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Yield and Nicotine Content of Flue-Cured Tobacco as Affected by Soil Nitrogen Mineralization^{*1}

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ABSTRACT

Nitrogen (N) supply is the most important factor affecting yield and quality of flue-cured tobacco (FCT). A field experiment and an *in situ* incubation method were used to study the effects of soil N mineralization in the later stages of growth on yield and nicotine content of FCT in Fenggang and Jinsha, Guizhou Province. The yield and market value of FCT at Fenggang were much lower than those at Jinsha. However, the nicotine content of middle and upper leaves was much higher at Fenggang than at Jinsha when the same rate of fertilizer N was applied, which might be due to a higher N supply capacity at the Fenggang site. At later stages of growth (7–16 weeks after transplanting), the soil net N mineralization at Fenggang (56 kg N ha⁻¹) was almost double that at Jinsha (30 kg N ha⁻¹). While soil NH₄-N and NO₃-N were almost exhausted by the plants or leached 5 weeks after transplanting, the N taken up at the later growth stages at Fenggang were mainly derived from soil N mineralization, which contributed to a high nicotine content in the upper leaves. The order of soil N contribution to N buildup in different leaves was: upper leaves > middle leaves > lower leaves. Thus, soil N mineralization at late growth stages was an important factor affecting N accumulation and therefore the nicotine content in the upper leaves.

Key Words: flue cured tobacco, nicotine content, soil N mineralization, tobacco quality, tobacco yield

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INTRODUCTION

Nitrogen is the most important mineral nutrient affecting the yield and quality of flue-cured tobacco (FCT) (Elliot, 1975; Collins and Hawks, 1994; Marchetti *et al.*, 2006). The N uptake of FCT occurs mainly at early stages of growth, with less uptake at later stages. The typical N uptake curve in North America showed that N uptake was low during the first 3 weeks after transplanting, but increased sharply after 3 to 8 weeks, and 80% of total N uptake occurred during the first 8 weeks after transplanting (Collins and Hawks, 1994). FCT demands high soil N levels at early growth stages, but tends to exhaust the N supply after reaching full leaf area (usually a short time after topping) at later growth stages in order to realize the maturity of different leaf positions at different times. Both excessive N supply and inadequate N supply may significantly affect the yield and quality of FCT (McCants and Woltz, 1967; Marchetti *et al.*, 2006). In order to meet the N demand pattern of FCT and produce high quality leaves, there are two clear strategies behind FCT production in North America: first, choose light textured soils, such as sandy soils, to plant FCT (Collins and Hawks, 1994; Tso, 1990; Chari *et al.*, 1994) in order to supply N by applying rational rates of fertilizer at early growth stages and have a very low soil N mineralization at later stages; and second, avoid application of manure to decrease N release at later growth stages. N

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release from manure is affected by soil temperature and moisture and it is difficult to predict the timing of the N release pattern (Chadwick *et al.*, 2000; Li *et al.*, 2004).

The physical and chemical characteristics of soils for planting FCT are quite complex in China and most of them are heavy textured soils (Li *et al.*, 2004). Based on the analysis of 13 704 soil samples collected from Yunnan, Guizhou, Hunan, Hubei, Henan, Anhui, Fujian, Guangdong, and Heilongjiang, the main FCT production areas in China, the organic matter (OM) content in 80% of soil samples was more than 15 g kg⁻¹. In contrast, the OM of soils used for planting FCT in North America was quite low, with 65% of soil samples lower than 7.5 g kg⁻¹ (Li *et al.*, 2004). Soil OM is the most important index reflecting the soil N supply capacity. The N supply pattern for FCT is easily managed by application of chemical N fertilizers (including rate, type, and time of application) when FCT is planted in low organic matter soils (Tso, 1990; Marchetti *et al.*, 2006). Fertilization in FCT production in China has been based on experience from other countries in recent years, including ensuring the proportion of NO₃-N in the basal fertilizer is more than 50% and that had better choosing KNO₃ as the type of N fertilizer for top-dressing, which should be applied during the first 3 weeks after transplanting (Liu, 2003). However, problems of high nicotine content and low flavor in the upper leaves are still serious (Ji *et al.*, 2001; Yin *et al.*, 2003). On the basis of analysis of 228 FCT samples collected from 17 provinces in 2002, the proportion of FCT samples with the nicotine content over 35 g kg⁻¹ in the upper leaves was more than 45.4%, which made the upper leaves less useful to the tobacco industry (Yin *et al.*, 2003).

