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Original Scientific paper

USE OF A CHLOROPHYLL METER AS A TOOL TO DIAGNOSE THE NITROGEN STATUS OF ORIENTAL TOBACCO

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ABSTRACT

The aim of this work was to assess the usefulness of non-destructive and quick measurements of leaf chlorophyll content with a Hydro N-Tester as indicator of tobacco nitrogen status. For the purposes of the study four nitrogen rates were tested in a stationary field trial. During the vegetation period leaf chlorophyll content was measured with a portable instrument Hydro N-Tester.
Chlorophyll content in the leaves ranged from 283 to 559 HNT units and was affected by leaf measurement date and N fertilizing rates. Chlorophyll meter readings of youngest, fully formed leaves (third or fourth leaf from the top) could be used to accurately discriminate N-deficient from N-sufficient treatment. Chlorophyll meter values were highly correlated with concentration of total N in tobacco leaves and yield of cured leaves. After further testing Hydro-N-tester values can be used as an indirect method for assessing the nitrogen nutrition of tobacco at different stages of vegetation.

Key words: tobacco, chlorophyll content, Hydro N-Tester chlorophyll meter

УПОТРЕБА НА ХЛОРОФИЛМЕТАРОТ КАКО СРЕДСТВО ЗА ОДРЕДУВАЊЕ НА НИВОТО НА АЗОТ КАЈ ОРИЕНТАЛСКИОТ ТУТУН

Целта на овој труд е да се процени корисноста од недеструктивните и брзи мерења на содржината на хлорофил во листот со помош на Hydro N-Tester како индикатор на нивото на азот во тутунот. За целите на истражувањето испитувани се четири дози на азот во стационирран полски опит. За време на вегетациониот период содржината на лисниот хлорофил е мерена со подвижен рачен инструмент Hydro N-Tester.

Хлорофил во лисјата се движи од 283 до 559 HNT единици, а врз него влијаат датумот на мерење на листовите и конценрациите на азотнот ѓубре. Мерењата на најмладите, целосно оформени листови (третиот или четвртиот лист од врвот) со хлорофилметар можат да се користат за да се направи прецизна разлика помеѓу N-дефицитарните од N-суфицитарните третирања. Вредностите од хлорофилотметарот се во корелација со концентрацијата на вкупен N во тутунските листови и приносот на сушените листови. По натамошно тестирање, вредностите добиени со Hydro-N-tester можат да се користат како индиректен метод за проценка на азотното прихранување на тутунот во различните фази на вегетација.

Клучни зборови: тутун,содржина на хлорофил, Hydro N-Tester за мерење на хлорофил

INTRODUCTION

Nitrogen management, rate and time of nitrogen application are critical factors in optimizing oriental tobacco yield and quality. Although a high nitrogen supply generally increases yield, it is necessary to achieve a balance between maximum yield and optimal quality TsoT.C (1999). The value of soil tests prior to transplanting to evaluate fertilizer-N requirements for tobacco has been well established in our country. Various plant tests (assessment of leaf chlorophyll, tissue total N and NO_3-levels) during the growing season are also used to estimate tobacco N status and to make fertilizer N recommendations.

Leaf chlorophyll concentration is strongly correlated with plant N concentration, which is a good predictor of yield potential because of the association between photosynthetic activity and leaf N Evens J.R. (1983). The traditional method of measuring leaf chlorophyll concentration involves wet chemical procedures. Recently chlorophyll meters were introduced to nondestructively measure relative chlorophyll content. The chlorophyll meter offers a possible alternative technique to estimate dryland winter wheat N status and determine the need for additional N fertilizer Follet et.all.(1992). According to MacKown and Sutton and Lin et al. (2007) chlorophyll meters can be used for evaluating nitrogen nutrition of tobacco plants. Lin et al. (2007) observed that chlorophyll meter readings varied with year, location, N rate, leaf position on the stem and part of the leaf. Castelli and Contillo (2009) in pot experiment with flue-cured tobacco varieties found that SPAD values were well-correlated with both total chlorophyll and total N leaf concentration, and one leaf stalk position alone is able to monitor the N-status of the whole tobacco plant during the first six weeks after transplanting, without distinction of year and variety effects.

