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POSSIBLE USE OF THE CHLOROPHYLL METER (SPAD - 502) FOR EVALUATING NITROGEN NUTRITION OF THE VIRGINIA TOBACCO

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ABSTRACT

The aim of the present study was to determine a correlation between nitrogen fertilisation and leaf chlorophyll content as measured using an optical instrument – a chlorophyll meter and an effect of different nitrogen fertilisation doses on the yield and quality of leaves of the 'Wiślica' cultivar of the Virginia tobacco. To this purpose, a field experiment was conducted, in which varied nitrogen fertilisation was applied. During the vegetation period leaf chlorophyll content was examined, using the chlorophyll meter, as well as leaf nitrogen content. After harvesting and drying the leaves, the yield was assessed in terms of quantity and then graded according to its quality. Statistical analysis carried out afterwards demonstrated that the increase in nitrogen fertilisation had resulted in the yield growth together with the deterioration of the raw product quality. The results of the measurements with the chlorophyll meter proved to be closely correlated with nitrogen content in leaf blades, which means that the instrument can be used for evaluating nitrogen nutrition of tobacco plants.

Key words: tobacco, chlorophyll meter, nitrogen fertilisation

INTRODUCTION

In order to obtain adequate quality tobacco yield, one must provide the plants with numerous necessary nutrients, of which nitrogen has the greatest consequence for their growth and development. Both deficiency and excess in nitrogen supply may be detrimental to the plants. Nitrogen considerably affects chemical constitution of raw tobacco product, which determines the latter's technological value and its degree of harmfulness for cigarette smokers [29,24]. Evaluation of nitrogen status in tobacco leaves requires tissue and soil laboratory analysis, which is time-consuming and costly.

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One of the first indicators of nitrogen deficiency is leaf yellowing, caused by transferring part of the nitrogen to newly formed plant organs and by chlorophyll decomposition. A close correlation between chlorophyll content in a plant and its nitrogen status enables researchers to make an evaluation on the basis of plant pigment content alone [7]. Quantitative assessment of leaf chlorophyll content using laboratory tissue tests has not had practical application.

On the other hand, an optical instrument called N-tester or chlorophyll meter provides a fast, non-destructive method of estimating chlorophyll content by measuring differences between light absorption at 650 nm and 940 nm [28]. Light with a wavelength of 650 nm is within maximum absorption range of chlorophylls *a* and *b*, whereas a wavelength of 940 nm corresponds to the far red, where chlorophyll spectrum is not transmitted. A measurement result is rendered in units called SPAD (an acronym from Soil Plant Analysis Division, the organization that has developed the instrument) [20]. Readings range from 0 to 800 SPAD, while in the older version - from 0 to 80 SPAD.

A close correlation ($r = 0.93$) was established between the measurements of chlorophyll content using the N-tester and those obtained by laboratory tissue testing, for soybean, rice and spring wheat leaves [22], potato ($r = 0.97$) [30], rice [14]. Also Dwyer et al. [8], Piekielek and Fox [25], Smeal and Zhang [27], Waskom et al. [31] confirm that the measurement results obtained using the SPAD optical method reflect the actual chlorophyll content in various crop plants. This pattern was also observed to occur for tobacco leaves from different layers of the plant [17].

Following up on the relations between nitrogen and chlorophyll content and SPAD readings, works were undertaken aiming to develop a field test for determining the crop N status and making nitrogen fertilisation recommendations according to measurements using the N-tester. Most endeavours of this kind focused on corn [5,7,8,25,26,27,31,32], where statistical analysis showed a close correlation between SPAD readings and nitrogen concentration in leaves. Similar research projects involved experiments with rice [13,14,19,23], potato [12,30] and winter wheat [1,2,3,9,10,11]. Coefficient values of correlation and regression between nitrogen concentration and chlorophyll meter-based measurements proved to be significant in those cases, too.

A preliminary study carried out by the present authors, as well as scarce reports on the subject from abroad, suggest the usefulness of the chlorophyll meter also in appraising nitrogen status in tobacco leaves [16,18,20,23]. Following from the premise that the relation between nitrogen fertilisation and N concentration and chlorophyll content is close and statistically corroborated, the undertaken research will pave the way for further works on test calibration, based on chlorophyll content measurements using the optical method, with respect to different tobacco cultivars.

