

## **EFFECT OF LONG-TERM PHOSPHORUS FERTILIZATION ON THE MINERAL COMPOSITION OF ORIENTAL TOBACCO**

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### **Abstract**

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The effects of the different phosphorus rates (0; 75 and 225 kg ha<sup>-1</sup>) on tobacco yield and concentration of macro- and micronutrients in leaves have been studied in a stationary field trial. A long-term fertilizer experiment with continuous tobacco cropping system was established on rendzina soil (Rendzic Leptosol) in 1966. Phosphorus was identified as a factor limiting cured leaf yield in continuous tobacco cropping system, established on a soil with low available P. With the increase of phosphorus fertilization rate the content of the phosphorus and calcium in leaves also increased. Zinc concentration was significantly greater in the lower and middle leaves that received no supplemental P fertilizer compared with those plots that did. Copper content of the leaves decreased with increase of phosphorus fertilizing level from 0 to 75 kg ha<sup>-1</sup>. Fertilization with 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> should be considered as optimal for producing high yield of sun-cured leaves.

*Key words:* tobacco, phosphorus fertilization, macronutrients, micronutrients

### **Introduction**

Optimum tobacco growth can only be achieved with adequate and well timed nutrient supply under favorable environments (Tso, 1990). Maintaining an adequate P concentration in the soil solution is necessary for improved yield and quality of tobacco. In general, soils used for tobacco production in Bulgaria are low in available phosphorus. Phosphorus deficiency results in stunted growth, poor leaf expansion and unusually dark-green leaves (Flower, 1999). In long-established areas of production, repeated application of large quantities of phosphorus fertilizer, coupled with low plant absorption and essentially no losses by leaching, have resulted in a substantial increase in the level of this element in the soil (McCants and Woltz, 1967). Only extremely large amounts of applied phosphorus or the use of phosphorus over a long period of time would result in high concentrations of P in soil solution and subsequent reduction in uptake of microelements (Racz and Haluschak, 1974). Moderate excess of

phosphorus usually causes no marked effect on tobacco. Extremely high levels of phosphorus can reduce yield and result in narrow thick leaves (Tso, 1999). Excessive phosphorus in soil depresses Zn uptake and reduces the level of Mg in the tobacco plant (Takahashi and Yoshida, 1957; Burleson et al., 1961). Reductions in micro-element uptake from highly phosphated substrates are mainly due to ion antagonism effects and not to soil micro-element-phosphorus interactions (Racz and Haluschak, 1974). The amount of added P had a direct effect on Ca, Mg, and B and an inverse effect on Zn and Cu concentrations in the tobacco leaves (Peterson et al., 1969). Lolas et al. (1978) reported that concentrations of N, P, K, Ca and Mg in cured leaves and chemical composition of flue-cured tobacco were not affected significantly by the P<sub>2</sub>O<sub>5</sub> rates.

This paper considers the results of the long-term phosphorus fertilization on oriental tobacco yield and concentration of macro- and micronutrients in leaves from different stalk position (lower, middle and upper leaves).

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## Materials and Methods

The effects of the different phosphorus rates (0; 75 and 225 kg ha<sup>-1</sup>) on tobacco yield and concentration of macro- and micronutrients in leaves were studied 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 (Rendzic Leptosol) in 1966. The experimental design was a randomized complete block replicated three times. In 2008 and 2009, oriental tobacco plants (*Nicotiana tabacum* L. cv. Plovdiv 7) were grown in the stationary field. The plot area was 6.25 m<sup>2</sup> (2.5 × 2.5 m). Tobacco seedlings were transplanted at a 0.5 × 0.12 m distance (166 000 plants ha<sup>-1</sup>). Cultural practices such as irrigation, weeding, pest and disease control were applied in accordance with the recommended practices for commercial plantations. After the last priming the plots were cleared of crop residues. The land was prepared for seedling establishment by hand cultivation. Urea, triple superphosphate and potassium sulphate were used as the source of N, P and K. Fertilizers were broadcast before transplanting and then were incorporated in the top soil layer. At the beginning of the experiment, the soil had a pH value of 8.5 and contained 3.01% humus, 15 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> soil and 400–500 mg K<sub>2</sub>O kg<sup>-1</sup> soil (Vartanyan, 1979).