The aim of this work was to assess the usefulness of non-destructive and quick measurements of leaf chlorophyll content with a Hydro N-Tester (HNT) as indicator of oriental tobacco nitrogen status.

MATERIAL AND METODS

For the purposes of the study four nitrogen rates (0, 25, 50 and 100 kg ha^{-1}) were tested in a stationary field trial. A long-term fertilizer experiment with continuous tobacco cropping system was established at Tobacco and Tobacco Products Institute – Markovo, Bulgaria on rendzina soil in 1966. The experimental design was a randomized complete block replicated three times. In 2006 oriental tobacco plants (Nicotiana tabacum L. cv. Plovdiv 7) were grown in the stationary field. The plot area was 6.25 m^2 (2.5 X 2.5 m). Nitrogen was applied as urea before transplanting. Tobacco seedlings were transplanted at a 0.5 x 0.12 m distance (166 000 plants ha^{-1}). All cultural practices were in accordance with those used by the growers for oriental tobacco production. Leaves were harvested in three primings and were sun-cured.

At the beginning of the experiment, the air-dried soil had the following characteristics in the top layer: pH - 8.5, total humus - 3.01%, available P_2O_5 - 15 mg kg^{-1} soil and available K_2O - 400-500 mg kg^{-1} soil (10).

Leaf chlorophyll content was measured with a Hydro N-Tester (HNT), a portable instrument measures the light transmittances of the leaf at 650 nm and 960 nm wavelength, red and near infrared chlorophyll absorption. Measurements were carried out during the following stages: rosette (35 days after transplanting - DAT), ripeness of basal leaves (56 DAT)

and ripeness of middle leaves (77 DAT) on thirty youngest fully formed leaves (third or fourth leaf from the top). At the same dates leaves were collected and tissue total N was analyzed using the Kjeldahl method.

The relationships between N rate, chlorophyll meter readings and selected dependent variables (yield of cured leaves, percentage of Ist grade, chlorophyll meter values and total N content) were studied by regression analysis.

RESULTS AND DISCUSSION

Yield of cured leaves was greatly affected by the rate of N application (Fig. 1). The average yield was 509 kg ha^{-1}, 733 kg ha^{-1}, 847 kg ha^{-1} and 905 kg ha^{-1} for the N_0, N_{25}, N_{50} and N_{100} treatment, respectively.

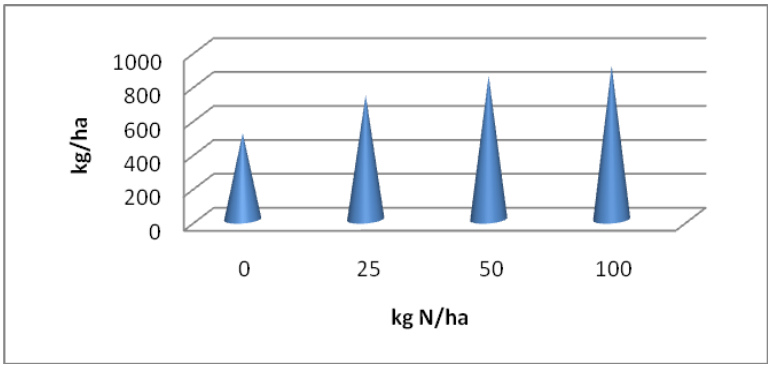

Figure 1. Effect of N rate on yield of cured leaves

The fertilizer N treatments resulted in diminishing rates of increase in leaf yield with increasing amounts of N fertilizer. The N rate of 25 kg N ha^{-1} increased cured-leaf yield by 44% compared to the unfertilized control. A further increase of nitrogen to 50 and 100 kg ha^{-1} resulted in an increase in leaf yield by 66 and 78%. The response in leaf yield (Y) to applied N (N) fitted quadratic model:

$Y = 513.4 + 9.793N - 0.059N^2$, $R^2 = 0.997$

Raw tobacco product quality was also influenced by N fertilizing rates (Fig. 2). The highest average percentage of Ist grade was obtained from the unfertilized plots. Leaf quality declined as nitrogen fertilization increased from 0 to 100 kg N ha^{-1}. The percentage of Ist grade was the lowest (6.3%) as a result of applying the largest nitrogen rate.

