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Nitrogen Fertilization for Optimizing the Quality and Yield of Shade Grown Cuban Cigar Tobacco: Required Nitrogen Amounts, Application Schedules, Adequate Leaf Nitrogen Levels, and Early Season Diagnostic Tests *

by

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SUMMARY

Nitrogen (N) fertilizers have a decisive influence on the yield and quality of tobacco. Yield, percentage of plant N, wrapper leaf quality, and nicotine content are all important quality characteristics in tobacco growing. This work is an attempt to provide a tool for optimizing mineral N nutrition for Cuban cigar tobacco, using a strategy that link**s** N supply with leaf N concentration and wrapper yield. Similar approaches developed worldwide have mainly involved Virginia and Burley tobacco types but not Cuban cigar tobacco. The objective of the current work is to identify the effects of fertilizer N levels and timing of application on each of the mentioned quality factors for shade grown Cuban cigar tobacco. Another purpose is to explore the usefulness of a quick method of assessing the N status of plants based on measuring leaf transmission at two different wavelengths (650 and 940 nm). The experiments were done in the main tobacco growing area of Cuba (Vueltabajo). In each experiment, nine separate treatments were used covering different levels and times of fertilizer N application. The same experiment was carried out in three different years (2005–2006, 2006–2007, 2007–2008) but as the results were similar only one set of data is described (2006–2007). The patterns of response to N fertilizer of all four quality measurements, including yield and wrapper leaf quality, were similar in the different replications of the experiments. The optimal fertilizer level was 140–190 kg N/ha (40% applied on days 8–10 after transplanting and 60% on days 18–20 after transplanting). The optimal N concentration of leaves taken at the central foliar level of the middle stalk position was 4.3–4.7% at harvest time. Leaf transmission measurements by means of the SPAD-502 Chlorophyll Meter in the early stages of growth were correlated with leaf chlorophyll and N concentration and provide an excellent guide for predicting Cuban cigar tobacco wrapper leaf yield. [Beitr. Tabakforsch. Int. 25 (2012) 336–349]

ZUSAMMENFASSUNG

Stickstoffdünger hat einen maßgeblichen Einfluss auf den Ertrag und die Qualität von Tabak. Ertrag, prozentualer Stickstoffgehalt der Pflanze, Deckblattqualität und Nikotingehalt sind alle bedeutende Qualitätsmerkmale im Tabakanbau. In dieser Arbeit wurde der Versuch unternommen, Wege aufzuzeigen zur Optimierung der mineralischen Stickstoffernährung für kubanischen Zigarrentabak mithilfe einer Strategie, bei der der Zusammenhang zwischen Stickstoffdüngung und der Stickstoffkonzentration im Blatt sowie dem Deckblattertrag ermittelt wird. Ähnliche Ansätze beschäftigten sich weltweit hauptsächlich mit Virginia- und Burley-Tabaken und nicht mit kubanischem Zigarrentabak. Ziel der vorliegenden Arbeit ist es, die Wirkung von Menge und Zeitpunkt bei der Stickstoffdüngung auf die oben beschriebenen Qualitätsmerkmale für beschattet angebauten kubanischen Zigarrentabak aufzuzeigen. Eine weitere Zielsetzung ist, die Nützlichkeit eines Schnellverfahrens zu untersuchen, das den Stickstoffstatus von Pflanzen mit Hilfe der Blatttransmission bei zwei verschiedenen Wellenlängen (650 und 940 nm) bestimmt. Der

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Versuch wurde in dem Haupttabakanbaugebiet Kubas (Vueltabajo) durchgeführt. In jedem Experiment wurden neun einzelne Behandlungen untersucht mit unterschiedlichen Mengen und Zeitpunkten der Stickstoffdüngung. Der Versuch wurde in drei verschiedenen Jahren (2005–2006, 2006–2007, 2007–2008) durchgeführt; da die Ergebnisse ähnlich waren, ist nur ein Datensatz (2006–2007) aufgeführt. Das Muster der Reaktion auf die Stickstoffdüngung war für alle Qualitätsmerkmale, inklusive Ertrag und Deckblattqualität, über die verschiedenen Wiederholungen des Versuches ähnlich. Die optimale Menge an Stickstoffdünger lag bei 140 bis 190 kg N/ha (40% der Menge 8–10 Tage nach Transplantation und 60% der Menge 18–20 Tage nach Transplantation). Die optimale Stickstoffkonzentration im Blatt, die im zentralen Bereich des mittleren Blattstands gemessen wurde, betrug zum Erntezeitpunkt 4,3 bis 4,7%. Blatttransmissionsmessungen mit dem SPAD-502 Chlorophyll Meter im frühen Wachstum waren mit dem Chlorophyllgehalt und der Stickstoffkonzentration im Blatt korreliert und bieten eine ausgezeichnete Hilfe, um den Deckblattertrag kubanischen Zigarrentabaks vorherzusagen. [Beitr. Tabakforsch. Int. 25 (2012) 336–349]

RESUME

Les fertilisants azotés ont une influence importante sur le rendement et la qualité du tabac. Le rendement, la teneur en azote, la qualité de la feuille de cape et le taux de nicotine sont des qualités importantes dans la culture du tabac. Ce travail a pour but l'optimisation de la nutrition minérale azotée du tabac Brun cubain pour cigare en utilisant une stratégie reliant l'apport en azote à la concentration d´azote des feuilles et le rendement en feuilles de cape. Des approches similaires ont été développées dans d'autres pays, principalement pour les tabacs Virginie et Burley, mais n'ont jamais été appliqué au tabac Brun cubains pour cigare. L'objectif de ce travail est l'étude des effets du niveaux de fertilisation azotées et de la date d´apport sur chacun des facteurs de qualité déjà mentionné précédemment pour le tabac Brun cubain pour cigare cultivé sous toile. Il s'agira ensuite de déterminer l´utilité d´une méthode simple et rapide pour évaluer la teneur d´azote des plantes en utilisant des mesures de la transmission des feuilles à deux longueurs d´onde (650 et 940 nm). Les expérimentations ont été menées dans la principale région cultivatrice du tabac à Cuba (Vueltabajo). Pour chaque expérience, neuf traitements isolés de différents taux de fertilisants azotés et de différents temps d'exposition on été réalisés. La même expérience a été menée sur trois ans (2005–2006, 2006–2007, 2007–2008); mais les résultats étant similaires, seuls les résultats d´une année sont décrits (2006–2007). Les profils de réponse aux fertilisantes azotés pour les quatre critères de qualités incluant le rendement et la qualité de la feuille de cape ont été similaires et reproductibles. Le taux optimal de la fertilisation azotée a été de 140 à 190 kg N/ha (40% ont été utilisés aux jours 8–10 après la transplantation et 60% aux jours 18–20 après la transplantation). La concentration optimale d´azote dans les feuilles récoltées au niveau foliaire médian a varié entre 4.3 et 4.7% au moment de la récolte. Les mesures de la transmission réalisées en utilisant le SPAD-502 chlorophyll meter dans les stades précoces de croissance furent corrélées avec la chlorophylle foliaire et avec la teneur en azote et constituent un excellent indicateur pour la prédiction du rendement de la feuille de cape du tabac Brun à cigare cubain. [Beitr. Tabakforsch. Int. 25 (2012) 336–349]

KEY WORDS: Cuban cigar tobacco, nitrogen fertilization, wrapper yield, SPAD-502 chlorophyll meter

ABBREVIATIONS

N	nitrogen
DAT	days after transplanting
SPAD value	reading from the Minolta chlorophyll meter SPAD-502
CV	coefficient of variation
SE	standard error
R^2	coefficient of determination
n	sample size for one treatment

INTRODUCTION

Improving crop N use efficiency (biomass production per unit of N in plant tissue) is a desirable goal in modern agriculture, which can be achieved by managing a number of external factors such as growing varieties of high yield potential and N use efficiency, and modifying the amount and application time of N fertilizer (1). The proper management of N nutrition includes the appropriate supply of the element, in the right amount and at the right time, using the most suitable chemical form, which in turn guarantees the most advantageous nutrient availability and assimilation at the whole plant level (2).