In March 2008, soils from the following treatments: N<sub>50</sub>P<sub>0</sub>K<sub>75</sub>, N<sub>50</sub>P<sub>75</sub>K<sub>75</sub> and N<sub>50</sub>P<sub>225</sub>K<sub>75</sub> were sampled for studying long-term effects of increasing P rates on soil fertility. Soil samples were collected from the upper layer (0–25 cm) of each plot. The following soil characteristics were determined: pH in water, humus according to Tjurin (Totev et al., 1987), available P – by the Egner-Riehm method, available K – in 2N HCl. Available Ca and Mg were determined by using 1N KCl (Tomov et al., 1999). A solution of 0.005 M DTPA+0.1 M TEA, pH 7.3 was used for extraction of the Fe, Mn, Zn and Cu mobile forms from soil.

In 2008 and 2009, the following measurements were taken: plant height and number of leaves per plant (as measured at flowering), dimensions of the middle leaves and yield of sun-cured leaves. The concentration of N, P, K, Ca, Mg, Fe,

Mn, Zn and Cu in mature leaves from different stalk position (lower, middle and upper leaves) was determined. All samples were washed with tap water to remove any adhering soil particles and rinsed afterwards with distilled water. Following drying at 75°C for 12 h samples were ground. Total nitrogen in the plants was determined by the Kjeldahl method. The preparation of plant samples for analysis of P, K, Ca, Mg, Fe, Mn, Zn and Cu was made by means of dry ashing and dissolution in 3 M HCl. Phosphorus was determined colorimetrically by the molybdovanadate procedure (Tomov et al., 1999). An atomic absorption spectrometer „Spektra AA 220” (Varian, Australia) was used for determination of K, Ca, Mg, Fe, Mn, Zn and Cu content in the soil and plant samples.

Results were analyzed using the SPSS statistical package and differences were assessed with the Duncan's multiple range test at the 0.05 probability level.

## Results and Discussion

Changes in pH values, humus, available P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O content in soil between the beginning of the experiment and 2008 are shown in Table 1. Soil pH varied from 8.20 to 8.25 among the treatments. After 42 years, the soil reaction was slightly decreased, which indicates its strong pH buffer capacity. Continuous phosphorus fertilization did not affect the soil pH. The humus content was 3.01% in 1966, when the experiment was started. In all treatments the humus content decreased by 13 to 16%. These results point that maintenance of humus content at the initial level is not possible through yearly mineral fertilizer application. The long-term fertilization with different P rates had no recordable influence on the soil humus content. The data for the 42-year period showed annual increases of available P<sub>2</sub>O<sub>5</sub> of 1.9 mg kg<sup>-1</sup> with the lower rate (75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and 4.3 mg kg<sup>-1</sup> with the higher rate. The soil available K content was the lowest in plots fertilized with 225 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. The soil Ca and Mg concentrations were enhanced by long-term fertilization with 225 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Different amounts of P fertilizers applied over 42 years resulted in a small differentiation in soil available

**Table 1**  
Main soil properties, determined in the study

Treatments	pH (H <sub>2</sub> O)	Total humus (%)	Available forms (mg kg <sup>-1</sup> )							
			P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	Ca	Mg	Fe	Mn	Zn	Cu
N <sub>50</sub> P <sub>0</sub> K <sub>75</sub>	8.21	2.52	24.3	577.0	3295	279	5.21	22.58	7.16	11.75
N <sub>50</sub> P <sub>75</sub> K <sub>75</sub>	8.20	2.63	96.2	660.2	3307	296	6.24	23.44	7.34	11.38
N <sub>50</sub> P <sub>225</sub> K <sub>75</sub>	8.25	2.57	195.0	510.6	4490	377	5.04	28.08	7.93	11.30
Initial soil	8.50	3.01	15.0	450.0	nd*	nd	nd	nd	nd	nd

\* – not determined

Fe, Mn, Zn and Cu content. Bogdanovic et al. (1999) have also found that the application of increasing P rates did not decrease the content of available Zn in the soil.

