrasco (1997) studied four N doses (60, 120, 180 y 240 kg/ha) on the same soil type on the variety ‘Habana 92’, and found that higher doses gave taller plants with central leaves wider and longer. A strong correlation between leaf length and wideness and wrapper yield from central and top levels of the plant was found.

$Y = - 86 + 2.20 X.$

$EE = 8.03; \; r= 0.92; \; r^2 = 0.85$

$Y = - 51.6 + 2.71 X.$

$EE = 8.64; \; r = 0.9; \; r^2 = 0.81$

Leaf surface

Leaf surface rises significantly with the rise of nitrogen doses, and the bigger response is located in the central and top level of the plant

Dry matter

Dry matter response to nitrogen doses between 140 and 280 kg/ha was positive and strong. No differences were detected. At the central plant level significant effect was detected, also for nitrogen distribution. The best result was obtained from distribution II.

Chlorophyll content at early stages of vegetative cycle

Chlorophyll content rose as a result of nitrogen application doses, with statistically significant differences between all the three. A quadratic correlation between nitrogen doses and spad values was founded, with a potential value for diagnostic purposes.

Regression curves between nitrogen doses and SPAD values. For both models: $p < 0.05$.
25 DAP: $Y = 30.6 + 2.2 * 10^{-2} X - 2.7 * 10^{-5} X^2$; EE = 0.05; r = 0.999; $r^2 = 0.998$. n=40
35 DAP: $Y = 28.4 + 5.0 * 10^{-2} X - 9.6 * 10^{-5} X^2$; EE = 0.19; r = 0.998; $r^2 = 0.996$. n=40

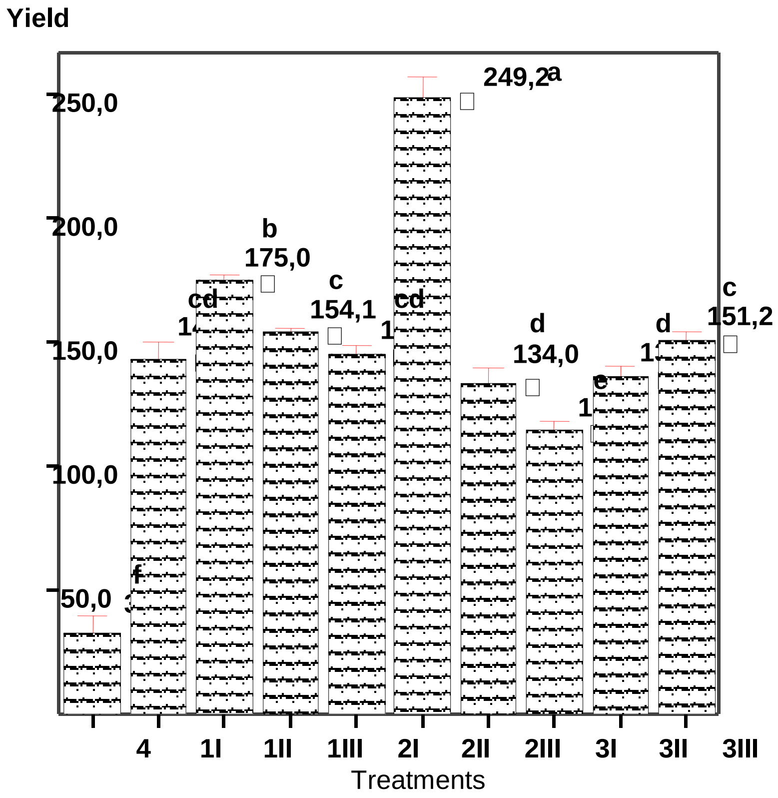
A correlation between SPAD values and wrapper yield was established:
25 DAP $Y = - 680\ 793.1 + 80\ 912.6\ X - 3\ 603.3\ X^2 + 71.3\ X^3 - 0.53\ X^4$. EE = 17.96; r = 0.50
35 DAP $Y = 97\ 255.6 - 12\ 651.7\ X + 613.1\ X^2 + 13.1\ X^3 + 0.10\ X^4$. EE = 15.24; r = 0.68. n=40.

Total leaf nitrogen at the harvesting time.

The applied nitrogen doses to the Soil positively affected total nitrogen content of tobacco leaf. Distributions II and III gave the highest results, without significant differences between them. Wrapper yield rose up to 140 kg/ha dose. Higher doses did not show a linear response. This is the reason to suggest 140 kg/ha N dose as recommendation for commercial fields of wrapper tobacco on this type of soil and technology. The more efficient plant response was to the nitrogen distribution 0-40%-60%.