Much of the work in determining fertilizer requirements is based on the difference between crop demand and the ability of the soil to provide the required mineral N. Some workers have attempted to estimate the time course of these factors and thereby arrived at more accurate estimates of requirement (3). Crucial to the development of such models is the relationship between critical % N and mass. Much attention has been given to this aspect and the results are indeed often used for advisory purposes (4, 5). These values are useful for defining macronutrient amounts in crop fertilization regimes and have been used thoroughly for tobacco (1, 6–8). The approaches mentioned above have mainly involved the more widespread Virginia and Burley types (9). The appraisals developed so far for cigar tobacco are still empirical as they do not successfully link soil N supply with plant element concentration and yield.

Cigar tobacco is grown in Vueltabajo for manufacturing the world's best quality cigars. It is the unique place in Cuba where all types of tobacco cultivated on the island are produced and contribute 50% of wrappers and 100% of fillers and binders in Habanos's whole production. The expensive hard-to-obtain wrapper leaves come from shade grown plants and are used as cover towards the end of manufacturing; they play a major part in Cuban cigars' excellence (10). All distinguishable characteristics of wrapper leaves are extremely sensitive to N nutrition (11). Tobacco is a crop of high economic value and fertilizer N supply is the most important field practice for its growth. Excessive application of macronutrients sometimes takes place with the objective of increasing yields but this may also reduce crop quality as well as cause soil and water pollution. On the other hand, plant growth is frequently limited in plantations by the lack of this important element (12, 13).

Overabundant N supply brought about that 33.46 kg N/ha were lost by leaching in the Vueltabajo region, which was equivalent to 95.5 kg/ha of NH_4NO_3. This leaching process has increased year by year as the nitrate (NO_3^-) levels in the surrounding water reached approximately 45 mg/L in some places, the maximum allowed in Cuba for human consumption (14). There are also levels of P and K slightly above 45 mg/100 g soil and 20 mg/100 g soil, resp. that are recommended as optimum in Vueltabajo´s soils. Excessive supplies of these nutrients are not absorbed by the plant, something particularly important for K because tobacco is considered an avid K consumer (IZQUIERDO, unpublished results).

N absorption should continue until harvesting time in cigar tobacco, so N must be available along the entire vegetative growth period. This is probably the biggest difference between cigar and Virginia tobacco types (9). ESPINO *et al.* (15) recommend three times banding application of N fertilizer in Cuba's tobacco crop season: at transplanting, 30%; 8–10 DAT (days after transplanting), 50%; and 18–20 DAT the remainder.

Theoretical and practical considerations suggest that it may be possible to cease application at transplanting. Firstly, it is known that the Vueltabajo region has a soil type with high potential for N leaching, especially in nitrate (NO_3^-) form (14). Moreover, little absorption of water and nutrients takes place during the acclimatization and rosette formation period after transferring plants to field conditions. Finally, it is known (16) that yield and quality are not impaired by making split banding: 50% at transplant and 50% at any time from the first to the fifth week after transplanting.

For the shade grown cigar tobacco variety 'Criollo 98' under a fertilization regime it has been demonstrated that belated applications of N fertilizer (later than 28 DAT) reduce the quality of the leaf because its veins become more pronounced and its blade becomes thicker and darker in color. There are some disadvantages as well during curing, among other undesired characteristics (17).

Measuring N in the plant may be used for indicating N requirements. The chlorophyll meter SPAD-502 (MINOLTA, Spectrum Technologies, Inc.) uses light transmission at two wavelengths. Chlorophyll absorbance is measured at 650 nm and non-chlorophyll absorbance at 940 nm. It also provides an index of leaf greenness. SPAD readings not only strongly correlate with chlorophyll and total N concentrations but also with photosynthetic activity, which are good predictors of crop yield and quality (18–20).

Several authors have utilized SPAD values and their relationship with yield as a test of nutritional status in tobacco (*Nicotiana tabacum* L.) (21, 22), in coffee (*Coffea arabica*) (23) and in corn (*Zea mays*) (24).

Other researchers have also exploited SPAD values for specifying the moment of leaf ripeness in Virginia tobacco (25).

The following hypothesis was evaluated: Crop maximal yield and quality are closely linked to optimal response of both plant growth and nutritional status, both of which are defined by the same favorable amount and distribution of N fertilizer source under field conditions. It was also necessary to assess whether high quality wrapper yield could successfully be predicted by the nutritional status at the beginning of the main vegetative growth period. Thus, an early, fast and reliable N diagnostic test would allow farmers to adjust crop fertilizer needs before the last cultivation step and the onset of rapid growth and N accumulation.

The objectives of the present work were to define both the leaf N sufficiency range at harvest and the optimal range for SPAD chlorophyll in the early stages of plant development and to evaluate plant growth biological markers as predictors of high quality wrapper yield and nicotine concentration as quality parameters in cured leaves.

MATERIAL AND METHODS

Plant material and experiment location

The experiment was conducted in an area belonging to the Tobacco Research Station located in the Vueltabajo region (Pinar del Río province, Cuba) during the 2005–2006, 2006–2007, and 2007–2008 crop seasons. The soil is classified as "Acrisol háplico" (FAO - UNESCO classification) (26). *Nicotiana tabacum* L. plants of the variety 'Criollo 98' were grown under shade (covered with one layer of cheese cloth over and besides the fields).

Experimental design and description of treatments

A randomized complete block design in two factor arrangement was used with nine treatments and four replications each. The two factors were N amount (kg N/ha) and N application time and rate during plant vegetative development. There were three levels of each factor (Table 1); a control treatment without N application was included. Mineral nutrients were always placed as band application mixing single fertilizers in the appropriate proportions according to recommended practices (15). The total quantity of macronutrients (P, K and Mg) was applied as follows: 72 kg P/ha, 189 kg K/ha, and 24 kg Mg/ha, respectively. Top dress was applied at transplanting (72, 60 and 24 kg/ha, resp.), while split side dress fertilizations (from 8 to 10 DAT and from 18 to 20 DAT) were performed for the remaining K (49 and 80 kg/ha, resp.).

The chemical forms, in which mineral nutrients were applied, were: ammonium nitrate (NH_4NO_3) with 35.0% N (equally as ammonium and nitrate); single super phosphate with 18% P; potassium sulfate (K_2SO_4) with 44.9% K, and magnesium sulfate ($MgSO_4$) with 20.2% Mg. The plots consisted of four rows, 0.84 m apart, and 21 plants per row, 0.35 m apart. Other suggested agronomic and cultural management practices were also followed (15).

