EFFECTS OF NITROGEN SOURCE AND SOIL ACIDITY ON NITROGEN USE EFFICIENCY AND GROWTH OF FLUE-CURED TOBACCO[1]

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Yield and quality of flue-cured tobacco (*Nicotiana tabacum* L.) are often reduced by $(NH_4)_2SO_4$ on acid soils. Urea is a less acid-forming source of ammonium than $(NH_4)_2SO_4$ and may be a better source of plant nitrogen than $(NH_4)_2SO_4$ under acid soil conditions. Our objective was to determine the effects of soil pH, N source, and Cl^- on the growth, N recovery, and N use efficiency of flue-cured tobacco under greenhouse conditions. In one experiment, 300 mg N kg^{-1} from four N sources [$NaNO_3$, $(NH_2)_2CO$, $(NH_4)_2SO_4$, and NH_4Cl] were added to a loamy sand soil (Typic Paleudult) at three pH levels (5, 7, and 9). ^{15}N-labeled N sources were used on three replications and nonlabeled sources were applied to six replications. In another experiment, the nonlabeled N sources were added to the soil at two pH levels (5 and 7) and Cl^- levels of all sources were brought to the level applied with NH_4Cl (760 mg kg^{-1}) using KCl. In both studies, one tobacco plant ('Speight G-28') was transplanted into each container. Nitrapyrin at 1 mg a.i. kg^{-1} was applied to all treatments.

Height, leaf number, and plant weight were highest for $(NH_2)_2CO$ and $NaNO_3$ at pH 5, for $(NH_4)_2SO_4$ and $NaNO_3$ at pH 7, and for $NaNO_3$ at pH 9. Production of hydroxyl ions from the hydrolysis of $(NH_2)_2CO$ may have countered acidity at pH 5 and improved its performance compared to $(NH_4)_2SO_4$. At pH 7, the acidic nature of $(NH_4)_2SO_4$ was countered by the higher soil basicity resulting in acceptable performance by this source. All NH_4^+ sources were inferior to $NaNO_3$ at pH 9, probably due to NH_3 volatilization and toxicity. Tissue N concentrations generally were inversely related to dry weight, but N use efficiency increased from 17.0 g leaf g^{-1} N for $(NH_2)_2CO$ and $NaNO_3$ at pH 5 to 27.2 g leaf g^{-1} N for $(NH_4)_2SO_4$ and $NaNO_3$ at pH 7. Apparent N recovery averaged 74% for $NaNO_3$ across all pH levels, but averaged only 54%, 41%, and 34% for $(NH_4)_2SO_4$, $(NH_2)_2CO$, and NH_4Cl, respectively. Chloride concentrations in leaves were above 8% for all sources at pH 5 when a constant rate of Cl^- was applied. This resulted in no toxicity symptoms for $NaNO_3$, slight symptoms for $(NH_2)_2CO$, and severe symptoms for $(NH_4)_2SO_4$ and NH_4Cl. Chloride levels were lower at pH 7, resulting in no symptoms for $NaNO_3$ or $(NH_2)_2CO$ and only slight symptoms for $(NH_4)_2SO_4$ and NH_4Cl. $NaNO_3$ was the best N source across all pH levels in this greenhouse study. However, the study suggests $(NH_2)_2CO$ may be an acceptable N source for tobacco under acid soil conditions while $(NH_4)_2SO_4$ may suffice at soil pH near neutrality.

Additional key words: ammonium, nitrate, ^{15}N, N sources, N-serve, mineral toxicities, *Nicotiana tabacum, urea.*

INTRODUCTION

Nitrate (NO_3^-) is usually the preferred form of nitrogen for flue-cured tobacco (*Nicotiana tabacum* L.) production because N supplied as the ammonium (NH_4^+) form may reduce yield (6) and leaf quality (7,9). The detrimental effects of NH_4^+ have been associated with conditions that inhibit nitrification such as high soil acidity (3,8), drought (14), and soil fumigation (6,11). Absorption of NH_4^+ by tobacco also increases chloride (Cl^-) concentration in cured tobacco leaves which, at levels above 1%, can result in leaf malformation (11) and reduced fireholding capacity, undesirable taste, and reduced smoking quality (15).

