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'CRIOLLO 98' TOBACCO: LEAF CHLOROPHYLL CONTENT AND COMMERCIAL QUALITY, AS INFLUENCED BY SOIL AMENDMENT WITH ACID PEAT

Tabaco 'Criollo 98': contenido foliar de clorofila y calidad comercial como respuesta a la enmienda edáfica con turba ácida

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ABSTRACT. Tobacco soils of the Rhodic Ferralsol group affected by alkalinity were amended with acid peat (0; 15; 30; 45 and 60 m^3 ha^{-1}) to reduce their pH and thus to increase the production of wrapper leaf for export cigars in the region of the Partido zone, located in Alquízar municipality, Artemisa province. The research was conducted for four consecutive crops at the UBPC "Felipe Herrera Acea" (Basic Unit of Cooperative Production) of the mentioned location, with the Cuban dark tobacco (*Nicotiana tabacum* L.) seed ´Criollo 98´. Indicators of the vegetable response were: the chlorophyll content [SPAD units, in crop] for each priming and the percentage of production with exportable quality, for every treatment. The photometric readings [SPAD units] for every priming and treatment were related with the vegetable response in terms of exportable commercial quality, for the calculation of chlorophyll critical levels [Cate & Nelson´s method]. Leaf chlorophyll variation as response to acid peat treatments follows quadratic models of $R^2 \geq 0,72$, although the greatest variation occurred between primings, in the opposite way to leaf harvesting: corona>centro gordo>centro fino>centro ligero>uno y medio>libre de pie and without relationship with the amendment. The wrapper production for export cigars increased by 10 % with respect to the 0 dose, being the [45 and 60 m^3 ha^{-1}] treatments the best statistically responses. The critical levels calculated for chlorophyll content in every priming ~first reference of its kind for the seed in the Partido zone~ match the technical maturity moment [suitable for harvesting] with the possibility of obtaining, at least, 30 % of the total production with exportable commercial quality.

Key words: alkalinization, *Nicotiana tabacum* L., Rhodic Ferralsol soils, SPAD units, Partido zone

RESUMEN. Suelos tabacaleros Ferralíticos Rojos alcalinizados, se enmendaron con turba ácida [15; 30; 45 y 60 m^3 ha^{-1}, además de un testigo], para disminuir pH y aumentar la producción de capa para puros de exportación del cultivar cubano de tabaco (*Nicotiana tabacum* L.) negro, 'Criollo 98', en áreas de la zona de Partido (UBPC "Felipe Herrera Acea"; municipio Alquízar, provincia Artemisa), durante cuatro campañas sucesivas. Los indicadores de respuesta vegetal fueron: contenido de clorofila [unidades SPAD; en cosecha] por piso foliar y porcentaje de producción con calidad exportable, por variante experimental. Se relacionaron las lecturas fotométricas [unidades SPAD] con la respuesta vegetal en términos de calidad comercial exportable, para el cálculo de niveles críticos de clorofila [método Cate y Nelson]. La variación de la clorofila foliar como respuesta a las dosis de turba ácida, se ajusta a modelos cuadráticos de $R^2 \geq 0,72$; pero, la mayor variación ocurrió entre pisos foliares, en orden contrario al de su recolección: corona > centro gordo > centro fino > centro ligero > uno y medio > libre de pie y sin relación con la enmienda. La producción de capa para puros exportables, aumentó un 10 % respecto al testigo, para las dosis estadísticamente de mejor respuesta [45 y 60 m^3 ha^{-1}]. Los niveles críticos de clorofila para cada piso foliar ~primera referencia para el cultivar en la zona de Partido~ relacionan el momento de madurez técnica [aptitud para cosecha], con la posibilidad de obtener, al menos, un 30 % de producción con calidad exportable.

Palabras clave: alcalinización, *Nicotiana tabacum* L., suelos Ferralíticos Rojos, unidades SPAD, zona de Partido

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INTRODUCTION

Chlorophyll is the main photosynthetic pigment of plants and, as such (1,2), is associated with the capture and transfer of light energy, which triggers the oxidation / reduction processes involved in the fixation of atmospheric CO_2 in the plant. The foliar content of chlorophyll depends on internal factors such as the age of the leaf and, in turn, the expression of its photosynthetic capacity together with the intensity of that process and the mobilization rate in the plant of the photosynthesis product; they also influence the genetic characteristics of the species (1-3).

The determination of chlorophyll foliar content, based on photometric measurements and favored by the technological boom of recent decades, has established itself as a precise, non-destructive and easy-to-execute alternative for monitoring growth and development under different growing conditions and management technologies, of plantations of species agricultural interest, as diverse as melon (*Cucumis melo* L.), soybean (*Glycine max* (L.) Merr.), oak (*Quercus petraea* (Matt.) Liebl.), cherry (*Prunus serotina* (Ehrh.) Borkh.), rice (*Oryza sativa* L.), tomato (*Solanum lycopersicum* L.), corn (*Zea mays* L.), peanut (*Arachis hypogaea* L.) and cotton (*Gossypium arboretum* L.) (1, 4-6).