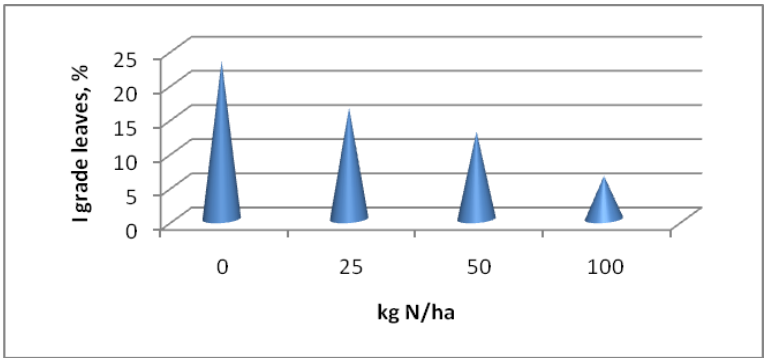

Figure 2. Effect of N rate on tobacco leaf quality

Model for linear relationships among nitrogen treatment rates (N) and changes in tobacco leaf quality (Y) was significant ($P < 0.05$):

$Y = 21.74 - 0.16N$, $R^2 = 0.964$

Given the above data we can point out that under our experimental climatic and soil conditions annual fertilizing with 50 kg N ha^{-1} led to obtaining relatively high yield and quality of the raw product of Oriental tobacco grown as monoculture.

Chlorophyll meter readings were in a highly significant positive correlation with total N of the tobacco leaves (Lin L. et.al. 2007). Kowalczyk-Juśko and Kościk (2002) also established a close correlation between nitrogen accumulation and chlorophyll meter readings for Virginia tobacco. Under the experimental conditions the correlation coefficients between chlorophyll meter readings and concentration of total N in tobacco leaves were 0.968, 0.818 and 0.927 for 35, 56 and 77 DAT, respectively. The correlation was statistically significant at the first date. At the subsequent stages the correlation was high but not significant.

Chlorophyll content in the tobacco leaves ranged from 283 to 559 HNT units (Table 1). Chlorophyll meter readings were the highest at the rosette stage (35 DAT). With the exception of the N_{25} treatment the lowest levels of chlorophyll meter readings were found in leaves measured at 56 DAT. Chlorophyll content was increased with an increase in nitrogen fertilizer level. Leaves from the unfertilized plots contained the least chlorophyll at all the measurement times. The average HNT values from the plots with an optimal nitrogen rate (50 kg N ha^{-1}) were from 385 to 534. Chlorophyll content in tobacco from 50 kg N ha^{-1} treatment was 534 HNT units during the rosette stage (35 DAT) when the last cultivation and application of sidedress N would be made. The values obtained from the unfertilized plots were much lower than the optimum N rate. The chlorophyll content at the 100 kg N ha^{-1} treatment ranged between 382 – 559 HNT units. Small differences in chlorophyll content were found between 25, 50 and 100 kg N ha^{-1} treatments during the first and second measurement. Greater differences between plots receiving supplemental N fertilizer were noted 77 days after transplanting.

Table 1. Impact of N fertilizing rate on chlorophyll content (in HNT units) and leaf total N concentration

Treatment kg N ha^{-1}	Chlorophyll content - HNT units			Total N, % dry weight		
	Days after transplanting			Days after transplanting		
	35	56	77	35	56	77
0	403	283	313	2.43	1.66	1.74
25	514	357	343	3.31	2.11	2.34
50	534	385	411	3.54	2.34	3.48
100	559	382	520	3.42	3.02	3.80

Regression equations describing the relationship between the N fertilizing rates and the chlorophyll meter values are presented in Table 2. Statistically significant linear dependency was noted between nitrogen treatments and chlorophyll content in the leaf tissues at 77 DAT.