The NH_4^+ source used to compare NH_4^+ to NO_3^- in most studies has been $(NH_4)_2SO_4$. This source is extremely acid-forming, and when it is used on acid soils it tends to further inhibit nitrification. Thus, high levels of NH_4^+ can remain in an acid soil environment for a significant portion of a tobacco growing season. Urea [$(NH_2)_2CO$] has been used successfully for tobacco (12) and was inferior to $NaNO_3$ in a dry year but superior in a wet year (14). Urea hydrolysis initially increases soil pH, resulting in a faster rate of nitrification compared to $(NH_4)_2SO_4$ on acid soils (8). Thus, $(NH_2)_2CO$ may be a better N source than $(NH_4)_2SO_4$ under acid soil conditions because nitrification proceeds at a faster rate.

The purpose of this greenhouse study was to investigate the interactions of N sources, soil pH, and Cl^- on the growth, N use efficiency, and N and Cl^- concentrations of flue-cured tobacco.

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MATERIALS AND METHODS

Loamy sand from the upper 20 to 30 cm of a Norfolk soil (Typic Paleudult) from the Upper Coastal Plain of North Carolina was used for two greenhouse experiments. The soil was screened through a 2 mm sieve and thoroughly mixed. The initial soil pH was 5.0. To attain desired pH levels for treatments, bulk volumes of soil were mixed with appropriate amounts of $Ca(OH)_2$, brought to 90% of field moisture capacity, covered with polyethylene, and allowed to equilibrate for 10 days. The soil was then dried to a moisture content that permitted handling and incorporation of fertilizers.

Fertilizers were incorporated with each bulk volume of soil to supply 75 mg P_2O_5 kg^{-1} as triple superphosphate, 200 mg K_2O kg^{-1} as K_2SO_4, 200 mg Mg kg^{-1} as $MgSO_4$, and 200 mg Ca kg^{-1} as $CaSO_4$. The nitrification inhibitor nitrapyrin (N-Serve) was mixed with the soil at 1 mg a.i. kg^{-1} to retard nitrification during the early stage of both experiments.

Treatments in Experiment 1 consisted of combinations of three soil pH levels (5, 7, and 9) and four N sources [$NaNO_3$, $(NH_2)_2CO$, $(NH_4)_2SO_4$, and NH_4Cl]. Control treatments consisting of no added N were also included. The rate of application was 300 mg N kg^{-1} for all N sources, and all materials were mixed with the soil as described previously. Treatments were arranged in a randomized complete block design with nine replications. In three replications, ^{15}N-enriched fertilizers [$NaNO_3$, 2.51 atom % ^{15}N; $(NH_2)_2CO$, 2.49 atom % ^{15}N; $(NH_4)_2SO_4$, 2.4 atom % ^{15}N; and NH_4Cl, 2.73 atom % ^{15}N] were used, while nonlabeled materials were used in the remaining replications.

Each experimental unit consisted of one flue-cured tobacco plant ('Speight-G28') transplanted into 5 kg of treated soil contained in a pot measuring 21.5 cm across the top by 20 cm deep. The pots were lined with a polyethylene bag to prevent leaching. Soil moisture was brought to 90% of field capacity gravimetrically, and subsequent watering to this level was done daily through plastic tubes inserted through the soil to the bottom of the pot. Plant height to the bud was

measured weekly and leaves were counted at harvest.

Three replications of the nonlabeled fertilizer treatments were harvested at 19 days after transplant, and the remaining three replications were harvested after 35 days. Plants of N-labeled treatments were harvested 60 days after transplanting. All samples (tops and roots) were dried at 70°C, weighed, ground to pass a 60-mesh stainless steel sieve, and analyzed for total N by flash combustion in a Perkin-Elmer PE 2400 elemental analyzer (5) and for Cl^- by potentimetric titration. Plant materials from the ^{15}N-labeled treatments were also analyzed for tissue concentrations of ^{15}N by mass spectroscopy (1). Nitrogen-use efficiency was calculated as the increase in top-leaf yield per unit of applied N compared to the nonfertilized check. Apparent fertilizer N recovery was calculated as increased N accumulated per unit of applied N compared to that accumulated by the check. Apparent fertilizer N recoveries were also calculated from ^{15}N analyses (2).