MATERIAL AND METHODS

The field experiment was conducted at the Zamość Tobacco Crop Testing Centre over 1996 - 1998. The experiment followed randomised split-plot design, repeated four times. The following doses of nitrogen were applied: 0 - control; 7.5; 15; 22.5; 30 and 45 kg of N per ha. The level of P and K fertilisation was constant and remained at 39.6 $P \cdot ha^{-1}$ and 124.5 kg $K \cdot ha^{-1}$. Nitrogen fertiliser doses of up to 22.5 kg $N \cdot ha^{-1}$ were applied immediately before transplantation, whereas as regards doses of 30 and 45 kg $N \cdot ha^{-1}$ the remaining amount was used 14 days after transplanting the 'Wiślica' cultivar of the Virginia tobacco.

The experiment was set up on brown soil of lessive origin, with a slight acid reaction, very rich in P and K and moderately rich in Mg and Fe. The area of individual plot was 27 m^2, the plants were spaced at intervals 90 cm by 40 cm. During the vegetation period chemical protection and standard cultivation procedures were carried out (including topping and suckering).

From the beginning of the stalk development stage onwards (approximately 35 days after the transplantation) to the end of the vegetation period, leaf chlorophyll content was measured using, by courtesy of Hydro Poland, an optical instrument, the N-tester SPAD - 502, a Minolta Co. Ltd. product. Measurements were carried out every 10 days on thirty newest, but fully formed leaves. The measurements were taken on the spot without removing the leaves. At the same dates portions of leaf tissue were collected to be analysed for nitrogen accumulation using the potentiometric sodium hypobromite titration method.

Leaves were harvested following the maturation of particular layers. Then the air-cured leaf yield was assessed and the percentage of light grades (I and II) was calculated. A large percentage of light grades in raw tobacco product is desirable because it enables the planter to claim a high price and indicates good technological value. The results were verified statistically using analysis of variance. Tukey's test at $\alpha = 0.05$ was used in order to estimate the significance of the differences.

In terms of weather conditions the time span of the study was quite diversified. Although the average air temperature in all the three vegetation seasons approached the multi-years average, wide fluctuations were recorded in particular months and decades. In the third decade of May in 1998 killing frosts occurred, which inhibited the growth process in the plants and negatively affected the establishment of the seedlings. Additionally, low level of rainfall was observed in the same period. In the second decade of July in 1996 thunder and hailstorms damaged tobacco leaves. The weather conditions in September in 1996 were not conducive to maturing leaves in the top layers either, as low air temperature was recorded, and killing frosts, accompanied by heavy rainfall (Table 1).

Table 1. Air temperature and rainfall over the tobacco vegetation season

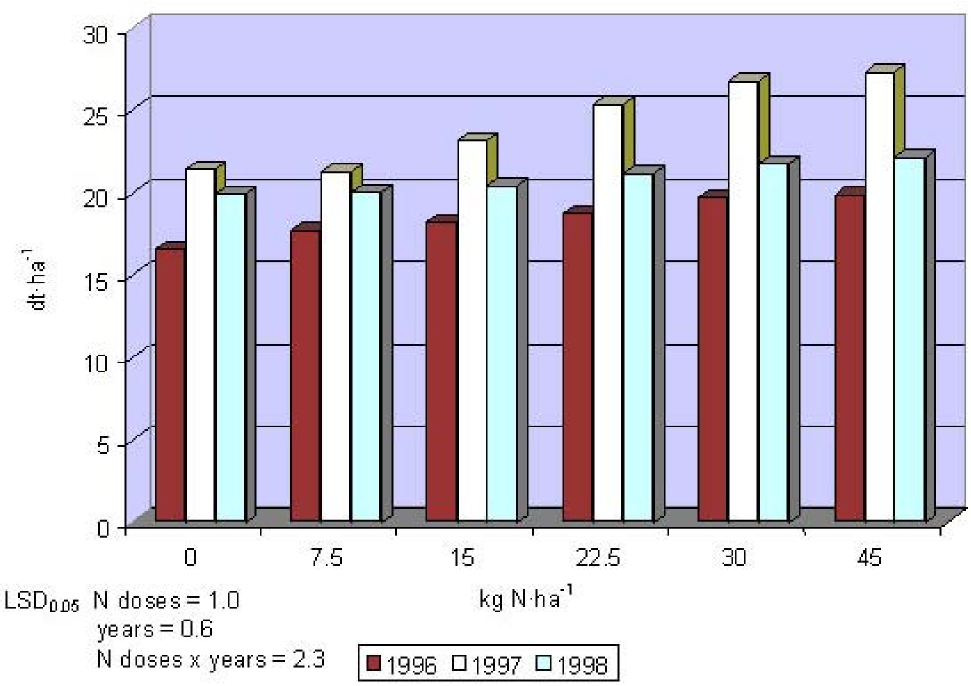
Month	Air temperature, °C				Precipitation, mm			
	1996	1997	1998	1951-2000	1996	1997	1998	1951-2000
May	16.1	14.4	13.7	13.1	122	70	54	62
June	16.7	16.8	17.9	16.4	17	28	124	81
July	16.5	17.6	18.0	17.9	120	201	115	91
August	17.3	17.6	16.5	17.0	54	93	67	81
September	10.2	12.7	12.8	13.1	94	83	41	52
Mean/Sum for May through September	15.4	15.8	15.8	15.5	407	475	401	367