Concentration of total N in tobacco leaves generally increased with increasing fertilizer N applied (Table 1). The total N concentration of the lamina tissue decreased between 35 and 56 DAT, and increased at 77 DAT. Therefore, patterns of nitrogen accumulation and chlorophyll synthesis during vegetation were similar. Kowalczyk-Juśko and Kościk (2002) reported that the highest N accumulation was observed during the first measurement and subsequent analyses showed lesser N concentration in the tobacco leaves. According to Mylonas (1984) nitrogen concentration in tobacco leaves decreased from the 4th to the 11th week after transplanting. The same author explained this pattern by the high leaf growth rate compared to that of roots for the period from the 4th to the 9th week and by diminishing available soil nitrogen in later growing season. The increased content of total nitrogen in leaves at the last measurement is likely to be related to favorable environmental condition (soil temperature and moisture) for a higher N mineralization in late growth period. There was significant linear interaction between N fertilizing rates and the concentration of N in the leaf tissues at 56 DAT (Table 2). Small differences in N concentration were found between 25, 50 and 100 kg N ha^{-1} treatments during the first measurement and the correlation coefficient was lower at this stage.

Table 2. Relationships between the N fertilizing rates and changes in the chlorophyll meter values and total N content

Dependent variables	DAT	Relationship	Correlation coefficient (r)
Chlorophyll content - HNT units	35	Y = 442.4 + 1.37x	0.852
	56	Y = 313.2 + 0.88x	0.792
	77	Y = 303.2 + 2.14x	0.994**
Total N, % dry weight	35	Y = 2.81 + 0.01x	0.712
	56	Y = 1.70 + 0.01x	0.996**
	77	Y = 1.92 + 0.02x	0.935

** Significant at 0.01 probability level

The yield response of cured leaves to HNT values measured with the chlorophyll meter was described by a linear model (Table 3). At 35 and 56 DAT there was strong significant correlation between chlorophyll meter readings and leaf yield, but there was no significant interaction between the chlorophyll meter values and

leaf yield at 77 DAT. The increase by 1 HNT unit produced a rise of 1.64 to 3.60 kg ha^{-1} in yield of cured leaves. Borges et al. (1) found an increase of 11.4-20 kg ha^{-1} in wrapper yield with the rise of the SPAD value by 1 unit. Negative relationship between HNT values and raw tobacco product quality assessed on the basis of the percent of I^{st} grade was observed (Table 3).

Table 3. Relationships between chlorophyll meter readings and yield of cured leaves and tobacco leaf quality

Dependent variables	DAT	Relationship	Correlation coefficient (r)
Yield of cured leaves, kg ha^{-1}	35	Y = -509.7 + 2.50x	0.985*
	56	Y = -518.0 + 3.60x	0.978*
	77	Y = 96.3 + 1.64x	0.745
Tobacco leaf quality, percent of I^{st} grade	35	Y = 62.8 - 0.10x	0.936
	56	Y = 61.0 - 0.13x	0.889
	77	Y = 43.8 - 0.07x	0.958*

* Significant at the 0.05 probability level

CONCLUSIONS

Chlorophyll content in tobacco leaves ranged from 283 to 559 HNT units and was affected by leaf measurement date and N fertilizing rates. Chlorophyll meter readings were highly correlated with concentration of total N in tobacco leaves and yield of cured leaves. Chlorophyll meter readings of youngest, fully formed leaves (third or fourth leaf from the top) could be used to accurately separate N-deficient from N-sufficient treatment. Soil tests prior transplanting of oriental tobacco have been taken to make fertilizer N recommendations. The chlorophyll meter offers a possibility to estimate tobacco N status and determine the need for additional nitrogen fertilizer before onset of rapid growth.

Additional studies with different cultivars of tobacco, as well as in different soil and climate conditions are necessary. Consequently, after more testing, Hydro-N-tester values can be used as an indirect method for assessing the nitrogen nutrition of oriental tobacco at different stages of vegetation.