Conclusions:

- For best yield and quality results in commercial production a more intensive, than the currents given recommendations, nitrogen fertilization is required.
- Higher yields are obtained from plots where morpho-agronomical parameters show a more intensive growth and development of tobacco plant.
- Due to lixiviation losses in light textured soils, late nitrogen applications are more effective than applications at planting time.



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EE = 2.836, CV = 35.80%. n = 4.

Tukey test (p<0.05).

Tobacco zone: Vuelta Arriba (Cabaiguan)

Variety: *Nicotiana tabacum* L. Var Sanctis Spiritus 96

Technology : Sun grown Soil type: Typic Eutropept

Design Field experiment (Two Years)

Treatments: Interaction between nitrogen dosages and its percentage of distribution in each application.

Nitrogen:	**0** **70 kg/ha** **140 kg/ha** **280 kg/ha**	Distributions	**40%-60%-0** **20%-75%- 5%** **30%- 70%-0**

For the development of the main plant, and for the ratoon 44kg/ha of Nitrogen were applied for all treatments. The rest of the nutrients (P, K, Mg) were applied according to the recommendations

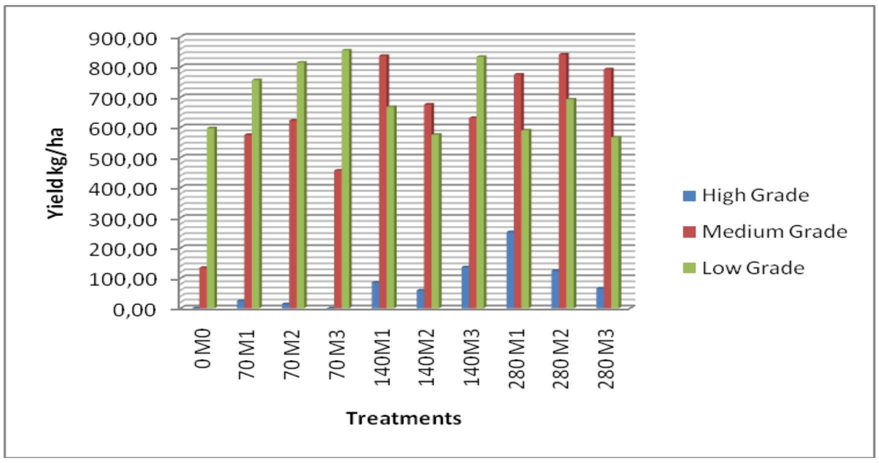
Nitrogen Current Recommendation: 102 kg/ha Distribution 0- 43%- 57%

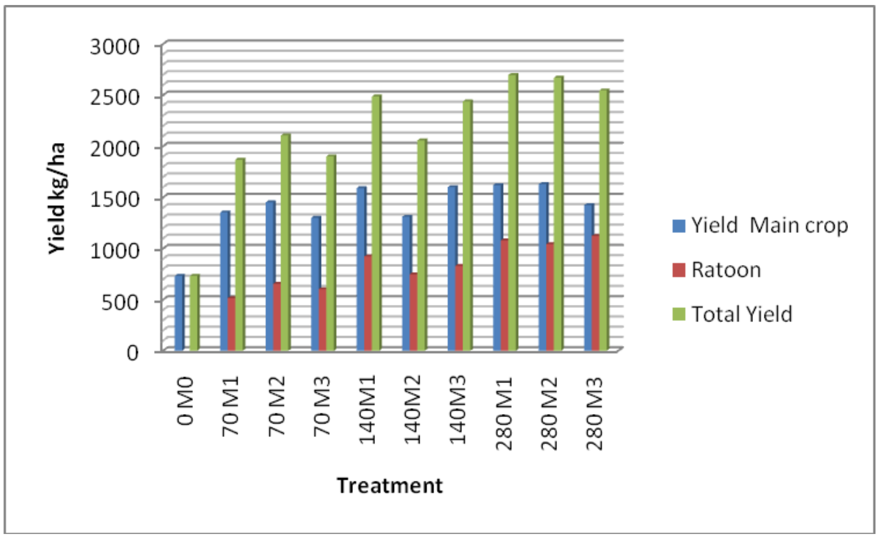
Results

Plant height (65 DAP) grew with the rise of nitrogen doses with distributions I and II. Leaf length was not significantly affected, but a trend to longer leaves with high nitrogen doses was observed. On the other side, leaf wideness was smaller without nitrogen and with the 70 kg/ha dose. A trend toward the reduction of growth parameters was observed for distribution III, but between distributions I and II there were not significant differences.