This is because, although the foliar chemical analysis (basically drying and preparation/digestion/ detection and quantification) it is still a fundamental tool in the study of soil/plant/fertilizer relationships whatever the nutrient management in use, there is also that to recognize the laborious and expensive that results as laboratory routine and the lack of immediacy between the identification of the situation that motivates the analysis and the availability of its results, for the corresponding decision making; in addition, the quantification of chlorophyll provides direct information on photosynthesis and primary productivity (7,8).

Thus, in the scientific literature there are already numerous studies on the relationship between the quantification of chlorophyll in leaves and foliar contents, mainly nitrogen (1,6) and to a lesser extent, magnesium and iron, not only in species of food interest but also in the study of species in extinction danger, which demand non-destructive methods of analysis (9) and in research on tobacco (*Nicotiana tabacum* L.) used as an energy crop (10).

The photometric determination (10,11) is based on the relationship between the transmittance of the leave, at two wavelengths: 650 nm, where absorption by photosynthetic pigments and 940 nm is characteristic, where this phenomenon does not occur. Based on the Lambert-Beer Law, said quotient, called the Chlorophyll Content Index is quantified in units SPAD (Soil Plant Analysis Division, acronym in English of the organization that developed the method).

Since chlorophylls *a* and *b* are the dominant pigments or those of greater absorption at 650 nm, it is assumed that the quantification in SPAD units is proportional to the foliar concentration of chlorophyll. Thus, the results in SPAD units have been positively correlated with the total content of chlorophyll, in a wide range of studies that include different species (11,12), which would explain that often, during the interpretation of data obtained with chlorophyllometers, there is a generic talk of chlorophyll content, as it happens in agriculture Cuban tobacco company (13).

In this way, the chlorophyllometers measurement becomes a method of foliar analysis, which detects the chemical species under study [chlorophyll], without interference or quantification of others that are not of interest, with an adequate relationship between the results it provides and the nutritional status of plants in various conditions (1-4,6,12,13), with accuracy and precision and quickly and easily. These are the validation criteria proposed by Bray (1948) and cited (14), on the requirements to be met by a reliable analytical method in soil analysis, extrapolated in this case to the foliar analysis.

In the case of tobacco, the chlorophyll analysis, in addition to reporting on the nutritional content of the leaves, is also useful to anticipate the possible response of this body in terms of commercial quality obtained (12), given the conditions to which it is submitted during the curing and beneficiation processes that follow the harvesting of each leaf layer and the existing relationship is known, between the state of technical maturity of the leaves aptitude for harvesting and its content of chlorophyll (13,15).

However, the result of the quantification of foliar chlorophyll is also influenced by the environmental conditions present at the time of taking the photoelectric readings ~ relative humidity; intensity of incident solar radiation and temperature, ~ as well as aspects of agronomic management such as planting density, the source of nitrogen supply to plants and the magnitude of the effects of pests on them (6,16).

Therefore, it is necessary that the relationships between chlorophyll foliar content and other indicators of plant response, such as agricultural yield and nutrient concentration in plant organs, be established specifically for species, varieties and conditions of agronomic management.

On the other hand, in Red Ferralitic soils of the Partido zone, Artemisa province, one of the most important for the Cuban tobacco industry, with status of Protected Designation of Origin (17), the production of black tobacco layer has been reduced capped for export cigars, due to alkalization processes whose effects led to evaluate the application of acid peat, as an adequate alternative, to lower the pH of the soils and improve the productive response (18).

As the edaphic amendment also influences the growth and development of the Cuban cultivated plants ‘Criollo 98’ under cover, its effect on the foliar chlorophyll contents can be described, throughout the harvest cycle and also, characterize the relationship between the foliar content of chlorophyll per crop soil harvested and the productive response exportable quality to the edaphic amendment with acid peat: to that objective the work carried out responded.

MATERIALS AND METHODS

The work was developed during four consecutive campaigns (tobacco harvest+profit) between 2012 and 2016, in the Basic Unit of Cooperative Production (UBPC) “Felipe Herrera Acea”, located in Alquízar, Artemisa province and belonging to the Tobacco Company “Lázaro Peña", From the Cuban Ministry of Agriculture.

The region climate is classified as tropical subhumid (19), with a rainy season with an average annual rainfall of 1500 mm and a little rainy, suitable for planting and growing tobacco, with accumulated between 300 and 350 mm per year. As for the thermal regime, average annual temperatures of 24,7 °C are recorded, with average temperatures, maximum of 29,8 °C and minimum of 19,7 °C.

The planting frame was 0,90 x 0,30 m and rooted postures of the black tobacco cultivar ‘Criollo-98’ were used, affected in the expression of its productive potential by the alkalization of tobacco soils in the Partido zone (18,20).

The preparation of the soils (Red Ferralitic grouping (21)) and the cultural attentions were carried out in accordance with the provisions of the Technical Instructions for the cultivation of covered tobacco in Cuba (22).

The treatments were arranged in plots of 320 m^2 and replicated four times in each tobacco harvest; they were distributed according to random block design. The treatments under study are presented in Table 1.

Soil samples were taken every year for pH analysis (H_2O) and to characterize the effect of the amendment on the classification of the soil according to its acidity (23), at the time of the transplant, and the acid peat was incorporated in the corresponding treatments. Four composite samples of 1 kg were taken per experimental variant.