Table 1. Fertilization of Cuban cigar tobacco plants (´Criollo 98´ var.) combining three amounts of fertilizer with three application times.

Treatment	Factor 1 Fertilizer amount (kg N/ha)	Factor 2 Fertilizer application schedule (time and % of total)		
		1st Application at transplanting, top dressing	2nd Application 8–10 DAT, side dressing	3rd Application 18–20 DAT, side dressing
1I	70	30%	50%	20%
1II	70	—	40%	60%
1III	70	—	60%	40%
2I	140	30%	50%	20%
2II	140	—	40%	60%
2III	140	—	60%	40%
3I	280	30%	50%	20%
3II	280	—	40%	60%
3III	280	—	60%	40%
4	0	—	—	—

Curing, fermentation, grade selection and yield calculation

After leaves were properly air cured they were subjected to natural fermentation (15) followed by grade selection and calculation of high quality wrapper yields (27). Relative wrapper yield in each plot was estimated as the percentage of maximal high quality wrapper yield in the experiment. The leaf N sufficiency range and the early SPAD chlorophyll reading optimal range calculated guaranteed a high quality wrapper yield equal to or more than 90% of maximal yield.

Soil sampling

Soil samples were taken from each plot before transplanting and after harvesting following the procedure described below. Four samples collected to a depth of 30 cm were mixed and homogenized. Samples were taken before the establishment of plants under field conditions and after harvesting all leaves. Samples were transported to the laboratory in black polyethylene bags, air dried and sieved through a 0.5 mm mesh sieve. Finally, they were stored in hermetic plastic pots for future analysis.

Chemical soil analysis

The following chemical soil determinations were made before transplanting and after harvesting: pH according to ISO (28), organic matter (29), total N by the NESSLER method (30). Also K and the exchangeable ions (Na, Mg, Ca) by the ammonium acetate method (31), and Zn, Cu, Fe and Mn (32) before planting.

Plant sampling, sample preparation and leaf growth biological markers

Four plants were randomly selected from the two central rows of each experimental plot for all observations and determinations. Leaf sampling at harvest was carried out beginning with the lowest pair of leaves and ascending successively from bottom to top. It was done at about three day intervals between each harvesting in order to allow proper leaf ripeness. All seven foliar levels from selected plants were transferred to the laboratory and leaf growth was determined immediately after harvest. Each plant was divided into three stalk positions: bottom (two lower foliar levels), middle (three central foliar levels) and top (two upper foliar levels). Leaves were washed with distilled water and dried by vigorous shaking (33). The pair of leaves for each foliar level was weighed and its area was calculated according to $A = L \cdot W \cdot 0.7$, where A is leaf area, L is length, W is width and 0.7 is a factor proposed by TORRECILLA and PINO (34).

The two upper leaves in the bottom stalk position, the two central leaves in the middle position and the two upper leaves in the top position were oven dried (60 °C) and weighed again. Afterwards, they were crushed in a stainless steel surface mill (IKA Labortechnik, Staufen, Germany) and stored in plastic pots for later chemical analysis.

Whole plant growth biological markers: plant height and stalk diameter

Plant height was determined (with 0.1 cm error) from the base to the shoot apex at 25 and 35 DAT and also after harvesting all leaves (85 DAT); the stalk diameter (with 0.1 mm error) between the two lowest internodes was determined at 85 DAT.

Markers of nutritional status: leaf total nitrogen (N) and chlorophyll

For the leaf total N determination (with 0.01% error on a dry weight basis) 0.1 g of dry powdered leaf material was weighed and subjected to KJELDAHL digestion (VELP Scientifica, Usmate, Italy). Subsequently, ammonia concentration was determined by the NESSLER colorimetric technique (30).

Early SPAD chlorophyll values

The third leaf (counting from the base to the shoot apex) from one plant in each plot was sampled for estimating the possible relationship between SPAD readings and real chlorophyll content at 25 DAT. This plant was not subjected to any other analysis. The sampled leaf was cleaned very carefully with distilled water and hand dried. Six readings using the chlorophyll meter SPAD-502 (with 0.1 unit error) (Minolta, Spectrum Technologies, Inc.) were performed in the right central region of the leaf with the apex directed towards the person, who carried out the determination. After that, a leaf disk (0.1 g) was sampled from near the spot where the six SPAD readings were taken and total chlorophyll concentration (% on a fresh weight basis) was chemically determined (35). The same procedure was in parallel applied in the field by taking SPAD values on the selected four plants of each plot. An identical routine was also followed at 35 DAT.

Late SPAD chlorophyll values (at harvest)

SPAD chlorophyll readings were taken at the apex, center and base of the leaf at both sides of the central vein. These six readings were averaged by leaf and a single value was obtained.

Nicotine determination

Nicotine content was obtained after grade selection applying steam distillation (36).

Statistical analysis (37)

As crop year effect was consistent for the selected variables (data not shown), only results from the 2006–2007 crop season are reported. Experimental data of each analyzed variable were subjected to the KOLMOGOROV-SMIRNOV test for checking normality. LEVENE's test was applied for examining the homogeneity of variances. The parametric two-way analysis of variance (ANOVA) test was also performed with all variables, in which main effects and factor interactions were evaluated, namely the N amount (at three levels) and N application along the vegetative growth period (at three dates). For those variables where factor interaction was significant the best level combination between factors was determined using one-way ANOVA evaluating treatment effects.

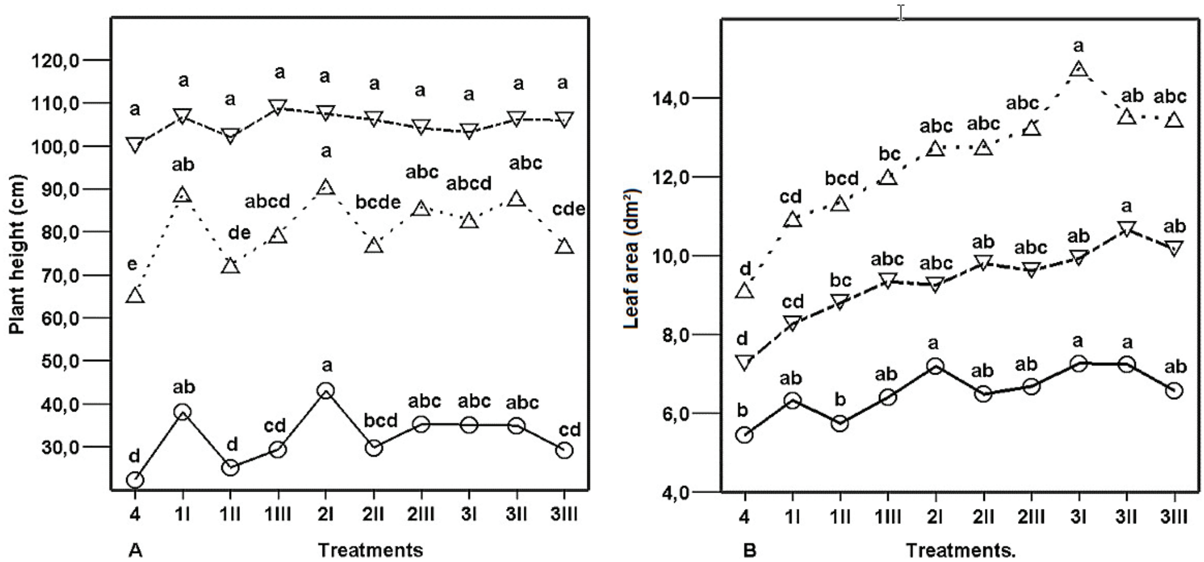

Figure 1. Growth markers for Cuban cigar tobacco plants. Means with different letters are significantly different according to TUKEY's HSD test (p <0.05).