Results by Alfonso *et al*. (1997) in the Cabaiguan Tobacco Research Station demonstrated a strong dependence of yield and quality of sun grown dark tobacco on N applications and small or null effects from rising doses of phosphorous and potassium. According to distributions, the best effect on yield of high and medium classes was obtained from distribution I. Distribution III gave the worse results.

Regardless of the ratoon crop a constant amount of nitrogen was applied. It seems that a better nitrogen balance in the soil allows higher yields, where the main crop was fertilized with 180 kg/ha N.





Conclusions:

- For best yield and quality results in commercial production more intensive nitrogen fertilization is required. Almost doubled, than current recommendations.
- Nitrogen applications, just before intensive growth stage is more effective on clayish soils.
- Nitrogen fertilizers show an effect on chemical composition of tobacco plant. Nitrogen content is the most affected, but cations are also highly dependant on nitrogen availability, due to the used fertilizer.

Green tobacco leaves analysis.
Sun grown tobacco.
Nitrogen levels affect nitrogen content of tobacco leaf. Without nitrogen fertilizers the N content shows values near the deficient limit, while the higher doses show values very high untypical for Cuban dark tobacco. Potassium, magnesium and calcium contents grow with the increase of nitrogen applications, for a better burning quality of the leaves. Values of microelements contents are strongly variable, probably due to raw material contamination by Mn & Zn containing fungicides. Nitrogen doses and application times show little effect on phosphorous content of tobacco leaves. Only early application of low amounts of nitrogen causes an important phosphorous content decrease.

Similar results were obtained for shade grown tobacco (CV. ´Habana 2000).

Conclusions

- Doses and distribution of N fertilizers affect the chemical composition of tobacco leaf sun and shade grown.
- Metallic cations content of tobacco leaves grows when nitrogen doses grow, improving burning quality of the leaves.
- Up to this moment is difficult to establish any relationship between nitrogen doses and microelements content, due to their high variability.

Tabla. Nitrogen doses influence on chemical composition of green tobacco leaf.

Treatments	%					ppm			
	N	P	K	Ca	Mg	Cu	Zn	Mn	Fe
1	2,25	0,123	1,763	1,314	0,330	23,75	75,79	88,78	167,18
2	2,52	0,189	2,406	2,085	0,257	18,75	55,67	82,69	102,52
3	2,47	0,202	2,050	1,645	0,231	17,16	53,61	82,89	132,98
4	3,09	0,186	2,295	2,188	0,257	19,38	51,48	68,09	141,55
5	2,82	0,201	2,270	1,872	0,237	18,12	57,23	67,29	219,59
6	2,91	0,202	2,154	2,124	0,269	20,62	56,63	81,67	148,71
7	3,54	0,192	2,143	2,109	0,267	19,70	55,34	36,28	115,42
8	3,33	0,194	2,303	2,321	0,423	19,26	64,16	71,28	242,34
9	3,44	0,203	2,221	2,455	0,649	21,74	79,34	125,98	147,52
10	1,92	0,184	1,598	1,102	0,211	18,18	64,23	72,19	187,13

Tobacco zone: Partido (La Habana Province)

Variety: *Nicotiana tabacum* L. Var Corojo 99

Technology: Shade grown Soil type: Typic Rhodudalf

Design Field experiment (Two Years)

Treatments: Interaction between nitrogen dosages and its percentage of distribution in each application.