Table 1. Treatments to evaluate the response of 'Criollo 98' tobacco to the amendment with acid peat, of Red Ferralitic soils of the Partido zone, Artemisa province

Treatment	Dose of acidic peat ($m^3\ ha^{-1}$)
1	0
2	15
3	30
4	45
5	60

The acid peat, coming from the peat bog “Caimanera”, located to the south of the Pinar del Río province (San Luis municipality), was incorporated to the soil manually in each year and per plot, before the transplant. The laboratory analyzes (6 composite samples) report as chemical characterization, average values of 0,77 % of P; 0,20 % of K; 0,04 % Na; 0,12 % Mg; 2,68 of Ca; 1,51 % of N and 21,87 % of organic matter, in addition to pH=5,20.

Response variables and information processing

a) Determination of chlorophyll content

A chlorophyllometer of the Japanese brand Minolta, model SPAD-502 was used. The measurements (SPAD units) were made immediately before the harvest of each leaf layer (successively and in ascending order, free standing, one and a half, light center, fine center, fat center and crown), directly in the limb of the leaves and at a point between the center and the apex of the same (22). 20 random readings were taken per experimental variant (five in each replica) in each campaign.

The lack of statistical differences between replicas of the same variant in each year and between years for each variant was verified. As information processing, a double classification variance analysis was carried out to determine the effect of the soil amendment on the aforementioned plant property and the Duncan test for the separation of statistically different means. The information of each variant under study is presented as the average value for the whole experimental period and for the foliar layer harvested.

Regression analyzes were performed a) to model with quadratic functions, the chlorophyll foliar content ratio/acid peat dose, for each leaf layer and b) to model the linear upward relationship, of the chlorophyll foliar content from free standing to crown, for each of the treatments included in the experimental scheme.

b) Percentage obtained from production with exportable commercial quality

In each campaign and for each replication by experimental variant, the percentage of production with exportable quality was calculated with respect to the total production obtained, based on post-harvest management according to the norms and specifications in force in Cuba (13,15,22).

Regression analysis was carried out between the productive response (% of exportable commercial quality) and the edaphic amendment (dose of acid peat), to model the tendency of the vegetal response. Likewise, the existence of a statistical difference between replicas was discarded for each variant in each year and between years, for the results of each variant. For the statistical processing analysis of variance of double classification the data from x to 2 arcoseno√x was transformed, where x is the percentage of layer production for export cigars, with respect to the total production obtained. The results that are presented also correspond to the average value of each variant under study, for the four experimental evaluation campaigns.

c) Functional relationship between foliar chlorophyll content and percentage of production with commercial exportable quality

From the methodological indications for the calculation of critical levels (24), the SPAD ratio of foliar chlorophyll/% exportable commercial quality was modeled mathematically, with the objective of establishing the critical value of the photometric determination for each leaf layer.

As the maximum reference value for calculating the percentage of Relative Performance (% RR), the average of the best responses obtained for the percentage of production with exportable quality was considered, according to the statistical evaluation of the replicas of the variants under study and for the total of campaigns (24,25).

Each proposed critical level was calculated from 20 pairs of values (SPAD/% RR) and it is accompanied by its corresponding coefficient of determination (R_2).

All data processing was done using the IBM SPSS Statistics version 20 software and the Microsoft Excel spreadsheet, in a Windows 7 environment. For each regression analysis, the corresponding analysis of variance was performed and the coefficient of determination was calculated and reported (R_2).

RESULTS AND DISCUSSION

The pH of the soil allowed to characterize it at the time of the transplant as slightly alkaline (range: 7,81 to 7,87 for the whole of the campaigns) in the plots without incorporation of acid peat and as neutral (range: 7,31 ± 0, 02 to 7,58 ± 0,02 for all campaigns) in the parcels where the soil amendment was made. The values of the photometric determination are shown in Table 2. There is an obvious response to the edaphic amendment, in terms of foliar content of chlorophyll, according to the aforementioned interpretation on SPAD units (11,12), which reaches its maximum values with doses of 45 and 60 m^3 ha^{-1} , according to the statistical test.

If it is considered that for tobacco in Cuba, it is assumed that SPAD values in leaves from 39 units indicate contents of the pigment associable to the state of optimum technical maturity (22,26), it can be affirmed that, according to the values that appear in the Table 2, each leaf layer was harvested opportunely, in terms of growth and development of the leaves and physiological state of the plant. The trend of the response obtained for each leaf layer can be described according to the quadratic equations of Table 3.

Table 2. Effects of the edaphic amendment on the content of foliar chlorophyll in tobacco (*Nicotiana tabacum* L.) black capped 'Criollo 98', cultivated in the zone of Partido, province Artemisa

Acid peat [m^3 ha^{-1}]	Chlorophyll [spad units] per foliage layer					
	Libre de pie (the base) 60 dat	Uno y medio (section directly above the base) 67 dat	Centro ligero (center of plant with thinnest leaves) 74 dat	Centro fino (center of plant with mid-sized leaves) 81 dat	Centro gordo (center of plant with thickest leaves) 86 dat	Corona (Crown) 90 dat
0	40^c	40,4^d	43,07^d	45,2^d	48,2^c	49,1^c
15	40,3^b	40,9^c	43,8^c	46,4^c	48,4^{bc}	49,3^c
30	40,5^b	41,4^b	44,2^b	47,15^b	48,6^b	49,7^b
45	40,8^a	41,9^a	44,8^a	47,6^a	49^a	50,2^a
60	40,8^a	41,9^a	44,8^a	47,5^a	48,9^a	50,15^a
E. S. $_{[X]\ [* = 0.05]}$	0,0782*	0,1386*	0,1536*	0,2075*	0,0777*	0,1073*

dat: days from transplant to collection; different letters in the same column indicate statistically significant differences according to the Duncan test for p≤0.05