(A) Plant height at early stages of plant development and after harvesting all plant leaves (85 DAT):
- ○ and solid line at 25 DAT: SE = 1.92; CV = 41.66%; n = 16
- Δ and dotted line at 35 DAT: SE = 2.67; CV = 22.05%; n = 16
- ∇ and dashed line at 85 DAT: SE = 2.09; CV = 11.33%; n = 16

(B) Leaf area at harvest time at different stalk positions:
- ○ and solid line at the harvest time of 48–54 DAT at the bottom stalk position (two foliar levels): SE = 0.28; CV = 26.45%; n = 16
- Δ and dotted line at the harvest time of 56–67 DAT at the middle stalk position (three foliar levels): SE = 0.57; CV = 34.03%; n = 16
- ∇ and dashed line at the harvest time of 70–76 DAT at the top stalk position (two foliar levels): SE = 0.32; CV = 21.95%; n = 16

How to interpret the graphical depictions of TUKEY's test results:
TUKEY's test compares all possible pairs of means in a data set and determines which means are significantly different from one another. In Figure 1, the results of six separate TUKEY's tests are graphically displayed in (A) for three different DAT and in (B) for three different harvest times. Within each (of the six) statistical comparison, the means marked with the same letter (whether single or in combination with other letters) are not significantly different.
For example, in (A) for 85 DAT (top line) all means show the letter "a" and, consequently, are not significantly different from one another. Again in (A) for 25 DAT (bottom line) the mean for treatment 4 shows the letter "d" and, therefore, is not significantly different from treatments 1II, 1III, 2II and 3III but significantly different from treatments 1I, 2I, 2III, 3I and 3II (all of which do not show the letter "d").

TUKEY's significant difference test was performed for establishing differences between means corresponding to main or treatment effects. TUKEY's test results are graphically depicted in Figures 1, 3 and 4. Advice regarding their interpretation is included in the caption of Figure 1. Regression and correlation analysis with each analyzed variable and the variables and yield components were performed for assessing predictors of N sufficiency in terms of early diagnosis of yield before harvesting. The F test ($p < 0.05$) was significant for all regression equations examined. A 5% significance level was used for all statistical tests using SPSS version 12.0 and CurveExpert 1.3 statistic platforms.

RESULTS

Soil analysis

All elements analyzed before transplanting were within the typical ranges reported (38) for the type of soil where the experiment was performed (Table 2). Organic matter was in a range from minimum to medium, while at the end of the crop season the value was slightly above the maximum reported in the literature. Na was below the minimum; Mg was between the minimum and medium values as was K. There were no statistically significant differences among the plots for N concentration, not only before starting the experiment but also at the end.

Table 2. Soil analysis before the transplantation and after the harvesting of Cuban cigar tobacco plants.

Analysis	Before transplantation	After harvesting
pH in KCl	5.07	5.12
Organic matter (%)	1.99	2.32
N (%)	0.096	0.099
K (meq/100 g of soil)	0.45	na
Ca (meq/100 g of soil)	2.8	na
Mg (meq/100 g of soil)	0.5	na
Na (meq/100 g of soil)	0.06	na
Zn (ppm)	5.01	na
Cu (ppm)	1.38	na
Fe (ppm)	11.24	na
Mn (ppm)	16.24	na

na: not analyzed
Results are shown as means of all experimental plots.

Whole plant growth biological markers: plant height and stalk diameter

Plant height

Plant height was determined three times during plant development: at 25, 35 and 85 DAT (when harvesting was completed). Early application of fertilizer with 140 kg N/ha (treatment 2I) resulted in optimal growth not only at 25 DAT but also at 35 DAT (Figure 1A). Plant height at 85 DAT was not influenced by the amount of N or its application time and rate. Plant height was not correlated with relative wrapper yield at any time point analyzed.

Stalk diameter

Supplying 70 kg N/ha (lowest amount) was sufficient for obtaining an optimal diameter regardless of application (data not shown). No correlation was seen with relative wrapper yield.

Leaf growth

The amount of N was the most important factor for obtaining the biggest leaf area at the whole plant level (Figure 1B). 140 kg N/ha (medium amount) guaranteed optimal leaf growth for all stalk positions while early N application generated the biggest leaf elongation only in bottom leaves. N application did not significantly enhance leaf growth at middle and top stalk positions.

Nutritional status biological markers: early season SPAD chlorophyll values and SPAD values at harvest

Early season SPAD chlorophyll values

Early season SPAD values were correlated with total chemically determined chlorophyll (Figure 2A), a high positive correlation coefficient was obtained.
The amount of N fertilizer applied to the soil was the most important factor for obtaining the highest SPAD readings at 25 DAT. 140 kg N/ha (medium amount) was sufficient while the N application schedule was less crucial. Plants receiving early N application (treatment I) had better nutritional status (Figure 3A). A quadratic type correlation was detected between SPAD values and the amount of N fertilizer, which was useful for nutritional diagnosis (Table 3, section 5). It is important to notice that the highest quantity of N fertilizer at transplanting (treatment 3I) did not produce negative effects in plant chlorophyll content. A correlation was also detected between early season SPAD readings and relative wrapper yields (Table 3, section 1).
The total amount of N fertilizer was more important than its application schedule for achieving the highest concentration of total chlorophyll at 35 DAT (Figure 3A). 140 kg N/ha (medium amount) produced optimal results. The application schedule did not have any effect. A strong quadratic type correlation of early season SPAD values with N amount (Table 3, section 5) and relative wrapper yield (Table 3, section 1) was detected. It should be emphasized that SPAD chlorophyll values were lower at 35 DAT than at 25 DAT, except for treatment 2II.

SPAD values at harvest (late SPAD values)

Late SPAD readings showed a positive correlation with chemically determined total N concentrations (Figure 2B). They were obtained at three foliar levels of the plant: the upper foliar level at the bottom stalk position, the central foliar level at the middle stalk position and the upper foliar level at the top stalk position (data not shown). In all cases the total quantity of N was the decisive factor for obtaining the highest SPAD values at harvest. 140 kg N/ha (medium amount) produced optimal results. The way in which N was applied to the plants had a significant effect on the upper foliar level at the bottom stalk position and the upper foliar level at the top stalk position while it did not show any effect on the central level at the middle stalk position. Regression equations were fitted for late SPAD values at each analyzed foliar level and the quantity of N supplied to the soil (data not shown).