The second experiment had the same cultural conditions and treatments as the first with these exceptions: the pH 9 treatment was eliminated; ^{15}N enriched fertilizers were not used; and Cl^- levels of all N sources, except NH_4Cl, were brought to 760 mg kg^{-1} with KCl, which equaled the Cl^- level of the NH_4Cl treatment. The experiment was terminated 35 days after transplanting. The Cl^- concentration of leaves was determined as previously described.

At harvest, roots were removed and the soil from each experiment was analyzed for extractable mineral N (NH_4^+ and NO_3^-) after extraction with 1 M K_2SO_4, using procedures previously mentioned.

The data were subjected to analysis of variance with the control treatment excluded from the analysis. Means were separated by LSD at the 0.01 level of probability.

RESULTS AND DISCUSSION

Physical Properties and N Content of Plants

In Experiment 1, the effects of soil pH and N source on plant height, weight, and N concentrations were similar over time; therefore, only data taken at 60 days are presented. At pH 5, plants treated with $(NH_2)_2CO$ were taller than those treated with other N sources, but height for $(NH_2)_2CO$ decreased with increasing pH (**Table 1**). Plant height for the other N source treatments was markedly greater at pH 7 compared with pH 5 and 9. Plants treated with NH_4Cl were considerably shorter than those treated with other N sources at all pH levels, and they were shorter and had fewer leaves than control plants at pH 9. Plants treated with $NaNO_3$ or $(NH_4)_2SO_4$ were similar in height at pH 5 and 7, but at pH 9, those treated with $(NH_4)_2SO_4$ were severely stunted compared to those treated with $NaNO_3$. The number of leaves at harvest was directly related to plant height and top weight (**Table 1**).

The superiority of $(NH_2)_2CO$ compared to $(NH_4)_2SO_4$ or NH_4Cl at pH 5 may be the result of an initial increase in soil pH associated with urea hydrolysis that partially offsets acidity generated by nitrification. Henry and Raper (4), using hydroponic culture, showed that NH_4^+-produced tobacco had characteristics similar to those associated with N stress at pH 4. However, Vessey et al. (13) did not observe these negative effects under similar conditions at pH 4.5. Thus, a critical root-zone pH effect for NH_4^+ may exist between pH 4 and 4.5. Although soil pH was not measured during the experiment, urea may have maintained soil pH above the critical pH for NH_4^+ while $(NH_4)_2SO_4$ may have lowered pH below the critical level resulting in less plant growth.

At pH 7, plants treated with $(NH_2)_2CO$ were smaller than those treated with $(NH_4)_2SO_4$ (**Table 1**). Volatilization of NH_3 from $(NH_2)_2CO$ may have reduced its availability to plants at pH 7 and 9. At pH 7, acidic effects of $(NH_4)_2SO_4$ were probably buffered by the soil, and this N source gave the tallest plants.

Total dry weight (tops and roots) and dry weight of tops were generally affected by N sources and soil pH in patterns similar to those for plant height (**Table 1**). Urea-treated plants had the highest dry weight at pH 5, whereas growth was severely depressed by NH_4Cl and $(NH_4)_2SO_4$. At pH 7, $NaNO_3$ produced the most dry weight even though plants receiving $(NH_4)_2SO_4$ were taller (**Table 1**). Ammonium sulfate was markedly superior to urea at pH 7. Dry weights of plants from all NH_4^+ sources were depressed at pH 9 compared to those for $NaNO_3$, and NH_3 volatilization may have resulted in insufficient N for the growth period.

The N concentration in leaves was significantly higher for plants treated with NH_4Cl or $(NH_4)_2SO_4$ compared to those treated with $(NH_2)_2CO$ or $NaNO_3$ at pH 5 and 7 (**Table 2**). Because growth was restricted by NH_4Cl and $(NH_4)_2SO_4$ at pH 5, compared to $(NH_2)_2CO$ or $NaNO_3$ (**Table 1**), the high N levels probably reflect a concentrating effect in small plants. The same reasoning may apply for NH_4Cl at pH 7. Nitrogen concentrations were lowest for plants treated with $(NH_2)_2CO$ at pH 5 and 7 (**Table 2**). This relatively low N concentration at pH 5 for urea was accompanied by a high dry weight, which may have diluted the N concentration compared to those measured for the other N sources. Variations in N

Table 1. Effect of soil pH and N source on tobacco height, leaf number, total dry weight, and top dry weight 60 days after transplanting.