Table 3. Ratio of foliar chlorophyll (SPAD units) vs edaphic amendment (m^3 ha^{-1}) per foliage layer harvested in tobacco (*Nicotiana tabacum* L.) black capped 'Criollo 98'

Foliage layer	Equation	R^2
Libre de pie	$y = -0,0002x^2 + 0,0238x + 39,995$	0,78**
Uno y medio	$y = -0,0003x^2 + 0,0457x + 40,357$	0,92**
Centro ligero	$y = -0,0004x^2 + 0,0535x + 43,066$	0,93**
Centro fino	$y = -0,0009x^2 + 0,0939x + 45,196$	0,97**
Centro gordo	$y = -0,0001x^2 + 0,021x + 48,163$	0,72**
Corona	$y = -0,0001x^2 + 0,0276x + 49,033$	0,83**

** Statistically significant for $p \leq 0.01$ according to Duncan's test

Note that the response to the edaphic amendment exhibits a unique pattern for the cultivar, regardless of when the photometric readings were taken, which was done between 60 and 90 days after the transplant and before harvesting each leaf layer.

The values calculated for the coefficient of determination of the quadratic models validate their choice to describe the trend of that plant response. They indicate that the doses of acid peat were correctly selected and, furthermore, that the decomposition rate of the amendment in the soil is not directly proportional to the incorporated amount thereof.

If there is no linear relationship between SPAD readings and soil amendment, it is because the chemical, physical, biological and physical-chemical processes that describe the acid/soil peat interaction and largely determine the effect of the amendment on the plant response, take place in regulated way, under the influence of external factors such as temperature, rainfall and agronomic management of plantations. A mineralization rate is reached, which at the same time provides nutrients, also satisfies the demand for energy, carbon and minerals of the successive populations of microorganisms present in the soil. For this reason, carriers of organic matter incorporated into soils as an amendment are considered slow-release fertilizers (27).

The effect of the edaphic amendment on the pH of the soil was evidenced in the fact that the amended soils passed from the classification as moderately alkaline, of the witness or non-amended variant similar to that of red Ferralitic soils degraded in western Cuba (28,29), to be classified as neutral, regardless of the amount of peat incorporated into the soil, which supports the previous interpretation on the acid/soil peat interaction.

But soil degradation is not only expressed in the elevation of the pH: other limiting effects of the productivity of this natural resource in the study area, such as compaction, poor drainage and the decrease of organic matter content, are manifested (28 , 30.31). These effects are counteracted with the edaphic amendment; peat, as a carrier of organic matter positively influences porosity, moisture retention and stability of aggregates; it also decreases bulk density and contributes nutrients to the soil (32), which was expressed in the obtained plant response.

It is also observed in Table 2 that, regardless of the dose of acid peat applied to the soil to reduce its pH, the values of the response variable increase between leaf levels, from free standing to crown and that the greatest differences occur between foliar layers for each level of amendment and not between the doses of peat handled, for each foliar layer. This trend is described with the linear regression models that appear in Table 4.

Table 4. Linear regression analysis in foliar content of chlorophyll [SPAD units] vs time [days] of harvest, from libre de pie to crown in tobacco (*Nicotiana tabacum* L.) black capped 'Criollo 98'

Acid peat (m^3 ha^{-1})	Equation	R^2
0	$y = 0,328\ x + 38,97$	0,96**
15	$y = 0,326\ x + 39,53$	0,98**
30	$y = 0,333\ x + 39,87$	0,98**
45	$y = 0,333\ x + 40,29$	0,99**
60	$y = 0,330\ x + 40,30$	0,99**

** statistically significant for $p \leq 0.01$ according to Duncan's test

There is a strong correlation between both variables, which does not depend either on the application or not of acid peat, nor on the value established for the edaphic amendment.

A response pattern is described in terms of foliar chlorophyll content throughout the harvest. Observe the values of the regression coefficient, for the agronomic management conditions created in the Partido zone: crown> fat center> fine center> center light> one and a half> free standing, which is the first reference of its kind for the cultivar 'Criollo 98', in it. The differences between leaf levels are essentially due to the fact that they are not collected simultaneously and as long as the plant cycle continues during the staggered harvest, factors such as the cover of the plantation, the consumption of nutrients, the unbundling and undoing, foliar fertilization with $Mg(NO_3)_2$ at 40 dat according to current technical instructions (22,33) affect the synthesis and/or redistribution of nutrients and substances that define the chemical composition (chlorophyll included) of the leaves remaining in the plant.

If it is considered that because the leaf is the main objective of production of the tobacco crop, the agronomic management of the plantations is directed to the plant accumulating as much biomass as possible in that organ (22,33), that the ability to harvest According to the foliar content of chlorophyll (26), it also means a plantation in optimum state of development and, the relationship between the photosynthetic capacity of the leaves and their nitrogen content (2), will be understood the role of continuous absorption and nutrient translation proper to the growth and development of the plants, in the increase of the foliar chlorophyll content, throughout the tobacco harvest.