Many studies have shown that nicotine accumulation in FCT leaves starts after transplanting and increases continuously until the leaves reach maturity. However, nicotine accumulation in the leaves occurs mainly at later growth stages, especially in the period after topping (Mumba and Banda, 1990; Hu *et al.*, 1999, 2000; Cao *et al.*, 1989). Most N taken up after topping is synthesized into nicotine unlike N taken up before topping (Hu *et al.*, 1999, 2000). Soil nitrogen mineralization at late growth stages may have important effects on nicotine content in upper leaves in China, because the soils planted with FCT have high OM content (Goenaga, 1987; Goenaga *et al.*, 1989). To test this hypothesis, a field experiment was conducted at two sites in Guizhou in 2002 to study the effects of soil N mineralization on yield, production level, and nicotine content of FCT.

MATERIALS AND METHODS

Experimental sites and soil chemical properties

The field experiment was conducted in 2002 in Fenggang and Jinsha counties in the main FCT production area of Guizhou Province, which has a sub-tropical monsoon climate. The Fenggang site is located at 27° 55' N and 107° 42' E and is 750 m above sea level. There are 1 238 h of annual solar radiation, with an average daytime temperature of 15.5–16.5 °C, an annual cumulative temperature above 10 °C on average of 4 580 °C, and an average annual precipitation of 1 240 mm, of which 658 mm occurs between May and August, the FCT growth period. Precipitation during the FCT growth period (May to August) in 2002 was 837 mm, representing 127% of the average precipitation in this period. The previous crop at Fenggang was paddy rice. Jinsha site is located at 27° 18' N and 105° 47' E and is 930 m above sea level. There are 1 098–1 346 h of annual solar radiation, with an average daytime temperature of 15.8–16.9 °C, an annual cumulative temperature above 10 °C on average of 4 512 °C, and an average annual precipitation of 1 070 mm, of which 616 mm occurs between May and August. The precipitation during the FCT growth period in 2002 was 831 mm, representing 135% of the average precipitation in this period. The previous crop was paddy rice.

The soil type is Perudic Ferrisol at both sites and the soil texture is heavy clay in Fenggang and loam clay in Jinsha. Some chemical properties of the soils are shown in Table I. The Fenggang site has higher soil fertility than the Jinsha site based on total N and OM contents.

TABLE I

Selected soil chemical properties at Fenggang and Jinsha, Guizhou Province

Site	Depth	pH (H ₂ O)	Organic matter	Total N	Olsen-P	NH ₄ OAc-exchangeable K
	cm		g kg ⁻¹			mg kg ⁻¹
Fenggang	0–20	5.32	33.2	2.34	8.33	171
	20–40	6.54	21.8	1.47	6.67	135
Jinsha	0–20	6.67	25.3	1.79	17.12	207
	20–40	7.06	12.6	0.92	6.05	83

Experimental design and management

The field experiment received the following five N application rates in order to investigate the local farming practice: no N fertilizer (control, N0), 75 (N1), 97.5 (N2), 120 (N3), and 142.5 kg N ha⁻¹ (N4). The N2 rate was the conventional N fertilizer application rate used by local farmers and the N:P₂O₅:K₂O ratio in this treatment was 1:0.73:2.34. The other treatments had the same amount of P₂O₅ and K₂O as that in N2. Plot size was 50 m² with 80 FCT plants, which were arranged at interval of 110 cm in line and 55 cm in row. All treatments had four replicates in a randomized complete block design. The N2 treatment had slightly larger plots, each containing 96 plants, to provide extra space for ¹⁵N micro-plots. The variety of FCT used was K326. Seedlings grown in small pots were transplanted in the field plots.

The basal N (ammonium nitrate, representing 42% of the total N rate), phosphorus (superphosphate), and potassium (potassium sulfate) fertilizer were applied in the middle of the ridge at 20 cm depth before transplanting. The seedling N (ammonium nitrate, representing 28% of the total N rate) fertilizer was applied to the seedling positions when transplanting. The top-dressing N and K (potassium nitrate) were applied in solution around the plants 15 days after transplanting. The fertilizers were applied in the same way at both sites and the ridges were covered with plastic film.