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In memoriam

Dr Iskra Hristovska

On 22.2.2013 we lost our dear colleague, senior research fellow in Department of tobacco protection from diseases, pests and weeds at Tobacco Institute-Prilep.

The news of her untimely death shook all the employees of Tobacco Institute-Prilep. We knew that she fights with a serious disease, but we hoped that she would win yet another victory in her life. Unfortunately, we lost forever a young person, full of energy.

Dr Iskra Hristovska was born on 5.3.1965 in Skopje, where she completed her elementary school and high school education. She graduated from the Faculty of Agriculture in Skopje in 1989. Throughout her education, she was a distinguished and successful student, with high average grades.

In 1989 she enrolled postgraduate studies in Genetics and selection of plants with seed production at The Faculty of Agriculture in Skopje and finished it with 9.20 average grade.

In 1990 she worked as teaching assistant at the Faculty of Agriculture – Skopje and received a scholarship from the Ministry of Science of the Republic of Macedonia.

In 1991 she took the course on genetic engineering in MANU (Macedonian Academy of Science and Arts). Together with a group of professors from the Faculty of Agriculture she was included in the project "Gene-Bank". Her good proficiency in English allowed her to follow the contemporary foreign literature and to take participation in projects.

On 10.3.1995 she defended her master thesis entitled "Investigation of the variability of some producing and quality characteristics of soybean (*Glycine hispida Max*.) in the region of Skopje". The same year she submitted a proposal for doctoral dissertation and in 1996 she started with the experimental part of the thesis.

In 1997 she took three months specialization on the methods for investigation of tobacco resistance to diseases at the Institute of Genetic Engineering in Kostinbrod- Bulgaria.

On 1.1.1998 she was employed at Tobacco Institute-Prilep, in Department for tobacco protection from diseases pests and weeds.

On 25.5. 1999 she defended the doctoral dissertation entitled "*Phytophthora parasitica var. nicotianae* and possible measures for its control" .

From 2004 to 2008 she was a member of the Commission on pesticides in the *Ministry of Agriculture, Forestry and Water Economy.*

She presented her research work at many scientific meetings, congresses and symposia and she is also an author of several research papers published in scientific journal *TUTUN/Tobacco*.

Dr Iskra Hristovska successfully combined her career and motherhood. She was a good colleague, open and honest person. She always had a positive attitude toward solving the working assignments. She remained optimistic until the last moments of her life.

In 1999 Dr Iskra Hristovska became our representative in Skopje, engaged on assignments related to the work of the Institute that have a big impact to tobacco economy.

With her scientific-research activity on diseases that attack tobacco, Iskra Hristovska gave a great contribution in solving the actual problems, and with her commitment she contributed to the development of tobacco science.

She became a modern researcher, but she stopped only a step before being promoted to the highest level in her profession. Death was an obstacle to her further creative work in the field of tobacco science. The most painful thing, however, is that her young life ended too early and separated her from her loved ones, from the joys of life.

Staff of the Scientific Tobacco Institute – Prilep express its deepest condolences to her family and gratitude to her contribution in the development of scientific thought and the prosperity of tobacco science.

INSTRUCTIONS TO AUTHORS

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Since the publication is of an international character, all manuscripts should be submitted in English. Authorswhose native language is not English should have their paperschecked by research workers from the related fields who have good proficiency in the English language. All manuscripts must be proofread prior to submission. Language and style of the manuscripts are responsibility of the author.

The publication presents:original scientific papers,review articles, short reports, professional

papers andother works related to tobacco science and practice.

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Tables should be numbered with Arabic numeralsaccording to their sequence in the text. The table title should be always above the table,centered, in 10 pt font, with one empty row between the title and the table and another one between the table and the text. Tables should be simple

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Reference to the Table, example: It could be seen from Table 1...., or: The nicotine content in tobacco is 0.98% (Table 4).

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Weybrew J.A., Wan Ismail W. A., Long R. C., 1983. The cultural management of flue-cured tobaccoquality.Tob. Sci. 27, 56-61.
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