The variation over time of chlorophyll foliar content has also been reported in other agricultural species, such as wheat [*Triticum durum* L.], soybean and safflower [*Carthamus tinctorius* L.] (3,5,34).

For tobacco it is also described in an increasing sense from the lower leaves to the upper ones (35,36), in relation to the growth of the leaves and the variation of its nitrogen content, to support the interpretation of the photometric reading, as an indicator of the nutritional status of the plant.

It is also associated with the chlorophyll content, with the absorption by the magnesium plant constituent element of the pigment molecule (37) and iron, micronutrient that participates in its synthesis (38), as well as phosphorus, while the energy obtained through photosynthesis and the metabolism of carbon compounds, it is stored and transported mainly in phosphorus compounds (39).

The results obtained with the edaphic amendment are consistent with reports on the use of acid peat in tests conducted at the Institute of Tobacco Research, for the correction of pH in Red Ferralitic soils of the Party area, with use of tobacco as an index plant (40); The uniform pattern of plant response and changes in pH, obtained throughout the four tobacco harvests included in this study, leaves no doubt about the favorable effect of the amendment, as the only factor of variation of the experimental scheme.

Bear in mind that the incorporation of organic matter carrier into the soil, together with the application of fertilization established for tobacco areas (22,33), conditions an organo-mineral-based nutrition, a practice of agronomic management of a conservationist nature and, as such, valid in the management of degraded soils as is the case (30,31), as well as favorable to the growth and development of the cultivated species (32, 41).

The plant response in terms of chlorophyll foliar content is the starting point or guarantee of the future commercial quality of the production obtained, because it conditions the response of the leaves, to the post-harvest stress that the successive healing processes under controlled temperature and humidity imply, of natural fermentation and of controlled humidification (22), which precede its selection and commercial classification as a layer for export, a layer for national consumption and casing for cigars, (13).

When this content is equal to or greater than 39 units SPAD, after its collection and benefit, the leaves reach a variable brown tone but without spots or streaks, thin thickness and remarkable elasticity, according to the current criteria in the Cuban tobacco agroindustry (15,42).

This relationship chlorophyll/quality for the cultivar 'Criollo 98' is expressed in the fact that the productive response to the edaphic amendment [X], in terms of production percentage with exportable commercial quality [Y], mathematically describes a similar trend to the determination of chlorophyll per leaf layer Table 3, according to the equation:

$$Y = -0{,}0026\ X_2 + 0{,}3286\ X + 22{,}83 R_2 = 0{,}98^{**}$$

In Table 5, information on the productive response is presented, as it is traditionally evaluated in the Partido zone (% production as a layer for export cigars). Note how the order of merit obtained for the doses of acid peat is repeated, which is observed in Table 2. The best productive response, statistically justified, is achieved with the doses of 45 and 60 $m^3\ ha^{-1}$ which also support the recommendation of the lower dose, as part of the agronomic management in the conditions studied.

Table 5. Effect of the edaphic amendment with acid peat on the commercial quality of the tobacco production (*Nicotina tabacum* L.) black capped 'Criollo 98' cultivated in the zone of Partido, Artemisa province

Acid peat [$m^3\ ha^{-1}$]	Exportable commercial quality production [%]	
	Real value	transformed value
0	23	$1{,}00^d$
15	26,9	$1{,}09^c$
30	30	$1{,}16^b$
45	33,2	$1{,}23^a$
60	33	$1{,}22^a$
E,E,χ [*= 0,05]	-	0,0198*

Different letters in the same column indicate statistically significant differences according to the Duncan test for p≤0.05

The amendment of the soils has made possible the improvement of the commercial quality of the production, something congruent with the Protected Designation of Origin status held by the Partido zone, which taxes around 60 % of the cigar layer for export in Cuba.

An increase of 10 % in the production of export quality layer in an area in which the agricultural yields for tobacco, according to the records of the UBPC "Felipe Herrera Acea" range between 1,0 and 1,5 t ha^{-1}

can represent from the percentages obtained from the treatment without amendment (23 %) and from the treatment with 45 m^3 ha^{-1} (33,2 %) a net production difference in favor of the amendment, estimated at 130 kg ha^{-1} of layer for export cigars.

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The information presented in Tables 2, 3 and 5 based the evaluation of the existing functional relationship, between leaf content of chlorophyll per leaf layer and % of production with exportable commercial quality obtained, to propose critical values of the photometric determination, per leaf layer. That evaluation appears in Table 6.