Source: Meteorological Bulletin [4]

RESULTS

The experiment established steady growth in leaf yield of the Virginia tobacco as a result of increased nitrogen fertilisation. A significantly higher yield was obtained after applying the dose of 15 kg N·ha^{-1} and each added dose led to its further increase (Fig. 1). The highest yield, 23.1 dt·ha^{-1}, was achieved in the plots subjected to the fertiliser dose of 45 kg N·ha^{-1}, which means a 19.7% increase compared to the control. A statistically significant growth in the yield as a result of applying the fertiliser doses of 30 and 45 kg N·ha^{-1} was observed in comparison to the dose of 15 kg N·ha^{-1}.

Fig. 1. Impact of N dose on tobacco leaf yield



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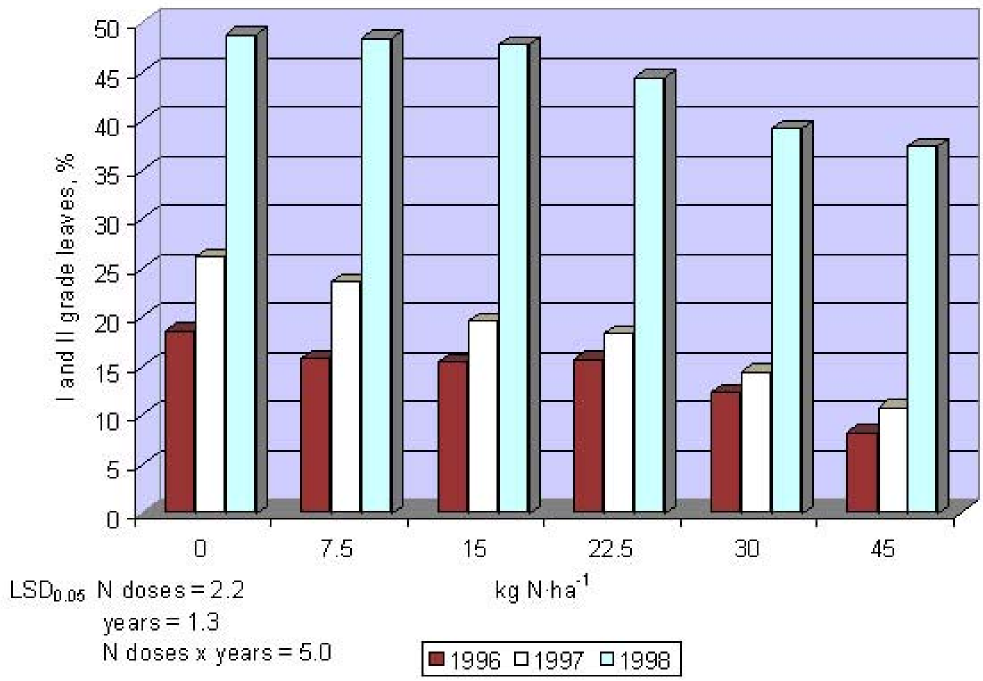
However, tobacco leaf yields were mostly affected by the weather conditions over the three vegetation seasons. The difference between the average yield obtained in the year most favourable to vegetation, that is 1997, and that of the year characterised by periodic droughts and unfavourable distribution of rainfall, 1996, reached as much as 30.8%. What was also noted was the relationship between the weather factor and the effect of nitrogen fertilisation. The total leaf yield resulting from the application of 45 kg N·ha^{-1} leaped in 1997 by 5.9 dt·ha^{-1} compared to the previous year. This indicates better assimilation of nitrogen from mineral fertilisers over seasons of favourable weather conditions.