Phosphorus application significantly increased plant height, number of leaves per plant and dimensions of middle leaves, where highest values were obtained at 225 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (Table 2). However, no significant differences were observed in studied characteristics between 75 and 225 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Tobacco yield was increased by phosphorus fertilization (Table 2). As the phosphate treatments with 0 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> differ significantly from each other, this demonstrates that phosphorus was a major limiting factor in this case. No significant yield differences were observed between 75 and 225 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, so continuous fertilization of Oriental tobacco grown as monoculture with very large amounts of phosphate fertilizers is not an effective cultural practice. Table 3 shows the macronutrient concentrations in the lower, middle and upper leaves as dependent on P fertilizing rate, averaged over the period studied.

Nitrogen concentration in the lower leaves did not change significantly ( $P > 0.05$ ) with increasing P level. Significantly higher N concentrations in middle leaves were observed at the 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> treatment as compared to those receiving supplemental P fertilizer suggesting a dilution of nitrogen concentrations by leaf dry matter accumulation. Similar trend was observed for total content of N in the upper leaves.

The P concentration in the upper leaves significantly increased with an increase in phosphorus fertilizer level. No significant differences were observed in P concentration in the lower leaves among treatments receiving supplemental P fertilizer. The P concentration in the middle leaves did not change significantly with increasing of phosphorus level from 0 to 75 kg ha<sup>-1</sup>. The concentration of P in mature leaves of 225 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> treatment is not high – from 0.1 to 0.2% and is lower than the values obtained by Mylonas (1984). The relatively low P accumulation in leaves under continuous fertilization with 225 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> can be explained by the fact that phosphorus uptake by plants depends not only on the levels of soil available phosphorus. Other factors such as soil moisture

and temperature, pH, lime and gypsum in the soil, amount of zinc and other metallic elements in the soil also have a strong influence on the absorption of phosphorus by plants (Traynor, 1980). Soil water content controls the acquisition of P both by favoring root growth and by allowing transport of inorganic P to the root surface by diffusion (Kirkby and Johnston, 2008). It is known that, except in extreme heat and drought condi-

**Table 3**  
Macronutrient concentrations of tobacco leaves as dependent on P fertilizing rate (2-year average)

Treatments	Lower leaves	Middle leaves	Upper leaves
N concentration (% of dry weight)			
N <sub>50</sub> P <sub>0</sub> K <sub>75</sub>	2.05a*	2.42a	3.15a
N <sub>50</sub> P <sub>75</sub> K <sub>75</sub>	2.05a	2.14b	2.86ab
N <sub>50</sub> P <sub>225</sub> K <sub>75</sub>	1.99a	2.00b	2.78c
P concentration (% of dry weight)			
N <sub>50</sub> P <sub>0</sub> K <sub>75</sub>	0.080b	0.085b	0.150c
N <sub>50</sub> P <sub>75</sub> K <sub>75</sub>	0.095a	0.085b	0.185b
N <sub>50</sub> P <sub>225</sub> K <sub>75</sub>	0.095a	0.121a	0.213a
K concentration (% of dry weight)			
N <sub>50</sub> P <sub>0</sub> K <sub>75</sub>	1.32c	1.60a	1.65a
N <sub>50</sub> P <sub>75</sub> K <sub>75</sub>	2.13a	1.73a	1.82a
N <sub>50</sub> P <sub>225</sub> K <sub>75</sub>	1.72b	1.82a	1.63a
Ca concentration (% of dry weight)			
N <sub>50</sub> P <sub>0</sub> K <sub>75</sub>	4.29c	3.33b	2.37a
N <sub>50</sub> P <sub>75</sub> K <sub>75</sub>	4.93b	4.22a	2.41a
N <sub>50</sub> P <sub>225</sub> K <sub>75</sub>	5.45a	4.35a	2.42a
Mg concentration (% of dry weight)			
N <sub>50</sub> P <sub>0</sub> K <sub>75</sub>	0.66a	0.63a	0.57a
N <sub>50</sub> P <sub>75</sub> K <sub>75</sub>	0.63a	0.61a	0.54a
N <sub>50</sub> P <sub>225</sub> K <sub>75</sub>	0.67a	0.62a	0.58a