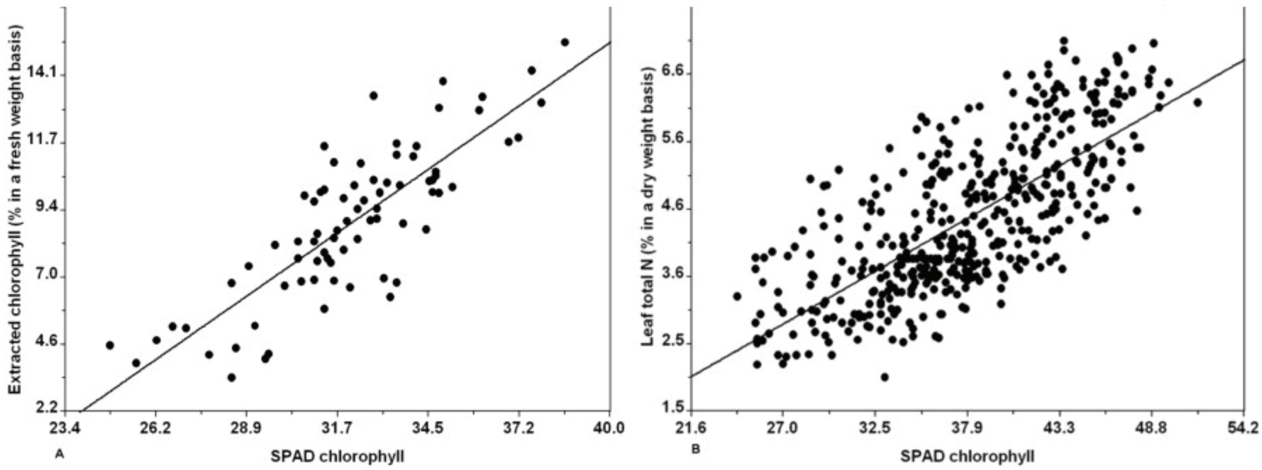

Figure 2. Measurements of the chlorophyll content of Cuban cigar tobacco leaves with the SPAD-502 chlorophyll meter.
(A) Relationship between chlorophyll extracted from leaf discs and SPAD readings at early stages of plant development (25–35 DAT). $y = -17.4 + 0.82\,x$; SE = 1.51; $R^2 = 0.69$; n = 4; $p < 0.05$.
(B) Relationship between leaf N content and SPAD chlorophyll readings at a late stage (harvest time) of plant development, utilizing data for the three foliar levels. $y = -1.15 + 0.14\,x$; SE = 0.78; $R^2 = 0.56$; n = 12; $p < 0.05$.

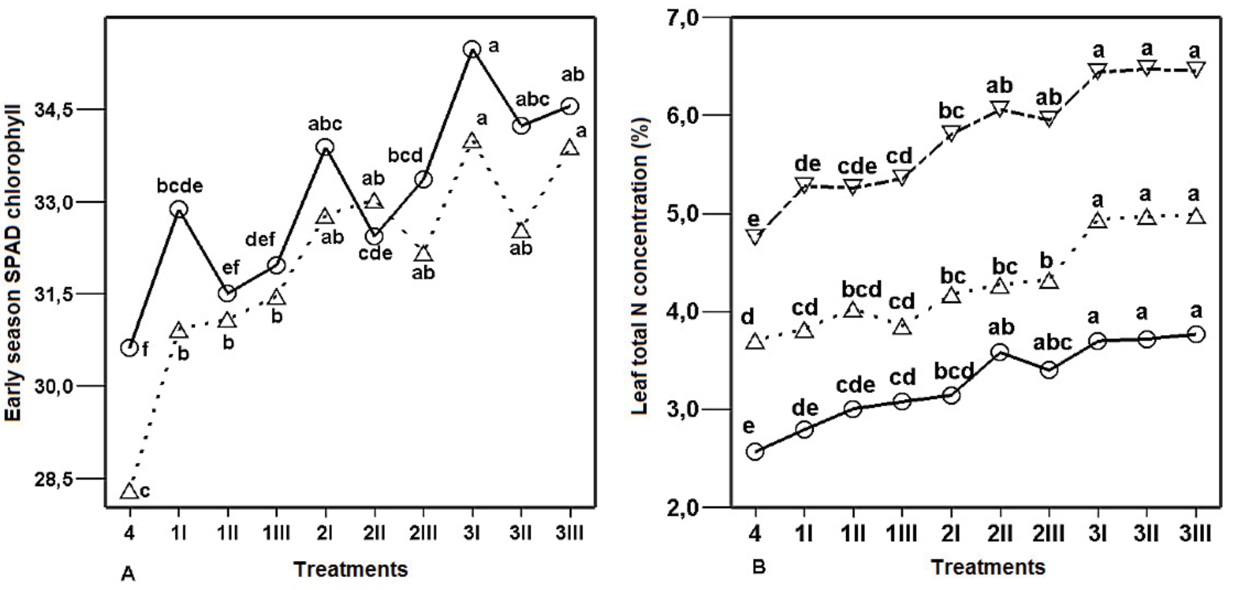

Figure 3. Effects of fertilizer treatment on the chlorophyll and total N content of Cuban cigar tobacco leaves. Means with different letters are significantly different according to TUKEY's HSD test ($p < 0.05$).
(A) SPAD chlorophyll in the third leaf from the shoot base during early developmental stages of the plant:
○ and solid line at 25 DAT: SE = 0.404; CV = 8.12%; n = 16
∆ and dotted line at 35 DAT: SE = 0.479; CV = 9.86%; n = 16
(B) KJELDAHL total N concentration in leaves at harvest time:
○ and solid line at the harvest time of 52–54 DAT in the upper leaves at the bottom stalk position: SE = 0.098; CV = 17.23%; n = 16
∆ and dotted line at the harvest time of 65–67 DAT in the central leaves at the middle stalk position: SE = 0.104; CV = 15.14%; n = 16
∇ and dashed line at the harvest time of 74–76 DAT in the upper leaves at the top stalk position: SE = 0.121; CV = 12.89%; n = 16

Leaf total nitrogen (N) sufficiency range and optimal fertilization range

Leaf total N concentration increased from bottom to top foliar levels. The quantity of N supplied to the soil was more important than its application schedule for reaching optimal leaf N concentrations at the whole plant level (Figure 3B). 140 kg N/ha supplied to the soil at intermediate (II) or late (III) time points were sufficient for obtaining optimal leaf total N concentrations at the upper foliar level in the bottom stalk position and the upper foliar level in the top stalk position. The N application schedule had no significant effects at the central foliar level in the middle stalk position. A quadratic response curve was fitted between relative wrapper yield and leaf total N concentration at harvest for the central foliar level at the middle stalk position, which allowed the calculation of a leaf N sufficiency range from 4.3 to 4.7% (Table 3, section 2). A strong and positive linear relationship was seen between leaf total N concentration and N fertilizer supply (Table 3, section 6), which permitted estimating the optimal fertilizer amount being between 140 and 230 kg N/ha.

Yield of high quality wrapper for premium cigar manufacturing

High quality wrappers generate the highest cash income. Therefore, high quality wrapper yield is a marker of crop quality (17). Of the main factors determining crop yield the combination of N amount and application schedule was decisive for whole plant yield response (Figure 4B).

Table 3. Regression equations for calculating optimal ranges of biological markers and fertilizer for Cuban cigar tobacco production ($p < 0.05$); n = 16 for equations in sections 3, 5 and 6; n = 4 for equations in sections 1, 2, 4 and 7.