N Source	Soil pH							
	5	7	9	Mean	5	7	9	Mean
	Height (cm)				Leaves/Plant			
Control[a]	25.0	32.5	36.0	31.2	12.3	15.0	17.3	14.9
NH_4Cl	30.2	54.3	32.5	39.0	15.0	20.0	15.0	16.7
$(NH_4)_2SO_4$	62.0	110.3	49.0	73.8	21.7	26.0	19.3	22.3
$(NH_2)_2CO$	73.7	67.2	56.3	65.7	25.0	22.7	20.3	22.7
$NaNO_3$	67.0	103.7	85.2	85.3	24.3	27.0	27.0	26.1
Mean	58.2	83.9	55.8		21.5	23.9	20.4	
LSD(0.01)		2.5		2.9		1.5		1.7
pHxSource		5.0				3.0		
	Top Weight (g/plant)				Total Weight (g/plant)			
Control[a]	6.4	9.8	13.3	9.8	8.9	14.2	14.5	12.6
NH_4Cl	7.7	34.2	24.1	22.0	10.2	43.8	30.4	28.1
$(NH_4)_2SO_4$	20.2	54.7	24.7	33.2	24.1	68.9	33.5	42.2
$(NH_2)_2CO$	42.4	37.4	27.5	35.7	53.5	46.8	37.6	46.0
$NaNO_3$	38.8	67.1	47.7	51.2	50.0	85.8	61.5	65.7
Mean	27.2	48.3	31.0		34.4	61.3	40.8	
LSD(.01)		2.2		2.5		2.9		3.3
pHxSource		4.3				5.8		

[a]Control not included in pH means or statistical analyses.

Table 2. Effect of soil pH and N source on N concentrations in leaves and stems at 60 days after transplanting.

N Source	Soil pH							
	5	7	9	Mean	5	7	9	Mean
	Leaf N (%)				Stem N (%)			
Control[a]	0.60	0.61	0.69	0.63	0.79	0.60	0.63	0.67
NH_4Cl	5.04	3.25	1.41	3.23	1.91	1.31	1.03	1.42
$(NH_4)_2SO_4$	4.41	2.75	1.72	2.96	1.70	1.11	1.01	1.27
$(NH_2)_2CO$	2.74	1.35	2.32	2.14	1.48	1.09	1.26	1.28
$NaNO_3$	3.25	1.83	2.90	2.66	1.85	1.14	1.82	1.60
Mean	3.86	2.29	2.09		1.74	1.16	1.28	
LSD(0.01)		0.31		0.36		0.16		0.19
pHxSource		0.55				0.30		

[a]Control not included in pH means or statistical analyses.

concentrations among soil pHs for $NaNO_3$ appeared inversely related to dry matter production. Concentrations of N in stems were considerably lower than those in leaves with less variation among treatments, although proportionally they followed similar patterns observed for leaf N concentrations (**Table 2**).

The accumulation of N by the above-ground portions of plants was generally lowest for NH_4Cl and highest for $NaNO_3$ (**Table 3**). However, $(NH_2)_2CO$ provided N accumulation levels similar to that of $NaNO_3$ at pH 5, and $(NH_4)_2SO_4$ gave the highest N accumulation at pH 7. Among the NH_4^+ sources, plants fertilized with $(NH_2)_2CO$ had the highest N accumulation at pH 5 while those treated with $(NH_4)_2SO_4$ accumulated the most N at pH 7.

Chloride Concentration of Leaves and Toxicity Symptoms

In Experiment 1, plants treated with NH_4Cl were shorter, weighed less, and developed fewer leaves than those treated with other N sources (**Table 1**). At pH 5 and 7, these plants also exhibited severe visual toxicity symptoms which were not apparent for other N sources. The symptoms were characterized by thickened leaves with margins rolled upward, and they appeared within one week at pH 5 and progressed to include an increasing yellow coloration with time. Symptoms at pH 7 appeared a few days later and were less severe than those at pH 5. No symptoms were observed on plants grown at pH 9.