Growth in leaf yield due to N fertilisation was accompanied by simultaneous deterioration of raw tobacco product quality. Quality of the raw product was assessed on the basis of the proportion of best grades I and II. The best quality raw product was obtained from the control. Due to the fertilisation dose of 7.5 kg N·ha^{-1} leaf quality declined insignificantly. A considerably decreased proportion of top grades leaves was brought about by the application of 15 kg N·ha^{-1}. Added fertiliser doses led to further deterioration in the quality (Fig. 2). The percentage of light grades was the lowest as a result of applying the largest N fertiliser doses, with the average value for the years 1996-1998 of 18.8%, and as such was significantly smaller compared to the other objects.

Fig. 2. Impact of N dose on tobacco leaf quality



The most decisive factor determining raw tobacco product was that of weather conditions during the consecutive growing seasons. The largest proportion of grades I and II (44.4% on the average) was achieved in 1998, when the overall pattern of weather phenomena during leaf maturation stages proved beneficial (despite the unfavourable conditions in the spring). The level of rainfall in August and September was low and insolation value did not diverge from the multi-years average, which resulted in the normal course of maturation processes in tobacco leaves. The proportion of light grades in the total yield in 1996 was 14.4%, i.e. three times lower than in 1998. Such a low quality of raw tobacco product had been mainly caused by damages due to hailstorms. While considering the influence of N fertiliser doses on raw product quality, it may be observed that it was at its lowest in 1998. The relative decline in quality resulting from the largest N dose in the same year was 30.1% compared to the control, whereas in the other years the quality went down by more than a half. This interaction has also been proved statistically.

Despite the fact that the largest yield was obtained as a result of applying the N fertilizer dose of 45 kg N·ha^{-1}, it was accompanied by the dramatically fall in the raw product quality. Taking into account quality traits of raw tobacco product, one can observe that the optimum N fertiliser dose for the Virginia tobacco is 22.5 kg N·ha^{-1}, which leads to a sufficiently high yield while preserving a relatively good quality of raw product.

The N fertilisation of the Virginia tobacco affected not only the yield and quality, but also its chlorophyll content as measured by the chlorophyll meter. Leaves of the plants from the control were found to contain the least