Time (DAT)	Phenological phase	Foliar level	Equations	R^2	Optimal range
Equations predicting relative yield of high quality wrapper (y)					
Section 1. Early season SPAD values (x)					
25	Main vegetative growth period	Third leaf from shoot base	$y = -3052.34 + 178.39\,x - 2.54\,x^2$	0.86	34–37[1]
35	Main vegetative growth period	Third leaf from shoot base	$y = -1528.11 + 90.06\,x - 1.24\,x^2$	0.90	33–39[1]
Section 2. Total N concentration in leaves at harvest (%) (x)					
65–67	Ripeness [7]	Central at middle stalk position	$y = -1372.36 + 652.45\,x - 75.59\,x^2$	0.77	4.3–4.7[2]
Section 3. Leaf length (cm) (x)					
65–67	Ripeness [7]	Central at middle stalk position	$y = -47.41 + 1.85\,x$	0.83	–
Section 4. Nicotine concentration in cured leaves (%) (x)					
65–67	—	Central at middle stalk position	$y = -1654.06 + 1363.33\,x - 266.09\,x^2$	0.83	2.5–2.7[3]
Estimated optimal amounts of fertilizer (kg N/ha) (x)					
Section 5. Early season SPAD values (y)					
25	Main vegetative growth period	Third leaf from shoot base	$y = 28.3 + 4.0 \cdot 10^{-2}\,x - 4.6 \cdot 10^{-5}\,x^2$	0.83	180–420[4]
35	Main vegetative growth period	Third leaf from shoot base	$y = 27.3 + 6.0 \cdot 10^{-2}\,x - 10^{-4}\,x^2$	0.71	120–300[4]
Section 6. Total N concentration in leaves at harvest (%) (y)					
65–67	Ripeness [7]	Central at middle stalk position	$y = 3.6 + 5.0 \cdot 10^{-3}\,x$	0.71	140–230[5]
Section 7. Nicotine concentration in cured leaves (%) (y)					
65–67	–	Central at middle stalk position	$y = 2.0 + 5.0 \cdot 10^{-3}\,x - 1.2 \cdot 10^{-5}\,x^2$	0.69	113–250[6]

[1] Optimal range of early season SPAD values, guaranteeing relative yields of wrappers equal or higher than 90% of maximal yield.
[2] Sufficiency range for leaf total N that produces relative wrapper yield equal or higher than 90% of maximal yield.
[3] Optimal range for nicotine concentration in cured leaves that allows obtaining relative wrapper yield equal or higher than 90% of maximal yield.
[4] Optimal range of fertilizer (kg N/ha) needed for attaining the optimal range estimated for SPAD values in section 1.
[5] Optimal range of fertilizer (kg N/ha) needed for attaining the estimated N sufficiency range in section 2.
[6] Quantity of fertilizer (kg N/ha) required for reaching the calculated cured leaf nicotine concentration range in section 4.
[7] Adequate time for harvesting leaves at a given foliar level.

Late application of 140 kg N/ha (medium amount) produced an optimal wrapper yield response at the whole plant level (treatment 2II).

High quality wrapper yield values were estimated for the three analyzed stalk positions (bottom, middle and top) (Figure 4A). There is no calculation for the bottom position because no wrappers were obtained in any treatment except in 2I. Treatment 2II achieved the biggest wrapper yield response at the middle position, while at the top position the maximal yield response was obtained by applying 140 kg N/ha, combined with application II or III (treatments 2II or 2III). The middle stalk position had the highest yield. 140 kg N/ha applied in treatment 2II produced the best high quality wrapper yield when not only whole plant results (Figure 4B) were taken into account but also the three analyzed stalk positions (Figure 4A). Supplying more N fertilizer caused a decrease of this yield component despite the fact that leaf growth was not affected (Figure 1B).

Nicotine

The amount of N fertilizer was crucial for achieving optimal nicotine concentration in cured leaves while the N application schedule had no significant effect (data not shown). 140 kg N/ha allowed optimal metabolite accumulation at the central foliar level in the middle stalk position. Nicotine concentration showed quadratic relationships with relative wrapper yield (Table 3, section 4) and the optimal range of nicotine concentration in cured leaves was from 2.5 to 3.0%. An optimal fertilizer range from 113 to 250 kg N/ha (Table 3, section 7) allowed reaching the above-mentioned optimal range of nicotine concentration in cured leaves.

DISCUSSION

Improving high quality wrapper yield is a major goal for farmers specializing in shade grown cigar tobacco. Therefore, an optimized N nutrition management program is important. Two main aspects were studied, the amount of N and its application schedule throughout the vegetative growth period.

This is the first preliminary attempt of taking a more functional approach to cigar tobacco N nutrition field experiments in Vueltabajo. The conceptual framework of the present assessment relies on the fact that crop yield responses are closely linked to crop growth and chemical composition (6), which can be modulated by N supply and application schedule during plant development.

Thus, consideration is first given to how the amount of N applied to the soil influences leaf growth, nutritional status and chemical composition. Their relationship with relative wrapper yield is specifically considered.

High quality wrapper yield affected by nitrogen (N) amount

The availability of N in the soil and its uptake and incorporation in the plant are permanently interrelated during crop development. Total N concentration in the 0–30 cm deep soil layer was not significantly different between experimental plots just before transplanting (Table 2). Increases in leaf total N concentration are, therefore, likely to depend on the amount of N supplied by fertilization.

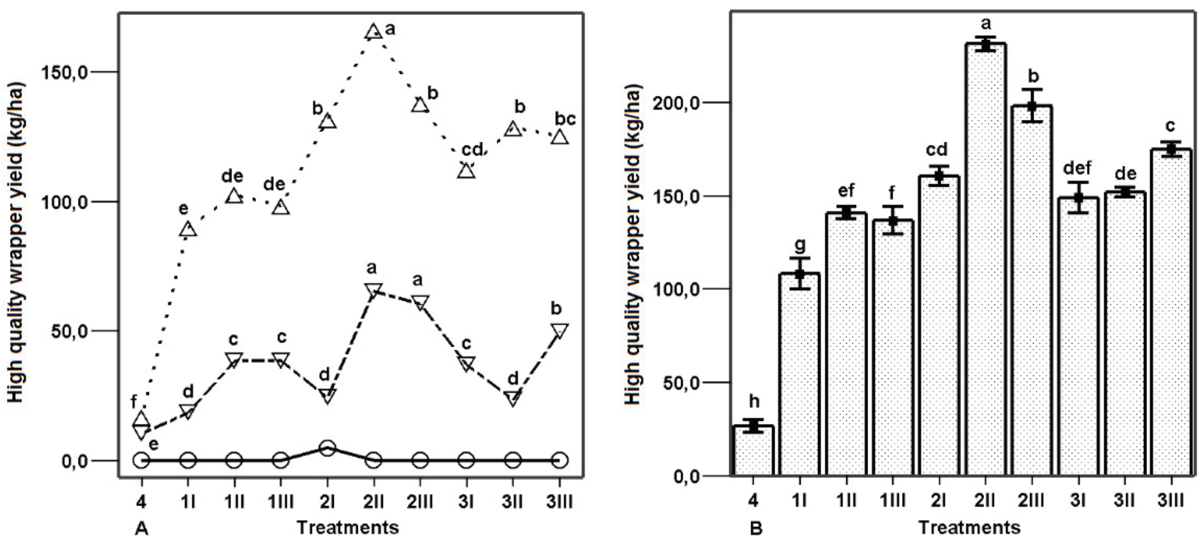

Figure 4. Effects of fertilizer treatment on the yield of high quality wrappers. Means with different letters are significantly different according to Tukey's HSD test ($p < 0.05$).
(A) Yield of wrapper leaf at different stalk positions:
○ and solid line at the harvest time of 48–54 DAT at the bottom position (two foliar levels): n = 4; SE and CV not calculated
Δ and dotted line at the harvest time of 56–67 DAT at the middle position (three foliar levels): SE = 2.868; CV = 38.81%; n = 4
∇ and dashed line at the harvest time of 70–76 DAT at the top position (two foliar levels): SE = 1.428; CV = 47.9%; n = 4
(B) Yield of high quality wrapper at the whole plant level: SE = 2.941; CV = 35.61%; n = 4