\* – Different letters within each column indicate that the means are significantly different ( $P < 0.05$ )

**Table 2**  
Plant height, number of leaves per plant, dimensions of the middle leaves (14-th leaf from the bottom) and yield of the oriental tobacco variety Plovdiv 7 (2-year average)

Treatments	Plant height (cm)	Number of leaves per plant	Dimensions of the 14 <sup>th</sup> leaf (cm)		Yield (kg ha <sup>-1</sup> )
			Length	Width	
N <sub>50</sub> P <sub>0</sub> K <sub>75</sub>	88.1	27.7	27.1	12.8	1460
N <sub>50</sub> P <sub>75</sub> K <sub>75</sub>	96.4	28.2	28.2	13.4	1635
N <sub>50</sub> P <sub>225</sub> K <sub>75</sub>	97.9	28.5	28.8	13.7	1692
LSD (0.05)	3.34	0.43	0.64	0.45	65.15

tions, no irrigation is recommended to produce quality Oriental tobacco. The experimental plots were irrigated when the available soil moisture was limiting tobacco growth (only two irrigations were applied in 2008 and 2009). Therefore, under our experimental conditions water deficit could be important factor, which reduces phosphorus uptake by tobacco. Mitreva and Apostolova (1986) obtained the highest yields of Virginia tobacco at low concentrations of phosphorus (0.12–0.13%) in the leaves at first priming. The same authors concluded that the concentrations of P in the leaves, providing optimum growth for tobacco plants, are lower in comparison to the other crops. Under the present experimental conditions, the concentration of P about 0.1% in the lower leaves can be assumed as sufficient to ensure the growth processes and to obtain satisfactory yields of oriental tobacco. That content of P in the lower leaves is achieved by fertilization with both phosphorus rates (75 and 225 kg ha<sup>-1</sup>).

Potassium content in the middle and upper leaves was slightly affected by the level of phosphorus fertilization. Among the three P fertilizing rates, 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> treatment led to highest K concentration in lower leaves and the differences between different treatments were significant (P < 0.05). According to Volodarskiy (1971) the concentration of K<sub>2</sub>O in the leaves of 3–5% (2.5–4.2% K) has beneficial influence on the burning properties of tobacco. The comparison of our data with these values shows that the leaf tissue concentration is relatively low in spite of the large quantities of mobile potassium in the soil in all treatments. One possible explanation for this contradiction could be found in the high content of calcium in the soil and its well-known function as an antagonist of K that decreases potassium absorption.

Calcium content in mature leaves was the highest in first priming (4.3–5.5%), fell in second priming (3.3–4.4%) and was the lowest in the upper leaves (approximately 2.4%). These discrepancies can be explained by the nutrient element immobility in the plant. The Ca concentration in the lower and middle leaves increased with the increase in phosphorus fertilizer levels. Increasing levels of P availability had no significant effect on calcium concentration in the upper leaves (P > 0.05).

Magnesium concentration in mature leaves varied between 0.54 and 0.67%. These values are similar to those reported by Drossopoulos et al. (1999). The good supply of plants with Mg was probably because tobacco was grown on a soil that had adequate levels of available magnesium. Takahashi and Yoshida (1957) have found that with a high level of phosphorus, the magnesium content of the leaf was reduced and Mg deficiency symptoms appeared. In our study, concentrations of magnesium in tobacco leaves were not significantly affected by P fertilization rate. Data on the micronutrient concentrations in tobacco leaves is presented in Table 4.