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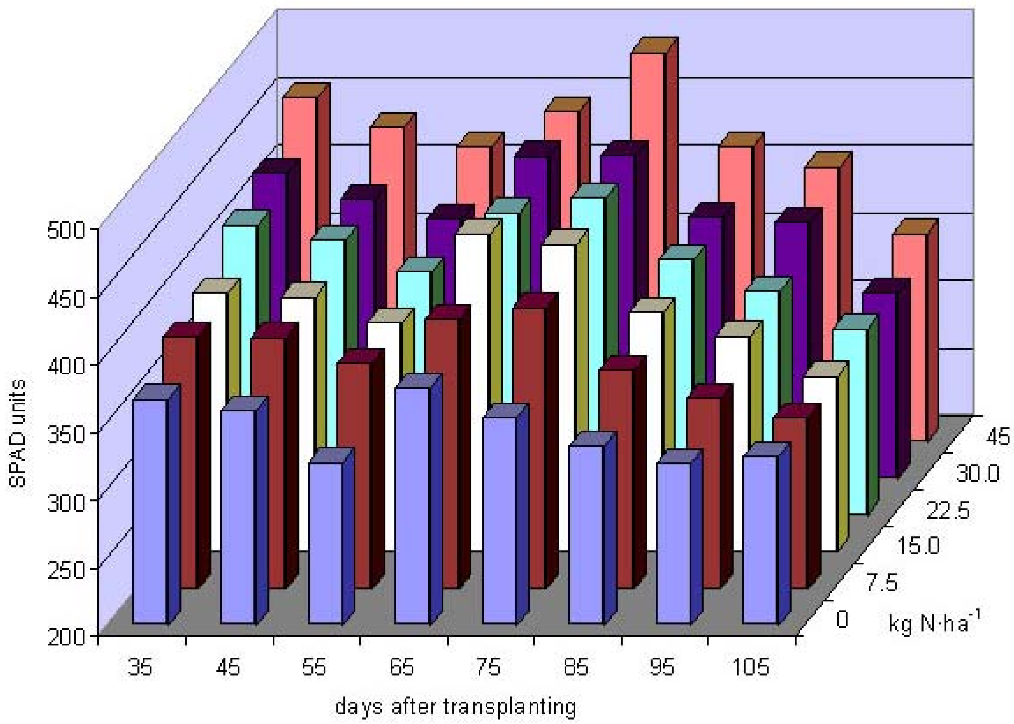
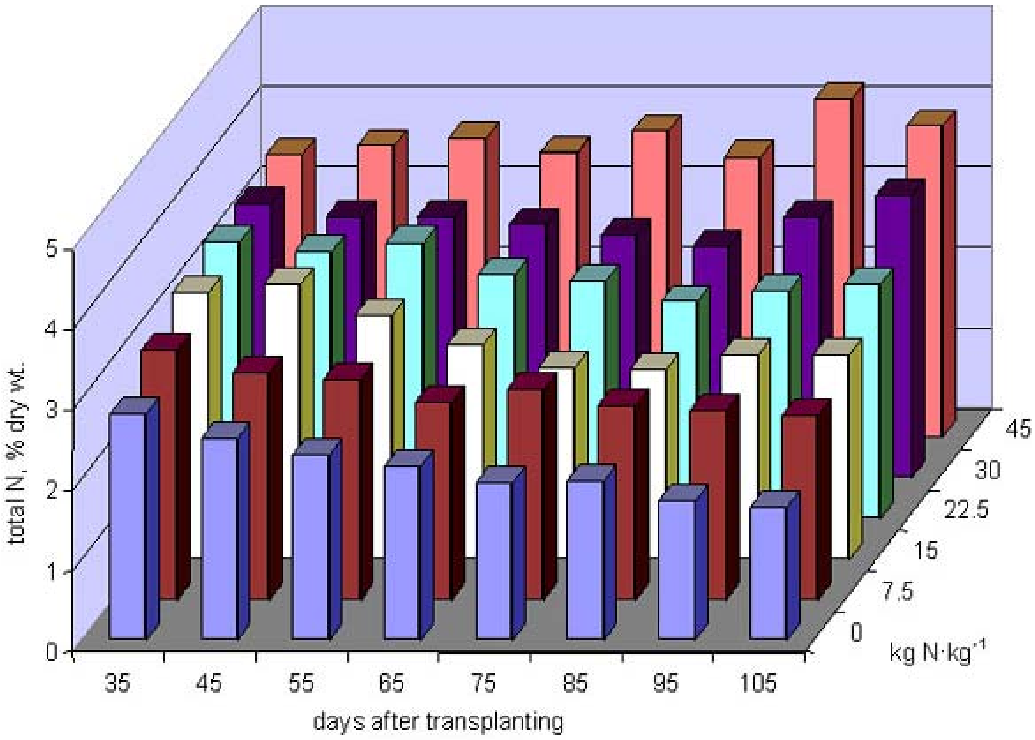
chlorophyll at all the measurement times (Fig. 3). Added nitrogen doses led to increased chlorophyll content in tobacco leaves. The trend was observed throughout the whole vegetation season; however, the last measurement indicated a looser relationship between the N fertilisation and chlorophyll content. For the plot treated with the 22.5 kg N·ha^{-1} dose, which in those soil and climate conditions may be regarded as optimum, the average chlorophyll content amounted to 390 SPAD units. The application of the N fertilisation doses of 30 and 45 kg N·ha^{-1} made the chlorophyll content exceed 400 SPAD units. Exceeding 400 SPAD units indicates excessive N fertilisation. Chlorophyll content in the Virginia tobacco leaves throughout the vegetation season ranged from 316 to 439 SPAD units. Maximum chlorophyll readings were obtained about 75 days after transplanting. Then the chlorophyll content gradually decreased till the end of the vegetation season.

Fig. 3. Impact of N dose on chlorophyll content in SPAD units over tobacco vegetation season



Chlorophyll content in tobacco leaves is connected with nitrogen concentration as the latter constitutes an element of this plant pigment. Chemical tissue analyses of leaves sampled from the same stem layers on which the chlorophyll meter measurements had been taken, corroborated the relation. The percentage of total N concentration in dried leaf blades increased as larger and larger N doses in mineral fertilisation were applied (Fig. 4). The pattern of nitrogen accumulation during the vegetation season deviated from the pattern of chlorophyll synthesis. The highest N accumulation was observed during the first measurement. Subsequent analyses showed lesser N concentration in the leaves. The lowest N concentration was noted 65 days after transplanting, and then it remained roughly at this level till the end of the vegetation season. The fact that the N concentration stayed roughly at the same level throughout the whole period from 65 days after transplanting onwards till the end of the vegetation season, while chlorophyll content in the leaves fell, points to the accumulation of nitrogen compounds other than pigments (e.g. proteins or alkaloids, including nicotine).