Table 6. Critical values of chlorophyll content per foliage layer (SPAD units), associated with obtaining not less than 30 % of exportable commercial quality production, and in tobacco plantations (*Nicotiana tabacum* L.) black capped 'Criollo 98' in the zone of Partido, Artemisa province

Foliar layer black capped tobacco 'CRIOLLO 98'	Foliar chlorophyll as an indicator of technical maturity	
	Minimum admissible value	R^2
Libre de pie (the base)	40,5	0,74
Uno y medio (section directly above the base)	41,2	0,80
Centro ligero (center of plant with thinnest leaves)	44,2	0,73
Centro fino (center of the plant with mid-sized leaves)	47	0,78
Centro gordo (center of the plant with thickest leaves)	48,7	0,74
Corona (Crown)	49,5	0,80

The range of recommended values is consistent with the already argued variation between harvests in the readings with the chlorophyllometer, which coincides with results in which variations in the determination of chlorophyll are reported, with the growth and development of the plants (9,36). At the critical level of foliar chlorophyll, it is recognized as useful for the nutrition management of cultivated species and as an alternative to the use of chlorophyll measurements in SPAD units (43).

The values obtained for the coefficient of determination are adequate with respect to the proposal of critical levels (24,44), at the same time as congruent with the versatility recognized to the aforementioned data separation procedure in two populations (25); constitute the first report of its kind for the cultivar 'Criollo 98' in the biophysical conditions of the Partido zone.

As such, they also contribute elements on the advisability of the chlorophyll photometric determinations use , as management part of other species of agricultural interest, in the same agroproductive scenario, based on evidence that the chlorophyll foliar content is an important indicator of physiological status of the plants and that their variation can be considered as a plant response to environmental stress (16,45) or in the existence of relationships between chlorophyll content in SPAD units on the one hand, and the water status of the leaves, the microbial activity, the level of humidity and the availability of nutrients, influenced by the processes that take place in the soils as a consequence of the tillage system put into practice, of the other (46).

It should be noted that the chlorophyll foliar critical levels that are proposed as a reference, are only achievable through an agronomic management of the cultivar that stands out for the strict compliance with the technological discipline (22,42), from the indispensable correction of the pH of soils, so they could be used equally, as quality indicators, in the stages of growth and development of the plantations, given the complex set of physiological processes and biochemical mechanisms, involved in the construction and maintenance of efficient photosynthetic systems in plants (47), which can be monitored directly or indirectly through these photometric readings.

CONCLUSIONS

- The changes induced by amendment with acid peat, in Red Ferralitic tobacco soils of the Partido zone affected in their agro-productivity by the alkalization, are expressed in increases of the foliar chlorophyll content and of the production with exportable quality, in sowings of the tobacco cultivar black 'Criollo 98'.
- The foliar layers of the cultivar 'Criollo 98' are ordered with respect to their chlorophyll content, in the opposite direction to their collection: crown> fat center> fine center> light center> one and a half> free standing.
- The application of 45 m^3 ha^{-1} of acid peat as an amendment before the transplant is sufficient to obtain the highest chlorophyll content for each leaf layer and the best commercial quality of the harvested tobacco.

- Critical values for chlorophyll content (SPAD units) are proposed in each leaf layer, associated with obtaining at least 30 % of production with exportable commercial quality and adequate to monitor the agronomic management of the plantations of this cultivar in the Partido zone of Alquízar municipality, Artemisa province.

RECOMMENDATIONS

- Studying the acid/soil peat interaction in incubation experiments, to know about other possible effects or uses of the edaphic amendment, in the agronomic management of tobacco.
- Socializing the use of certain critical levels in the management of tobacco plantations in the Partido area.
- Carrying out similar studies for the determination of the chlorophyll foliar critical level in other tobacco cultivars and agroecosystems.