The transplanting date at Fenggang was May 13, topping was 62 days (leaving 18 leaves), and final harvesting was 126 days after transplanting. The transplanting date at Jinsha was May 1, topping 52 days (leaving 18 leaves), and final harvest 113 days after transplanting. The plastic film was removed 39 days after transplanting. Other field management details followed local standard practice (Liu, 2003).

The ¹⁵N micro-plot experiment was conducted at Fenggang site in combination with the field experiment. ¹⁵NH₄¹⁵NO₃ and K¹⁵NO₃ (¹⁵N abundance of 5.26%) were applied as N fertilizer sources to the middle row of the N2 treatment (16 plants). A micro-plot frame was inserted extending 10 cm above the ground surface and 30 cm below down to the sub-soil to minimize the risk of interference between labeled and unlabeled N fertilizers. All other field management practices were the same as in the main field experiment.

Soil net N mineralization was measured *in situ* using the approach described by Hart *et al.* (1996). Soil sub-samples in 0–20 cm depth were taken from 3 positions on the ridge of the control plots. After thorough mixing of the sub-samples, a portion of the composite sample was placed in a cool box and analyzed for NO₃-N and NH₄-N immediately on arrival at the laboratory. Another portion was placed in a polythylene bag, buried in the top 20 cm of the soil profile under aerobic conditions at one sampling location, incubated *in situ* for one week, and analyzed for NO₃-N and NH₄-N. This procedure was repeated at weekly intervals after transplanting until the final harvest. Soil net N mineralization each week was calculated by subtracting the initial N_{min} (NO₃-N + NH₄-N) from the final N_{min} after incubation.

Sampling and analysis methods

Soil samples from the top 20 cm layer were taken from all plots before fertilizer application, 3, 5, 7,

9, 11, and 13 weeks after transplanting, and in the end after the final harvest, respectively, for analysis of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$. The $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ were extracted from 12.00 g fresh soil samples by shaking for 1 h with 0.01 mol L^{-1} CaCl_2 solution (about 10:1 volume/dry soil weight) on a rotary shaker (180 r min^{-1}) followed by filtration. The extracts were then analyzed using a TRAACS 2000 continuous flow analyzer (Bran and Luebbe, Norderstedt, Germany).

Two plants were harvested from each ^{15}N micro-plot 3, 5, 7, 9, 11, and 13 weeks after transplanting and after the final harvest, respectively. The plant samples were divided into upper, middle, and lower leaves, stems, and roots. Being oven-dried and weighed, the plant samples were finely ground ($< 0.25 \text{ mm}$) for analysis of Kjeldahl-N and ^{15}N abundance (Finnigan Mat 251 isotope ratio mass spectrometer, Germany).

The leaves in each plot were collected after maturity for measurement of yield and market value after curing and standard samples were analyzed for nicotine content (Xiao *et al.*, 1997). Market values were expressed as Chinese Yuan (RMB).

Data processing and statistical analysis

The following formulae were used in the analysis of the data:

$$\% \text{Ndff} = (\text{atom } \% ^{15}\text{N excess of plant} / \text{atom } \% ^{15}\text{N excess of fertilizer}) \times 100 \quad (1)$$

$$\text{Ndff} = \% \text{Ndff} \times \text{N uptake by plant} \quad (2)$$

$$\text{Ndffs} = \text{N uptake by plant} - \text{Ndff} \quad (3)$$

where %Ndff is the percentage of N derived from fertilizer, Ndff is the N derived from fertilizer, and Ndffs is the N derived from soil.

The data were subjected to analysis of variance (ANOVA) using the SAS software package, version 6.12 (SAS Institute, 1997).

RESULTS AND DISCUSSION

Yield, market value, and nicotine content in upper and middle leaves as affected by N supply

FCT showed a poor yield response to applied N at the Fenggang site (Fig. 1a). There were no significant differences among the applied N treatments, but the yields in the N3 and N4 treatments were significantly higher than those in the control. The yield in the N treatments ranged from 2059 to 2261 kg ha^{-1} . The market value did not differ significantly among the N treatments (13 745–17 595 RMB ha^{-1}), but was significantly higher than that in the control (Fig. 1b). The nicotine content of the upper leaves at Fenggang was 45.2–56.3 g kg^{-1} , much higher than the standard international value of high quality FCT leaves (15–35 g kg^{-1}) (Mumba and Banda, 1990). The nicotine content of the middle leaves was 25.7–33.4 g kg^{-1} across all N treatments and was within the accepted range except for the control treatment (43.0 g kg^{-1}). Considering the yield, market value, and nicotine content, an application rate of 75 kg N ha^{-1} was suitable under the conditions at the Fenggang site, but the nicotine content in the upper leaves (45.2 g kg^{-1}) was high even at this relatively low fertilizer N application rate.