Chloride concentrations in leaves at harvest were high for the NH_4Cl treatment and decreased from the bottom to top leaves (**Table 4**). At pH 5 and 7, Cl^- concentrations were similar at respective stalk positions, but those at pH 9 were about one-half as high. The greater degree of toxicity expressed at pH 5 compared to pH 7 with similar Cl^- concentrations suggests that either pH or some other factor was associated with the toxicity expression. The N concentration in leaves was much higher at pH 5 compared to that at pH 7, which suggests that differential absorption of NH_4^+ may have resulted in a more severe NH_4^+ x Cl^- interaction for plants grown at pH 5. Leaf Cl^- concentrations from the other N sources and pH combinations were less than 1 percent.

Table 3. Effect of soil pH and N source on N accumulation by above-ground portions of plants 60 days after transplanting.

N Source	Soil pH 5	7	9	Mean
	g/plant			
Control[a]	0.04	0.06	0.09	0.06
NH_4Cl	0.36	0.96	0.33	0.55
$(NH_4)_2SO_4$	0.74	1.42	0.46	0.87
$(NH_2)_2CO$	1.03	0.49	0.52	0.68
$NaNO_3$	1.15	1.11	1.24	1.17
Mean	0.82	0.99	0.64	
LSD(0.01)		0.11		0.13
pHxSource		0.23		

[a]Control not included in pH means or statistical analyses.

Table 4. Effect of soil pH on Cl^- concentration of leaves from plants fertilized with NH_4Cl and harvested 60 days after transplanting.

Stalk Position	Soil pH 5	7	9
	%		
above 11th leaf	4.4	4.3	3.7
8-10th leaf	5.9	8.2	4.5
5-7th leaf	9.1	11.0	5.8
1-4th leaf	14.0	13.2	6.9
stalk	3.2	2.0	1.8

Experiment 2 confirmed that observed toxicity symptoms were related to the presence of both NH_4^+ and Cl^- and soil acidity (**Table 5**). Relatively high Cl^- concentrations caused no visual toxicity in the control or in the presence of NO_3^- at either soil pH. However, toxicity symptoms were present for all NH_4^+ sources at pH 5 and for NH_4Cl and $(NH_4)_2SO_4$ at pH 7. The toxicity symptoms for $(NH_2)_2CO$ were less severe than for other NH_4^+ sources. The increase in pH associated with the hydrolysis of urea may have reduced the net acidity generated from this source. Acidity appears to enhance the severity of toxicity expression.

Nitrogen Use Efficiency

Nitrogen use efficiency of fertilizer is defined as the increase in leaf yield per unit of applied N and can be calculated from the following relationship:

$$\text{N Use Efficiency} = \frac{\text{Treatment yield} - \text{Control yield}}{\text{N Rate}} \quad (1)$$

At pH 5, both urea and $NaNO_3$ had relatively high and similar levels of N use efficiency (**Table 6**). Due to depressed growth, N use efficiency values for NH_4Cl and $(NH_4)_2SO_4$ at pH 5 were very low. At pH 7, $NaNO_3$ and $(NH_4)_2SO_4$ gave the highest efficiency levels attained in the experiment, while those for urea and NH_4Cl were about one-half as high. All NH_4^+ sources gave low efficiency values at pH 9 while that for $NaNO_3$ was high.

Apparent N recovery was calculated by the relationship:

$$\text{Apparent N recovery} = \frac{\text{Treatment N uptake} - \text{Control N uptake}}{\text{N applied}} \times 100 \quad (2)$$

and from the amounts of ^{15}N recovered from the labeled fertilizer using the method of Buresh et al. (2). Both methods

Table 5. Effect of soil pH and N source at high fertilizer Cl^- levels on Cl^- concentrations of leaves and toxicity symptom expression 35 days after transplanting.

N Source[a]	pH 5 Cl^-	pH 5 Sym. Severity[b]	pH 7 Cl^-	pH 7 Sym. Severity
	%		%	
None	9.4	0	7.2	0
NH_4Cl	10.4	6	10.0	5
$(NH_4)_2SO_4$	10.8	5	8.5	3
$(NH_2)_2CO$	9.1	3	5.8	0
$NaNO_3$	8.0	0	5.0	0

[a]KCl applied to all treatments except NH_4Cl to supply Cl^- equivalent to that from NH_4Cl (760 mg kg^{-1}).
[b]Visual rating: 0-no symptom, 6-severe symptom.