**Table 4**  
**Micronutrient concentrations of tobacco leaves as dependent on P fertilizing rate (2-year average)**

Treatments	Lower leaves	Middle leaves	Upper leaves
Fe concentration (mg kg <sup>-1</sup> dry matter)			
N <sub>50</sub> P <sub>0</sub> K <sub>75</sub>	368.1a*	228.9a	148.3b
N <sub>50</sub> P <sub>75</sub> K <sub>75</sub>	374.2a	222.5a	180.6a
N <sub>50</sub> P <sub>225</sub> K <sub>75</sub>	328.6a	192.1b	157.4b
Mn concentration (mg kg <sup>-1</sup> dry matter)			
N <sub>50</sub> P <sub>0</sub> K <sub>75</sub>	58.4a	52.2b	49.7a
N <sub>50</sub> P <sub>75</sub> K <sub>75</sub>	55.3a	56.3ab	51.7a
N <sub>50</sub> P <sub>225</sub> K <sub>75</sub>	59.7a	60.7a	51.1a
Zn concentration (mg kg <sup>-1</sup> dry matter)			
N <sub>50</sub> P <sub>0</sub> K <sub>75</sub>	90.4a	79.7a	65.6a
N <sub>50</sub> P <sub>75</sub> K <sub>75</sub>	62.4c	54.9b	65.7a
N <sub>50</sub> P <sub>225</sub> K <sub>75</sub>	74.9b	62.2b	58.7a
Cu concentration (mg kg <sup>-1</sup> dry matter)			
N <sub>50</sub> P <sub>0</sub> K <sub>75</sub>	22.6a	22.3a	18.1a
N <sub>50</sub> P <sub>75</sub> K <sub>75</sub>	10.6b	12.8b	14.4a
N <sub>50</sub> P <sub>225</sub> K <sub>75</sub>	10.6b	14.4b	15.4a

\* – Different letters within each column indicate that the means are significantly different (P < 0.05)

Iron content in mature leaves was lower than the values reported by Apostolova (1985). The iron concentration in lower leaves was not significantly influenced by P addition. Concentration of iron in middle and upper leaves at 225 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was significantly lower than at 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

The content of Mn in mature leaves was from 49.7 to 60.7 mg kg<sup>-1</sup>. These values are similar to those reported by Apostolova (1985) for tobacco grown on alkaline soil. Concentration of manganese in lower and upper leaves was not affected by phosphorus fertilization level. In the middle leaves, the Mn concentration increased with increasing P level.

The zinc content in tobacco leaves ranged between 54.9 and 90.4 mg kg<sup>-1</sup> and was higher than the values observed by Golia et al. (2009). The same authors reported statistically significant negative correlations between Zn concentrations in Oriental tobacco leaves and soil pH. High levels of zinc in oriental tobacco leaves under alkaline soil conditions in our experiment can probably be explained by the high content of available Zn in the soil. Zinc concentration was significantly (P < 0.05) greater in the lower and middle leaves that received no supplemental P fertilizer compared with those plots that did. Among the three P fertilizing rates, 225 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> treatment led to lowest

Zn concentration in upper leaves but the differences between treatments were not significant. The results of Bogdanovic et al. (1999) similarly demonstrate that the uptake of Zn by corn plants was significantly higher in the unfertilized check plot than plots fertilized with increasing P rates.

Concentration of Cu in mature leaves ranged from 10.6 to 22.6 mg kg<sup>-1</sup>. Observed values were within the ranges reported by Golia et al. (2009). Copper content of the leaves decreased with the increase of phosphorus fertilizing level from 0 to 75 kg ha<sup>-1</sup>. These results differ from the ones of Racz and Haluschak (1974) who have found that the Cu concentration in wheat was not greatly influenced by added phosphorus. The Cu concentration in the leaves did not change significantly with increasing of phosphorus level from 75 to 225 kg ha<sup>-1</sup>.

## Conclusions

Phosphorus is a factor, limiting cured leaf yield in continuous tobacco cropping system, established on a calcareous soil with low available soil P. No significant yield differences were observed between 75 and 225 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, so continuous fertilization of Oriental tobacco grown as monoculture with very large amounts of phosphate fertilizers is not an effective cultural practice. With the increase of phosphorus fertilization rate the content of the phosphorus and calcium in leaves also increased. Zinc concentration was significantly greater in the lower and middle leaves that received no supplemental P fertilizer compared with those plots that did. Copper content of the leaves decreased with increase of phosphorus fertilizing level from 0 to 75 kg ha<sup>-1</sup>. Under our experimental climatic and soil conditions annual fertilization with moderate phosphorus rates (75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) should be considered as optimal for improving the soil P fertility, for maintaining a proper P-supply level in the root zone and for producing high yield of sun-cured leaves. The data obtained can be helpful in developing nutrient management plans for oriental tobacco grown on alkaline soils with low available soil P.

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