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BIBLIOGRAPHY

1. Bielinis, E, Jozwiak ,W, Robakowski ,P. Modelling of the relationship between the SPAD values and photosynthetic pigments content in *Quercus petraea* and *Prunus serotina* leaves. Dendrobiology. 2015;73:125-34. doi:10.12657/denbio.073.013
2. Latsague M, Sáez P, Mora M. Efecto de la fertilización con nitrógeno, fósforo y potasio, sobre el contenido foliar de carbohidratos, proteínas y pigmentos fotosintéticos en plantas de *Berberidopsis corallina* Hook.f. Gayana. Botánica. 2014;71(1):37-42. doi:10.4067/S0717-66432014000100007
3. Wang G, Bronson KF, Thorp KR, Mon J, Badaruddin M. Multiple leaf measurements improve effectiveness of chlorophyll meter for *Durum wheat* nitrogen management. Crop Science. 2014;54(2):817. doi:10.2135/cropsci2013.03.0160
4. Peña MT, Thompson RB, Martínez-Gaytán C, Gallardo C, Giménez M. Sistemas ópticos de monitorización del estado del nitrógeno en melón. Actas de Horticultura. 2012;60:820-4.
5. Saadpanah A, Rokhzadi A, Mohammadi K. Growth response of soybean to the application of *Bradyrhizobium japonicum* and foliar methanol spraying in field conditions. International Journal of Biosciences. 2013;3:128–34.
6. Xiong D, Chen J, Yu T, Gao W, Ling X, Li Y, *et al.* SPAD-based leaf nitrogen estimation is impacted by environmental factors and crop leaf characteristics. Scientific Reports. 2015;5(1). doi:10.1038/srep13389
7. Correndo A, García F. Concentración de nutrientes en planta como herramienta de diagnóstico: Cultivos extensivos. Archivo Agronómico [International Plant Nutrition Institute]. 2012;14:1 – 8.
8. Lin C, Popescu SC, Huang SC, Chang PT, Wen HL. A novel reflectance-based model for evaluating chlorophyll concentrations of fresh and water-stressed leaves. Biogeosciences. 2015;12(1):49-66. doi:https://doi.org/10.5194/bg-12-49-2015
9. Ruttanaprasert R, Jogloy S, Vorasoot S, Kesmala T, Kanwar RS, Holbrook CC. Relationship between chlorophyll density and spad chlorophyll meter reading for Jerusalem artichoke (*Helianthus tuberosus* L. SABRAO Journal of Breeding and Genetics. 2012;44(1):149-62.
10. Altuna A. Evaluación del uso de variedades comerciales de tabaco transformadas con el gen de la tiorredoxina f como posible cultivo energético para la producción de bioetanol. [España]: Universidad Pública de Navarra; 2012. 132 p.
11. Pereyra MS, Davidenco V, Núñez SB, Argüello JA. Chlorophyll content estimation in oregano leaves using a portable chlorophyll meter: relationship with mesophyll thickness and leaf age. Agronomía & Ambiente. 2014;34(1-2):77-84.
12. Ligoska A, Kowalczyk-Jusko A, Koscik B. Possible use of the chlorophyll meter [SPAD-502] for evaluating nitrogen nutrition of the Virginia tobacco. Electronic Journal of Polish Agricultural Universities. Series Agronomy. 2002;1(05).
13. Izquierdo A, Borges A. Diagnóstico del momento de recolección del tabaco negro variedad 'Corojo'99' mediante el índice de madurez técnica. Cuba Tabaco. 2010;11(1):3-9.
14. Arozarena NJ. Criterios para el manejo sostenible de la nutrición vegetal en la agrotecnología zeopónica [Tesis de Doctorado]. [La Habana, Cuba]: Instituto de Investigaciones Fundamentales en Agricultura Tropical "Alejandro de Humboldt"; 1999. 102 p.
15. Valladares Díaz R. Instructivo para el acopio y beneficio del tabaco negro tapado. La Habana: Agrinfor; 2003.
16. Huang J, Zhang J. Changes in the photosynthetic characteristics of cotton leaves infested by invasive mealybugs tended by native ant species. Arthropod-Plant Interactions. 2016;10(2):161-9. doi:10.1007/s11829-016-9418-z
17. MINAG. Reglamento de la Denominación de Origen Protegida Habanos y de las restantes denominaciones de origen tabacaleras cubanas. [Resolución no. 201/2009]. La Habana ,Cuba; 2009. 30 p.
18. Ricote O, Trémols AJ, Arozarena NJ, Trujillo A. Recuperación de la calidad de suelos tabacaleros Ferralíticos Rojos en la provincia de Artemisa: la oportunidad de la turba ácida. En: XX Congreso Científico del Instituto Nacional de Ciencias Agrícolas (INCA). Mayabeque, Cuba; 2016. [CD-Rom]. Memorias.
19. Hoyos R, Gutiérrez Tde J, Peñate M. Estudio de las características agroclimáticas de la zona de Partido en el occidente de Cuba, aplicado al cultivo de tabaco negro tapado. Cuba Tabaco. 2010;11(1):10-23.