Table 6. Effect of soil pH and N source on N use efficiency of fertilizer 60 days after transplanting.

N Source	Soil pH 5	7	9	Mean
	g/g			
NH_4Cl	0.6	11.6	5.4	5.9
$(NH_4)_2SO_4$	5.9	27.1	5.9	13.0
$(NH_2)_2CO$	17.2	14.9	3.2	12.7
$NaNO_3$	16.7	27.3	15.1	19.7
Mean	10.1	20.2	7.4	
LSD(0.01)		1.2		1.4
pHxSource		2.4		

of determining fertilizer N recovery gave similar results and were equally effective (**Table 7**).

Fertilizer N recovery averaged 74 percent for $NaNO_3$ with no significant variation among soil pHs (**Table 7**). In contrast, soil pH greatly affected N recovery from other sources being highest for urea and $(NH_4)_2SO_4$ at pH 5 and 7, respectively. The effect of pH on fertilizer N recovery by NH_4Cl was similar to that for $(NH_4)_2SO_4$, but amounts recovered were only about 0.6 as high. Recovery was generally related to the effects of soil acidity among sources on dry matter, discussed previously.

At harvest, extractable soil N levels measured in the nonfertilized controls were subtracted from those for the N source treatments to provide a measure of extractable fertilizer N remaining in the soil. The amount of recoverable fertilizer N was low for all N sources and pH combinations (**Table 8**). Smallest amounts were recovered at pH 9, intermediate amounts at pH 7, and highest amounts at pH 5. Generally, the greatest amount of N was found in the NH_4Cl-treated soil while the remaining N treatments were comparable for amounts of extractable fertilizer N.

By summing the amount of soil extractable N from fertilizer and the amount taken up by plant tops, the recovery of applied N can be estimated. The N not accounted for is assumed to be lost by volatilization, tied up by roots, or fixed by the soil. Leaching of N was not a factor because pots were lined with plastic. Because the soil contained little clay, and that present was primarily kaolinitic, NH_4^+ fixation was minimal. Roots were not analyzed, but root mass varied from 2.5 to 18.7 g dry weight among treatments (**Table 1**) and they could have tied up some of the missing N.

For the $NaNO_3$ treatment, 80-90% of the applied N was accounted for among soil pHs. This was expected because leaching was not a factor in the plastic-lined pots. The highest amount of N accounted for came from the $(NH_4)_2SO_4$ treatment at pH 7 (**Table 8**). At pH 9, less N was accounted for among the NH_4^+ sources than at the other pH levels. This suggests that significant NH_3 volatilization occurred in the pH 9 treatments. We also suspect a large amount of NH_3 was lost in the urea treatment at pH 7. The relatively low N recoveries for the NH_4Cl and $(NH_4)_2SO_4$ treatments at pH 5 are harder to explain, but due to the lower pH they are unlikely to be attributable to losses of N via NH_3 or denitrification.

Table 7. Effect of soil pH and N source on apparent fertilizer N recovery as determined by calculation and by ^{15}N method.

N Source	Soil pH 5	7	9	Mean	5	7	9	Mean
	Calculation				^{15}N-Method			
	%				%			
NH_4Cl	21	60	16	32.3	31	57	18	35.3
$(NH_4)_2SO_4$	47	91	24	54.0	52	87	26	55.0
$(NH_2)_2CO$	66	29	29	41.3	63	31	31	41.7
$NaNO_3$	74	70	77	73.7	71	74	78	74.3
Mean	52.1	62.3	36.4		54.3	62.2	38.3	
LSD(0.01)	7.6	7.6		8.7				
pHxSource	15.1							

Table 8. Effect of soil pH and N source on extractable N from fertilizer at harvest and the percent of total applied N accounted for by plant and soil.

N Source	Soil pH 5	7	9	5	7	9
	ext. N			N accounted for[a]		
	g/pot			%		
NH_4Cl	0.40	0.30	0.02	48	80	17
$(NH_4)_2SO_4$	0.30	0.11	0.00	67	98	24
$(NH_2)_2CO$	0.29	0.13	0.02	85	37	30
$NaNO_3$	0.22	0.11	0.01	89	77	77

[a]Does not include N in roots. Total N applied was 1.5 g/pot.

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