Fig. 4. Impact of N dose on nitrogen content (% of dry weight) over tobacco vegetation season



The relation between chlorophyll content as measured by the chlorophyll meter and nitrogen concentration is often emphasized in the literature on the subject thanks to its practical value as a possible indicator of nitrogen nutrition of crops. The Pearson's coefficient of the correlation between index of leaf greenness SPAD and N accumulation, estimated for the whole data sampling period in three years was positive (r = 0.548), with the significance level $\alpha = 0.05$. However, coefficient values estimated on the basis of results obtained from consecutive measurements during a vegetation season were actually much higher (Table 2).

Table 2. Coefficient values of correlation between chlorophyll meter readings and N content over vegetation season

Number of days off transplanting	35	45	55	65	75	85	95	105
Correlation coefficient* (r)	0.944	0.956	0.967	0.903	0.882	0.891	0.821	ns

*** r significant at $\alpha = 0.05$; ns – r non-significant at $\alpha = 0.05$**

The first three chlorophyll readings were closely correlated with total nitrogen accumulation in the leaves. At the subsequent stages, however, the correlation became increasingly looser, and the last reading showed no correlation between chlorophyll content and N concentration. This finding corroborates the before-mentioned supposition that it was the other nitrogen compounds that accumulated in the leaves. Hence once cannot overlook the fact that evaluation of nitrogen status on the basis of chlorophyll meter readings is possible only at the early stages of the vegetation. This, however, does not preclude the practical value of the chlorophyll meter because tobacco topdressing, against which chlorophyll meter measurements are considered, is carried out during the first 2 to 5 weeks after transplanting.

Linear relationship between chlorophyll meter readings and nitrogen content, leaf yield, and raw product quality has been captured by the equations presented on the respective figures. Coefficient of determination R^2 between chlorophyll content in SPAD units and nitrogen concentration (Fig. 5) and leaf yield (Fig. 6) proved to be significant (R^2 = 0.6936 and 0.5869, respectively). However, negative relationship between SPAD leaf greenness index and raw tobacco product quality (Fig. 7), assessed on the basis of the percent-age of grades I and II, proved to be insignificant. This should be attributed to the law quality of raw product in one of the years of the experiment (1996), which occurred irrespectively of nitrogen fertilisation and consequently, of chlorophyll content.

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Fig. 5. Relationship between total N content and chlorophyll meter readings