The upper foliar level at the top stalk position had on average 1.34 times the N content of the central level at the middle stalk position, which in turn had 1.31 times the N content of the upper level at the bottom stalk position (data not shown). Coefficients of variation below 20% were found for leaf total N estimates at the three foliar levels tested. This is crucial for reliable plant tissue macroelement analysis (1).

A supply of around 190 kg N/ha permitted reaching maximal relative wrapper yield at the central foliar level in the middle stalk position (based on the correlation between relative wrapper yield and N amount shown in Figure 5). Therefore, an optimal fertilizer range from 140 to 190 kg N/ha is recommended as necessary for reaching optimal leaf N sufficiency levels though the optimal amount of fertilizer was actually estimated to be 140 to 230 kg N/ha (Table 3, section 6).

Middle stalk positions represented approximately 74.9% of the whole plant high quality wrapper yield in this experiment (data not shown). This is the reason why the optimal N fertilizer range was estimated by using the N sufficiency level in the central foliar level at the middle position along the shoot axis. Leaf total N concentration in the central foliar level at the middle stalk position increased slightly (0.005%) when banded N supply rose by 1 kg/ha (Table 3, section 6). Several authors studying N nutrition in cigar tobacco agree that the central leaves at the middle stalk position supply the highest share of high quality wrapper yield (17, 39, 40).

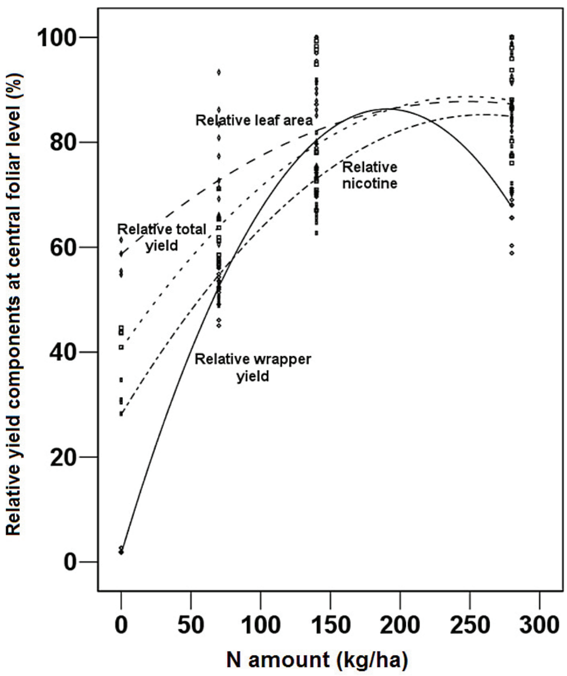

Figure 5. Regression curves for various relative yield components at the central foliar level in the middle stalk position of Cuban cigar tobacco plants and the total N fertilizer amounts used. n = 4 for all curves.
Dashed line = relative leaf area: $y = 55.67 + 0.23\,x - 5.0 \cdot 10^{-4}\,x^2$; SE = 8.49; $R^2 = 0.54$
Solid line = relative wrapper yield: $y = 1.59 + 0.89\,x - 2.0 \cdot 10^{-3}\,x^2$; SE = 7.09; $R^2 = 0.92$
Dotted line = relative total yield: $y = 40.64 + 0.37\,x - 7.0 \cdot 10^{-4}\,x^2$; SE = 8.75; $R^2 = 0.75$
Dashed/dotted line = relative nicotine: $y = 28.09 + 0.44\,x - 8.0 \cdot 10^{-4}\,x^2$; SE = 9.00; $R^2 = 0.80$.

This may be a consequence of more alpha-keto acid molecules being available at the middle stalk position for ammonium (NH_4^+) assimilation, which enhances leaf growth and nutritional status (41).

Current recommendations specify fertilizer levels between 120 and 140 kg N/ha (40). However, a higher amount of N fertilizer is suggested. The increase of relative wrapper yield by 7.03% (based on the correlation between relative wrapper yield and N amount shown in Figure 5) means a yield rise of 5.28 kg/ha (data not shown) when the mean optimal amount of N fertilizer reported here is compared to the mean currently used.

Guerra *et al.* (17, 39) report similar results as they find a high quality wrapper yield decline not only with decreased N fertilizer supply but also with excessive N amounts.

Leaf growth and chemical composition are the major determinants of yield. It is well known that N supply improves leaf growth to a greater extent than leaf photosynthesis (42). Increasing the suboptimal N amount from 0 to approximately 140 kg/ha, a relative rise of wrapper yield was observed, combined with a concomitant rise in relative leaf area (Figure 5). A simultaneous elevation of the relative nicotine concentration and the relative total yield was also detected. N supply in the optimal range of 140–230 kg N/ha allowed reaching maximal relative wrapper yields (around 190 kg N/ha) while the other relative yield components increased slightly up to the 230 kg N/ha upper limit. Additional N application above the optimum led to declining relative wrapper yields without decreasing the other yield components.

A relatively strong positive linear relationship was observed between leaf length and relative wrapper yield (Table 3, section 3). An increment of 1 cm in leaf length produced a relative wrapper yield increase of 1.85%, which represents nearly 2.5 kg/ha more of high quality wrapper yield (data not shown). This relationship is based on the current grade selection. Once cured leaves are grouped according to certain visual characteristics, they are sorted by length. More valuable wrapper leaves are those with bigger length (27). This finding defines for first time in the Vueltabajo region how leaf growth impacts on relative wrapper yield.

A quadratic equation was developed describing a relationship for predicting relative wrapper yield for the full range of cured leaf nicotine concentrations (Table 3, section 4). Optimal cured leaf nicotine concentration levels and the quantity of fertilizer needed for producing them are also reported (Table 3, section 7). Metabolite concentration was only influenced by the amount of N. Several authors agree with this statement because they found that nicotine concentration rose with the quantity of N applied (43). Other research (44) disagrees showing that cured leaf nicotine concentration is equally affected by the amount of N as by its application schedule. Anyway, split application with a very late second fertilization is suggested.