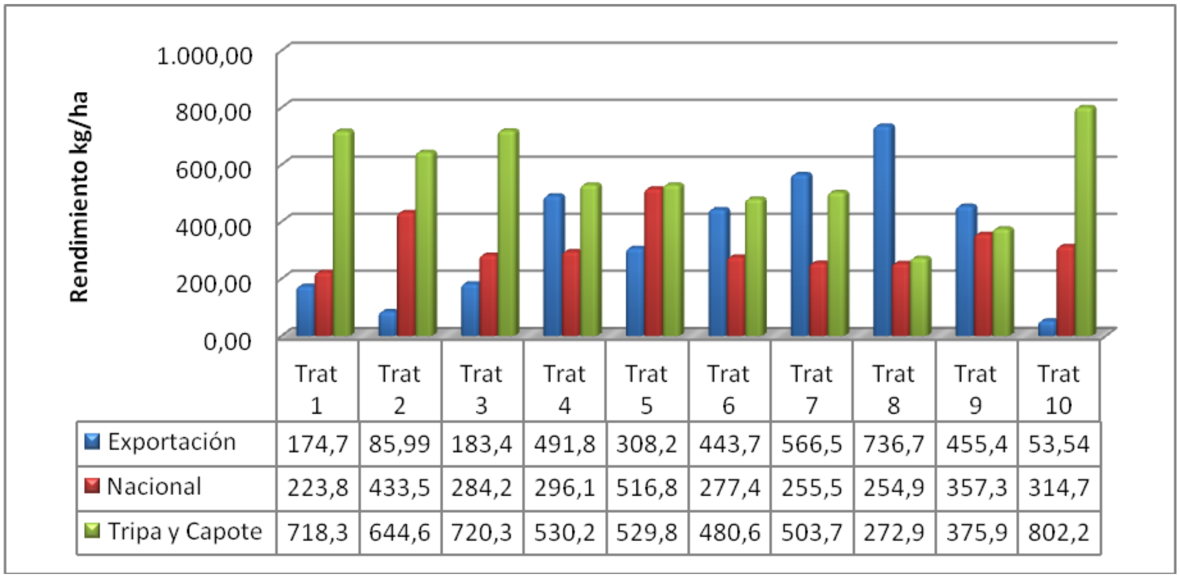
Nitrogen:		Distributions	
	0		30%-50%-20%
	70 kg/ha		15%-60%-35%
	140 kg/ha		10%-40%-60%

The rest of the nutrients (P, K, Mg) were applied according to the recommendations.

Nitrogen Current Recommendation 140 kg/ha Distribution 36%- 32%- 32%

Results

Among analyzed growth parameters plant height was not significantly affected by treatments, surely because plant height topping controls in a stronger way than nitrogen can do. Leaf length and wideness and fresh weight had significant differences between treatments, but the effect was due to the difference between control (no fertilizers) and all the other treatments.



	Trat 1	Trat 2	Trat 3	Trat 4	Trat 5	Trat 6	Trat 7	Trat 8	Trat 9	Trat 10
🟦 Exportación	174,7	85,99	183,4	491,8	308,2	443,7	566,5	736,7	455,4	53,54
🟥 Nacional	223,8	433,5	284,2	296,1	516,8	277,4	255,5	254,9	357,3	314,7
🟩 Tripa y Capote	718,3	644,6	720,3	530,2	529,8	480,6	503,7	272,9	375,9	802,2

Similar results were obtained by Gonzalez & Sacaria (1997) with the dark tobacco variety ‘Habana 2000’ grown under shade.
With regard to the vegetative growth, moderate nitrogen applications allow better growth velocity (Collins, 1982). Obtained leaves were wider and their weight by surface unit is reduced, as a result of leaf thickness reduction. Diaz *et al*. (1983) report that nitrogen applications are needed for production of wide and think leaves which may be used as wrapper.

Similar results were obtained by Diaz et al., 2000 and Venegas et al., 2002, reporting that high nitrogen fertilization promotes yields of broad, high quality tobacco leaves. Specifically for Cuban conditions it was shown that tobacco cultivated without fertilizers may reduce yield by half.
Perez & Cabrera, (1984) demonstrated the strong effect of rising nitrogen doses on tobacco yield up to a certain threshold.

Conclusions:

- For best yield and quality results in commercial production a more intensive (almost doubled), than current recommendations, nitrogen fertilization is required.
- Early nitrogen application is more effective for intensive vegetative growth of tobacco plant on soils with a compact subsurface horizon where lixiviation of nitrates is not a dominating process.

General conclusions:

- The result of the project allowed the development of more functional basis for tobacco nitrogen fertilization of Cuban dark tobacco.
- The validation in commercial fields of the conclusions of this work shows an important potential for yield and quality improvement of Cuban dark tobacco.
- Identification of nutritional patterns and typical symptoms of nutritional deficiencies for Cuban dark tobacco allows growers to establish monitoring techniques of commercial tobacco plantations. (From the results of the project a Guide for Identification of Tobacco Nutritional Diseases has been designed and now is in editorial process)
- Greenhouse techniques (hydroponic and pots culture) for nutritional studies on Cuban dark tobacco were established and validated.