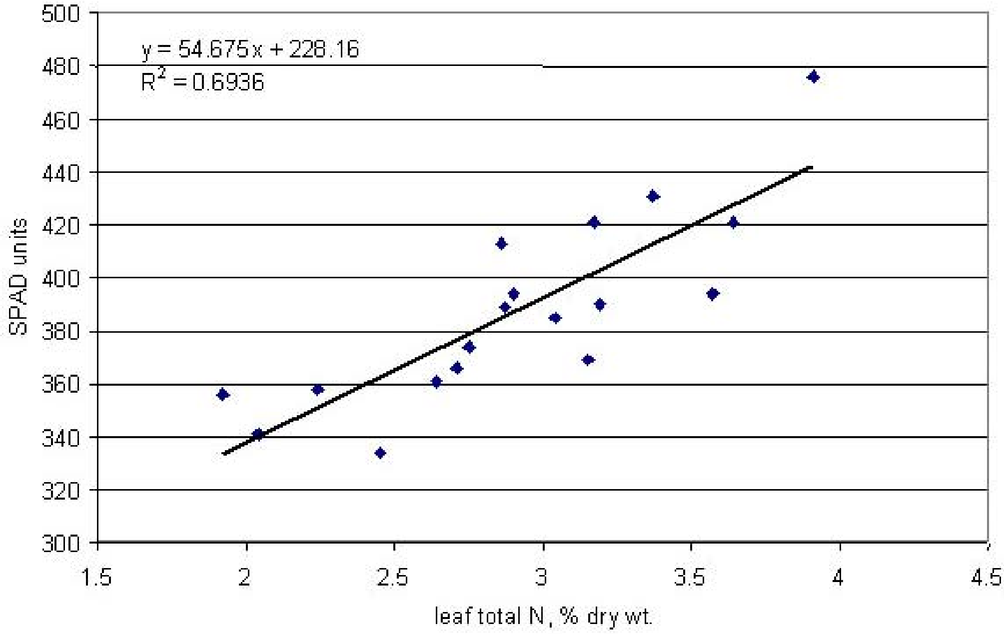

Fig. 6. Relationship between tobacco leaf yield and chlorophyll meter readings

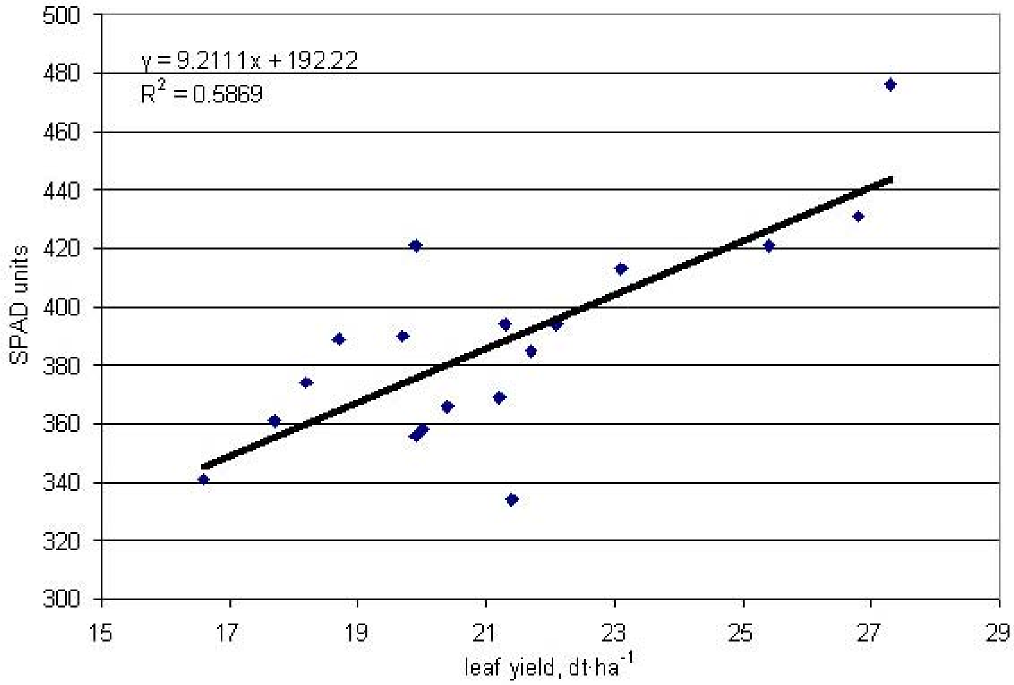

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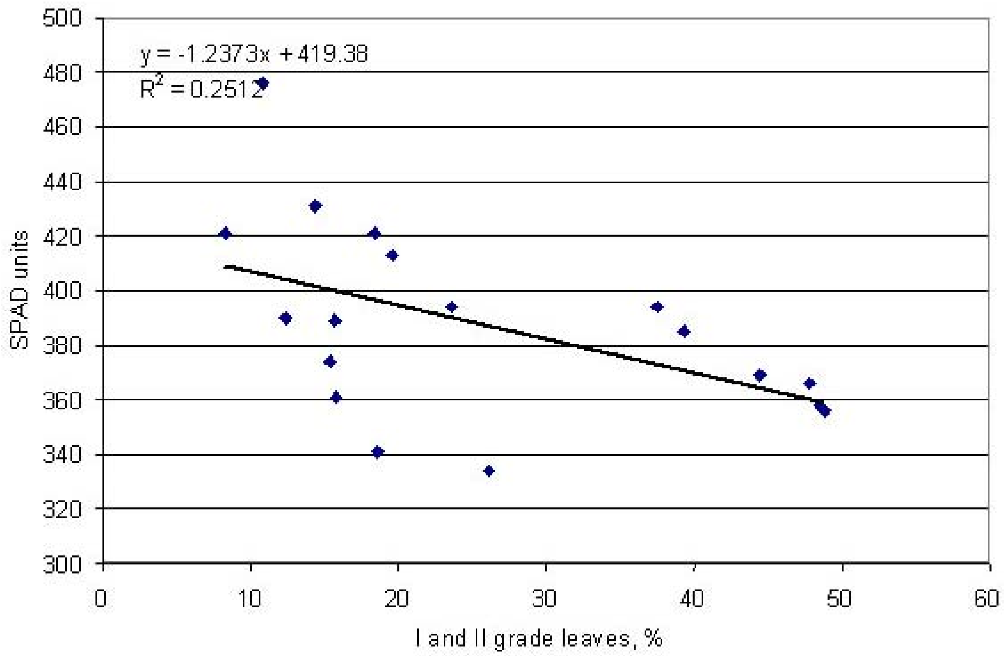
Fig. 7. Relationship between chlorophyll meter readings and tobacco leaf quality