The reported range of N sufficiency at the central foliar level is preliminary because it represents an instant view at the plant nutritional status at the final stage of central leaf development without enough time for correcting N deficiencies. Furthermore, plant tissue total N analysis is time-consuming and generally requires sample preparation and specialized equipment. However, it is of great value for obtaining the optimal range of N fertilizer in a way that successfully links soil N supply, leaf nutritional status and high quality wrapper yield. It would, however, appear to be more practical to use the critical N concentration approach, which allows the precise determination of N sufficiency in a dynamic way. Furthermore, the usefulness of this approach is emphasized by the fact that the relationship between critical N concentration and crop biomass may be insensitive to changes in the aerial environment (45). Nonetheless, the practical applicability of critical concentration in shade grown cigar tobacco is questionable because ripened leaves are successively removed from bottom to top stalk positions as they ripen, which affects the absorption of light by the photosynthetic pigments in the individual leaves and thus the N content of the whole plant. Moreover, one month of the three needed for shade grown cigar tobacco production has to be used for harvesting leaves.

Nitrogen (N) application schedule effects on high quality wrapper yield

It is important to notice that SPAD chlorophyll diminished from 25 to 35 DAT except in treatment 2II (Figure 3A). A similar trend was reported by MacKown and Sutton, (22). This treatment produced not only optimal leaf growth at the middle and top stalk positions (Figure 1B) but also the highest high quality wrapper yield (Figures 4 A and B). The early bottom leaves benefit from the effect of early N application, which explains the fact that early fertilized plants with medium N supply showed not only improved plant height and nutritional status at 25 and 35 DAT but also the strongest bottom leaf growth at harvest (Figures 1A and B, Figure 3A). However, the plants, which received N fertilizer belatedly, showed optimal growth of middle and top leaves (Figure 1B) and optimal nutritional status of the whole plant at harvest (Figure 3B), which in turn produced the largest high quality wrapper yield (Figures 4A and B).

As for plant height, a similar trend was observed at the early stages of plant development (25 and 35 DAT; Figure 1A). Early fertilized plants (application I) made contact with N earlier than late fertilized ones. Consequently, they had more time for N absorption and assimilation until 25 or 35 DAT, which guaranteed better N uptake. This explains why plants, which received N belatedly (applications II and III), had the lowest plant height compared to plants, where N was provided since transplantation. When height was measured after harvesting all plant leaves (85 DAT; Figure 1) no differences were observed. The practice of topping to a fixed number of leaves may be an important factor in explaining this behavior. Every cigar tobacco plant was topped to seven pairs of leaves (14 leaves).

Root growth is the most important and common distinguishable feature for characterizing the first two phenological phases of cigar tobacco, i.e. acclimatization and rosette formation (10, 44). The role of nitrate as a positive regulator of its own absorption, reduction and assimilation could explain the results obtained. Factors like N spatial and chemical availability, the number and activity of N transport systems, and the nitrate concentration during the induction of the synthesis of N transport systems are involved in the regulation of N uptake at the cellular level (45, 46, 47).

Leaf nutritional diagnosis at early stages of development. Estimation of additional nitrogen (N) requirements

Early SPAD values at the optimal level at 25 and 35 DAT permitted achieving a high quality wrapper yield of nearly 90% of the maximal response (Table 3, section 1). Correlations were established of early SPAD values with leaf chlorophyll and total N levels (Figures 2A and 2B), which allowed their quantitative determination using single and easy field measurements.

Predictions of relative wrapper yield by means of early SPAD measurements were made using quadratic equations (Table 3, section 1). Small changes in SPAD values were associated with large changes in relative wrapper yield. Therefore, the margin of error in obtaining representative values needs to be minimized. Yield response to SPAD values became linear ($y = -421.7 + 15.3x$; $R^2 = 0.66$; $p < 0.05$) by eliminating points with relative wrapper yields equal or higher than 90% at 25 DAT. An increase of about 15.3% in high quality wrapper yield (20 kg/ha, data not shown) was linked with the rise of the SPAD value by 1 unit. The same procedure at 35 DAT also allowed to develop a linear equation ($y = -205.9 + 8.6x$; $R^2 = 0.77$; $p < 0.05$). The increase by 1 SPAD unit produced a rise of approximately 8.6% in relative wrapper yield (11.4 kg/ha, data not shown). Plots that received additional N fertilizer above the optimum were found to be less useful for nutritional diagnosis because SPAD values showed only a weak correlation with leaf total N (20, 48, 49).

Kowalczyk-Juśko and Kościk (21) explained that the correlation between SPAD readings and leaf N concentration diminishes as the crop season advances. Therefore, late periods of vegetative development show the lowest correlations. Probably, N compounds other than chlorophyll and proteins of the light-harvesting complex can accumulate in leaves as plant development proceeds. Therefore, crop nutritional status evaluations based on SPAD readings are possible only in early stages of vegetative development. It was decided to sample third leaves, counting from base to shoot apex, because lower foliar levels are capable of showing N deficiency symptoms earlier than the other ones (11, 50). SPAD readings were always taken in the same blade region for guaranteeing the highest possible data homogeneity. Samples were retained as chlorophyll measurements are non-destructive.

About 35 DAT is the time of final cultivation work when the soil between rows is turned. This helps to maintain humidity, control weeds and aerate the soil and is the last opportunity for a complementary application of N fertilizer to deficient plants. To calibrate the SPAD readings in accordance with local field conditions (variety, climate, etc.) well-fertilized reference plots receiving sufficient N amounts should be established (19, 51). The most commonly used approach is to calculate an N sufficiency index (expressed as a percentage) as the quotient of average SPAD values from the trial and reference plots. This index is regressed against the relative yield and, subsequently, a threshold value is obtained. Below this cut-off point the additional application of N fertilizer is needed (20).

In order to determine the exact amount of additional N it is necessary to measure the mineral N in the soil at the start of the main vegetative growth period to the depth of rooting in both test and reference plots. This permits the rough calculation of how much of the total amount of N applied is still available. The difference between the levels of mineral N in the test and reference plots would be the recommended amount of additional N. It is, therefore, necessary to include soil measurements of the actual mineral N and the mineralization of organic N during the growing season (52, 53). This permits not only the fine-tuning of additional N sidedress applications but also the use of the equations defined here for making predictions of the final yield on other soils using the same variety and tobacco type. Furthermore, SPAD measurements could also be used for meeting the N demand on every part of the field employing a high frequency of readings and remote sensing (19, 20). Taking the results as a whole, high quality wrapper yields can be successfully predicted by means of early SPAD readings. These can also reveal whether or not a tested field has received a sufficient amount of N. On the other hand, the actual level of mineral N in the soil to the depth of rooting can be used for estimating the amount of supplementary N sidedress application.

The variety, quality and uniformity of transplanted seedlings and the climatic conditions are factors affecting the utility of this diagnostic technique (22, 54). Further experiments are needed to better define crop and environmental factors limiting the performance of the SPAD meter and to develop strategies how to best overcome these limitations.

CONCLUSIONS

Nitrogen (N) fertilizer supplied to the soil in a range from 140 to 190 kg N/ha (40% applied at 8–10 DAT and 60% at 18–20 DAT) produced the best high quality wrapper yield. This amount and application schedule allowed achieving a leaf N sufficiency level of 4.3–4.7%. Relative wrapper yield was successfully predicted by optimal levels of leaf chlorophyll SPAD values in the early stages of plant development. There is a relative increase of wrapper yield (1.85%; 2.5 kg/ha) for every 1 cm increase in leaf length. The N fertilizer amount impacted on relative wrapper yield through its influence on plant growth, nutritional status and chemical composition. The apportionment of N fertilizer application during crop growth influences relative wrapper yield, probably through its effect on nutrient availability in the soil during plant vegetative development.

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