20. Monzón L, Trémols AJ, Álvarez L, Díaz M, Reyes D. Estrategia varietal para enfrentar la degradación química de los suelos de la zona tabacalera de Partido. En: XX Congreso Científico del Instituto Nacional de Ciencias Agrícolas (INCA).Mayabeque, Cuba; 2016. [CD-Rom]. Memorias.
21. Hernández, A, Pérez J, Castro, N, Bosch,D. Clasificación de los suelos de Cuba 2015. Ediciones INCA; 2015. 91 p.
22. Colectivo de autores. Instructivo Técnico para el Cultivo del Tabaco Tapado en Cuba. AGRINFOR; 2012. 148 p.
23. Norma Cubana. Calidad del Suelo. Determinación de pH [NC ISO 10390]. Oficina Nacional de Normalización (NC); 1999. 11 p.
24. Cate RB, Nelson LA. A simple statistical procedure for partitioning soil test correlation data into two classes 1. Soil Science Society of America Journal. 1971;35(4):658-60. doi:10.2136/sssaj1971.03615995003500040048x
25. Mangiafico SS. Cate-Nelson Analysis for bivariate data Using R-project. Journal of Extension [Internet]. 2013 [citado 16 de marzo de 2018];51(5). Disponible en: https://www.joe.org/joe/2013october/tt1.php
26. Borges A, Izquierdo A, Hernández B, León Y. .La clorofila foliar como criterio para recolectar el tabaco Negro *Nicotiana tabacum* L. Var. «Corojo 99» cultivada bajo tela. Cuba Tabaco. 2007;8(1):3-9.
27. Lino A, Arozarena NJ, Dibut B, Ríos Y, Croche G, Fernández J, *et al*. Cultivo Protegido sobre suelos Ferralíticos Rojos. II) Alternativas nutrimentales de menor impacto ambiental para el cultivo de tomate (*Lycopersicon esculentum*, Mill) y pepino (*Cucumis sativus*, L). Agrotecnia de Cuba. 2006;30(2):1-12.
28. Hernández A, Morales M, Borges Y, Vargas D, Cabrera JA, Ascanio MO, *et al*. Degradación de las propiedades de los suelos Ferralíticos Rojos lixiviados de la" Llanura Roja de La Habana", por el cultivo continuado. Algunos resultados sobre su mejoramiento. Mayabeque, Cuba: Ediciones INCA; 2014. 156 p.
29. Febles-González JM, Vega-Carreño MB, Do Amaral-Sobrinho NMB, Tolón-Becerra A, Lastra-Bravo XB. Good Soils in Extinction: Degradation of Red Ferralitic Soils in Western Cuba. Soil Science. 2014;179(6):304. doi:10.1097/SS.0000000000000070
30. Ricote-Jorge Ó, Monzón-Herrera L, Villalón-Hoffmann A. Degradation of Red Ferralitic (Rhodic Ferralsol) tobacco soils in the Artemisa province, Cuba. Acta Agronómica. 2017;66(1):88-94. doi:10.15446/acag.v66n1.52771
31. 31. Cánepa Ramos Y, González T, J A, González Mederos A, Hernández Jiménez A. Situación actual de los suelos tabacaleros de la empresa Lázaro Peña de la provincia Artemisa. Cultivos Tropicales. 2015;36(1):80-5.
32. Dey A, Dasgupt S, Bhattarcharya S, Chaitanya AK, Pati S, Pal B. Soil physical fertility and performance of potato crop as affected by integration of organic and inorganic fertilizers in new alluvial soil of West Bengal. Journal of Crop and Weed. 2015;11(1):132-7.
33. Instituto de Investigaciones del Tabaco - MINAG. Guía para el cultivo del tabaco. San Antonio de los Baños (La Habana): Instituto de Investigaciones del Tabaco; 2011. 59 p.
34. Khaki-Moghadam A. Growth and yield parameters of safflower (*Carthamus tinctorius*) as influenced by foliar methanol application under well-watered and water deficit conditions. Environmental and Experimental Biology. 2015;13:93–97.
35. Hoyos V. Respuesta fisiológica y de producción del tabaco tipo Virginia bajo diferentes planes de fertilización en Campoalegre y Garzón, Huila [Tesis de Magister en Ciencias Agrarias]. Facultad de Agronomía/Universidad Nacional de Colombia; 2013. 116 p.
36. Wang Q, Xi L, Ren Y, Ma X. Determination of tobacco leaf maturity degree based on computer vision technology. Transactions of the Chinese Society of Agricultural Engineering. 2012;28(4):175-9.
37. Cakmak I, YazİcÍ AM. Magnesium: a forgotten element in crop production. Better Crops with Plant Food. 2010;94(2):23-5.
38. Zevada, KJ. Aplicación de nitrógeno y magnesio para estimular el contenido de clorofila y los parámetros de crecimiento en chile jalapeño (*Capsicum annuum* L.), bajo condiciones de invernadero. [Trabajo de Diploma]. [Obregón, México]: Instituto Tecnológico de Sonora; 2005. 84 p.
39. Aznar Antoñanzas Y. Caracterización fisiológica del Bitter Pit: aspectos nutricionales, fenológicos y de diagnóstico [Internet] [Tesis de Doctorado]. Estación Experimental del Aula Dei Zaragoza; 2001 [citado 14 de marzo de 2018]. Disponible en: https://digital.csic.es/handle/10261/2852
40. Ricote O, Trémols AJ. Efecto de la aplicación de turba ácida sobre el pH de un suelo Ferralítico Rojo y el crecimiento de plántulas de tabaco negro. Cuba Tabaco. 2013;14(2):65-8.
41. Dubey M, Agrawal KK, Ahirwar AD, Ahirwar SK. Rice-berseem cropping system influenced a remarkable effect on growth of different soil micro organisms in different rice based cropping systems. Plant Archives. 2015;15(1):115-8.
42. Espino E. El Habano: de la semilla al puro en 539 pasos. Madrid: GAVD; 2014. 92 p.
43. Villar D, Ortega R. Medidor de clorofila. Bases teóricas y su aplicación para la fertilización nitrogenada en cultivos. Agronomía y Forestal. 2012;(12):4-9.
44. Rodríguez S. Algunas consideraciones necesarias en la aplicación del método de Cate y Nelson para correlacionar el análisis de suelo con la respuesta de la planta. Ciencia y Técnica de la Agricultura (Serie Suelos y Agroquímica). 1980;3(2):5-20.
45. Percival GC, Keary IP, Noviss K. The potential of a Chlorophyll Content SPAD Meter to quantify nutrient stress in foliar tissue of sycamore (*Acer pseudoplatanus*), english oak (*Quercus robur*) and european beech (*Fagus sylvatica*). Arboriculture & Urban Forestry. 2008;34(2):89-100.
46. Mirassón1 HR, Faraldo ML, Fioretti MN, Miravalles M, Brevedan RE. Relaciones entre el índice de verdor y el nivel hídrico foliar en trigo con diferentes sistemas de labranza. Phyton (Buenos Aires). 2010;79(2):183-7.
47. Terashima I, Araya T, Miyazawa S-I, Sone K, Yano S. Construction and Maintenance of the Optimal Photosynthetic Systems of the Leaf, Herbaceous Plant and Tree: an Eco-developmental Treatise. Annals of Botany. 2005;95(3):507-19. doi:10.1093/aob/mci049

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