DISCUSSION

Increased leaf yield of the Virginia tobacco as a result of added N fertilisation and concomitant deterioration of leaf quality, observed by the authors of the present study, was also reported by other researchers [24,15,29,21]. In an experiment carried out in North Carolina (USA), 4 doses of N fertilisation were applied, ranging from 50 to 123 kg N·ha^{-1}. After topping leaves were collected from the bottom, middle and top layers, which were examined for chlorophyll content using the N-tester, and then tissue-tested in the laboratory for nitrogen accumulation. Similarly to the experiment in the present study, statistical analysis demonstrated significant correlation between SPAD readings and total nitrogen content in leaf blades [33]. In Kentucky an experiment was conducted in 1993, whose aim was to determine a relationship between selected traits of burley tobacco and nitrogen fertilisation [20]. The findings showed that added N fertilisation doses led to increased chlorophyll content as established by tissue-testing and SPAD readings as well as higher N concentration in leaf blades. This corroborates the results obtained in the present study. Models for linear relationships between these traits were significant (r^2 = 0.60 and 0.66) The content of the TN86 chlorophyll did not exceed 38 SPAD units, even when treated with the excessive N fertilisation dose of 336 kg N·ha^{-1}, which indicates that chlorophyll content in burley tobacco leaves is lower than in the Virginia tobacco, the latter including as it does the ‘Wiślica’ cultivar.

In the studies conducted on the ‘Wilga’ cultivar of the Virginia tobacco, it was observed that SPAD chlorophyll readings rose in the period from the second measurement onwards to the maturation phase of the top layer leaves, and after that it gradually fell, matching the pattern revealed in the experiment analysed in the present study [16,18]. N-tester readings were closely, positively related to N fertilisation dose, leaf yield as well as nicotine and protein content in tobacco leaves of the ‘Wilga’ cultivar. Chlorophyll content in the leaves treated with the optimum mineral fertilisation dose ranged from 330 to 380 SPAD units during the plant growth stage that is at the time of applying complementary mineral fertilisation.

The present study took into account only one tobacco cultivar. However, particular crop types are widely diversified in terms of leaf pigmentation intensity, variations occurring even within one type. Bezduszniak et al. [3] point out the difference in leaf chlorophyll content for wheat cultivars. The issue of the influence of a cultivar type on SPAD leaf greenness index is raised also by Schepers et al. [26] and Waskom et al. [31] in their studies of corn. These researchers emphasise the necessity of allowing for differences between cultivars when evaluating nitrogen status of crops using SPAD readings. Hence, recommendations for nitrogen fertilisation based on SPAD readings depend on further investigations of chlorophyll content in tobacco leaves sampled from different cultivars.

CONCLUSIONS

1. Increased N fertilisation led to the growth of leaf yield of the Virginia tobacco. At the same time the decline in raw tobacco quality, as measured by the percentage of light grades (I and II), was observed. The optimum N fertilisation dose in soil and climate conditions approaching those of the discussed experiment was 22.5 kg $N \cdot ha^{-1}$.
2. Increased N fertilisation affected chemical constitution of leaves – chlorophyll and total N content in leaf blades went up.
3. The results of chemical tissue tests and measurements taken using the chlorophyll meter indicated a close correlation between accumulation of nitrogen and SPAD readings, with the correlation becoming looser as the tobacco plants aged.
4. Estimation of nitrogen nutrition needs of tobacco plants on the basis of SPAD readings requires calibrating a test grounded in chlorophyll measurements using the optical method, with the allowance made for different types and cultivars of tobacco, as well as in varied environmental conditions. The presented research shows that the SPAD-502 chlorophyll meter may be a valuable tool for non-destructive evaluation of nitrogen content in tobacco leaves.

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