There was a more pronounced FCT yield response at Jinsha to the application of N (Fig. 1c). There was no significant difference among the N2, N3, and N4 treatments (2 683–2 907 kg ha^{-1}), but yields were significantly higher than those in the N1 and N0 treatments. The market value in the N2 treatment (25 950 RMB ha^{-1}) was significantly higher than that in the other N treatments (Fig. 1d). The nicotine content of the upper leaves at Jinsha was 19.9–33.3 g kg^{-1} in the N treatments, and increased with increasing N application rate except for the control treatment, in which the nicotine content was actually higher than that in the N1 treatment. Taking into account the yield, market value, and nicotine content,

Fig. 1 Yield, market value, and nicotine content in middle and upper leaves at Fenggang (a and b) and Jinsha sites (c and d). Bars with the same letter(s) are not significantly different at the 0.05 level by least significant difference (LSD).

97.5 kg N ha⁻¹ was a suitable N application rate under the experimental soil and climate conditions at Jinsha. However, the nicotine content of the upper leaves was below 35 g kg⁻¹ in this medium to high N application rate in contrast to the effective N application rate at Fenggang.

FCT quality is affected by many factors, including climate, soil, plant variety, planting technique, and curing technique, but the soil conditions form the basis for producing high quality FCT (Collins and Hawks, 1994; Tso, 1990; Hu *et al.*, 2000), especially the pattern of soil N supply. Because the yields, market values and nicotine contents were so different at the same N fertilizer rates at the two sites, it seemed likely that the soil N supply pattern (especially soil N mineralization after topping) might be markedly different between the sites. The yield in the control treatment at Fenggang was much higher than that at Jinsha and there was a poor yield response to fertilizer N at Fenggang, which simply reflected a higher residual soil N status before the start of the experiment at Fenggang than at Jinsha.

Dynamics of soil NO₃-N and NH₄-N in FCT growth season

Soil NO₃-N and NH₄-N contents in the top 20 cm of the soil profile (the main soil rooting zone) were measured frequently during the FCT growing season at both sites to understand the intensity of N supply at different growth periods (Fig. 2). In general, soil NO₃-N was far higher than NH₄-N at all growth stages, and NO₃-N was the main source of N supply to the plants. Soil NH₄-N showed an increasing trend with increasing N application rate, and NH₄-N in all treatments was exhausted with FCT growth and decreased to a fairly low level (about 3 mg kg⁻¹) about 7 weeks after transplanting at both sites. Nevertheless, soil NH₄-N was quite low 7 to 9 weeks after transplanting and before topping at Fenggang, but remained at higher concentrations before topping (7 weeks after transplanting) at Jinsha.

Soil NO₃-N sharply decreased from 3 to 5 weeks at Fenggang and subsequently remained low from

Fig. 2 Dynamics of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in the top 20 cm of the soil profile at different N application rates during the flue-cured tobacco growth period at Fenggang and Jinsha.

5 to 9 weeks (at topping). This may indicate that the soil N supply was inadequate before topping. One explanation for the low soil $\text{NO}_3\text{-N}$ levels may be that $\text{NO}_3\text{-N}$ was leached with high intensity rainfall (270 mm) during this period. At Jinsha soil $\text{NO}_3\text{-N}$ remained high before topping (week 7), and decreased to quite low levels after topping. This N supply pattern may have met the plant demand for N. Under conditions of inadequate $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ supply at early growth stages at Fenggang, the high nicotine content of the upper and middle leaves at this site may have resulted from higher rates of soil N mineralization after topping.

Soil net N mineralization at different FCT growth stages at both sites

Soil net N mineralization at all growth stages was much higher at Fenggang than at Jinsha (Fig. 3). Net N immobilization occurred during the first and second weeks after transplanting at Jinsha, most likely because rice straw (C:N = 58:1) was incorporated into the soil 3 weeks before transplanting. Net N immobilization often occurs immediately after mixing high C:N straw with soil (Ju *et al.*, 2004). Soil net N mineralization during the whole growing season at Fenggang was 88 kg N ha^{-1} , but only 37 kg N ha^{-1} at Jinsha (Fig. 3). Soil net mineralization in the later 7–16 week stages at Fenggang (56 kg N ha^{-1}) was almost double that at Jinsha (30 kg N ha^{-1}).

Plant N uptake derived from soil or fertilizer

The ^{15}N labeled N fertilizer was applied to the $97.5 \text{ kg N ha}^{-1}$ treatment in order to estimate how much of the N taken up by the plants was derived from soil or fertilizer at Fenggang (Fig. 4, Table II). N uptake derived from fertilizer was significantly higher than that from soil 7 weeks after transplanting under the experimental conditions. N uptake derived from fertilizer did not increase after week 7, but

Fig. 3 Soil net N mineralization during the flue-cured tobacco growth period at Fenggang and Jinsha, Guizhou Province.

Fig. 4 N uptake of flue-cured tobacco from soil or fertilizer during the flue-cured tobacco growth period at Fenggang.

that derived from soil increased sharply, indicating that N uptake after topping (week 9) was mainly derived from the soil at Fenggang.

TABLE II

N uptake derived from soil (Nd_{fs}) or fertilizer (Nd_{ff}), expressed as a percentage of total N uptake by leaves, at different positions during the flue-cured tobacco growth period at Fenggang

Leaf position	N source	Weeks after transplanting				
		7	9	11	14	18
		%				
Lower	Nd _{ff}	54.87	48.63	41.03	- ^{a)}	-
	Nd _{fs}	45.13	51.37	58.97	-	-
Middle	Nd _{ff}	50.47	42.22	28.96	27.88	-
	Nd _{fs}	49.53	57.78	71.04	72.12	-
Upper	Nd _{ff}	47.16	34.57	27.59	21.37	19.10
	Nd _{fs}	52.84	65.43	72.41	78.63	80.90

^{a)}Leaves were already harvested.

The proportion of N uptake derived from soil or fertilizer changes during the growing period (Goenaga, 1987; Goenaga *et al.*, 1989; Prasada Rao *et al.*, 1992; Tian and Yang, 1990; Yang *et al.*, 1991; Guo *et al.*, 1997). N uptake is mainly derived from fertilizer at early growth stages, but it is mainly derived from the soil at later stages if the soil has a high N supply capacity. In this study N uptake derived from fertilizer comprised 19% of total N uptake, and that derived from soil represented 81% at final harvest. It can be concluded that N taken up by FCT plant in later stages at Fenggang is mainly derived from soil N mineralization, while soil NH₄-N and NO₃-N are almost exhausted by plant uptake or by leaching 5 weeks after transplanting (Fig. 2a, c). Some studies have shown that a high proportion of N uptake after topping may be used to synthesize nicotine (Hu *et al.*, 1999), thus the high N uptake derived from soil N mineralization in later stages is the main explanation for the high nicotine content in FCT leaves, especially in the upper leaves.

The leaves are harvested at different times according their degree of maturity in FCT production. The ¹⁵N tracer results showed that the later the leaves harvested, the higher the proportion of N uptake derived from the soil (Table II). N uptake derived from soil by lower leaves at the final harvest comprised 59% of total N uptake, but that by middle and upper leaves represented 72% and 81%, respectively. The N uptake from soil as a proportion of total N uptake in the middle and upper leaves was much higher than that derived from fertilizer. The order of the soil N contribution to total N uptake of leaves

at different positions was: upper > middle > lower leaves. Other studies conducted using ^{15}N -labeled ammonium nitrate in Perudic Ferrisols in Guizhou also showed that N in the upper leaves was mainly derived from soil N (Tian and Yang, 1990; Yang *et al.*, 1991; Guo *et al.*, 1997). Therefore, the soil N supply may have been the major factor affecting N accumulation and thus the nicotine content in the upper leaves.

Selecting the appropriate soil, improving the control of the soil N supply pattern, and designing rational crop rotations in order to decrease the N supply at later stages are the key measures for decreasing the nicotine content and increasing the quality of the upper leaves in FCT production.

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