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STUDIES ON THE BITING TASTE OF THE BURLEY TOBACCO LEAVES (NUTRITIONAL PHYSIOLOGY OF JAPANESE TOBACCO)

III. EFFECTS OF THE FORM AND THE AMOUNT OF THE SUPPLIED NITROGEN AND PHOSPHORUS ON THE QUALITY OF THE BURLEY TOBACCO

By

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Introduction

As already reported (9), the biting taste of burley tobacco depends upon the high content of the total nitrogen and the low content of the soluble sugar, due to the earlier harvest of immature leaves by the heavy application of the nitrogenous fertilizer for heavy crops. To improve this inferiority, the accerelation of the maturity, as though earlier transplanting to get the ealier change from the stage of the vegetative growth to that of the reproductive growth, seems to be important for burley tobacco cultivation (9, 12, 14).

Since the content of available phosphorus is generally low in the soil of the burley tobacco cultivating region in northeastern Japan (10), the effect of the phosphorous application could be clearly observed. As the effect of phosphorus on the accerelation of the maturity was generally observed in the cereal crops, the experiments upon the phosphorus supply in relation to the amount and forms of nitrogen supply were advance.

In the present report, the results of the experiments on this problem are dealt with.

Materials and Methods

The water culture method was adopted for raising the tobacco plants, using a four litres porcelain container as the cultivating vessel. The burley tobacco seedlings were transplanted on June 6, 1952 to this vessel and cultured carefully. The composition of the culture solution was as follows.

P_2O_5	N	K_2O	CaO	MgO	Fe_2O_3
high phosphorus supply (*h*P) 40	nitrate nitrogen NO_3-N 40	40	40	10	3
	ammonia nitrogen NH_4-N 32 NO_3-N 8	40	40	10	3
low phosphorus suplly (*l*P) 2.5	nitrate nitrogen NO_3-N 40	40	40	10	3
	ammonia nitrogen NH_4-N 32 NO_3-N 8	40	40	10	3

In addition to these macroelements, 1 ppm of manganese and boron were supplied, as the used tap water contained sufficient amount of other micronutrients. The culture solution was renewed by the drip method and the hydrogen ion concentration was adjusted to pH 5.8 every morning and afternoon.

Plant growth

The results of the culture experiment were very satisfactory. The tobacco plants are unable to develope under supply of ammonia nitrogen only, so for NH_4-N plots a small amount of nitrogen was added to promote the growth of the early stage.

Since the end of June, the differences in the plant growth between the plots were observed to be remarkable, the NO_3-N plants showed excellent development and the color of the leaves were healthly green, on the contrary, the growth of the NH_4-N plants were quite inferior and the color of the leaves were dark, and in general the growth was somewhate retarded. As the growth

Table 1 Weight of dry matter (g per plant) Seedling (^{5}/IV) : 0.31

	Leaf and stalk			Leaf			Stalk		Root		
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	14/VII	8/VIII	20/VIII
NH_4-*l*p	1.55	14.39	21.79	Upper Lower Total	4.13 6.62 10.75	6.34 8.46 14.80	3.64	6.99	0.35	4.22	4.97
NH_4-*h*P	1.40	11.41	26.01	Upper Lower Total	4.59 4.12 8.71	5.95 9.21 15.16	2.70	10.85	0.40	3.24	5.63
NO_3-*l*P	2.25	16.75	27.13	Upper Lower Total	5.40 7.67 13.07	8.46 10.13 18.59	3.68	8.54	0.53	4.51	6.05
NO_3-*h*P	2.12	30.17	60.19	Upper Lower Total	8.61 12.88 21.49	15.52 20.44 35.96	8.68	24.23	0.66	7.22	12.51

progressed, the plants of NO_3-*h*P plot showed most vigorous development and NO_3-*l*P plot came next, and then NH_4-*l*P plot. The data of the individual yields are shown in Table 1. At the time of the last harvest, the plants of both *h*P plots matured and flowered, on the contrary, the plants of both *l*P plots were still immature and had green under leaves.

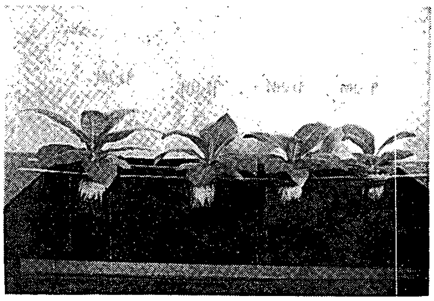

Plate 1. 45 days after the transplanting.

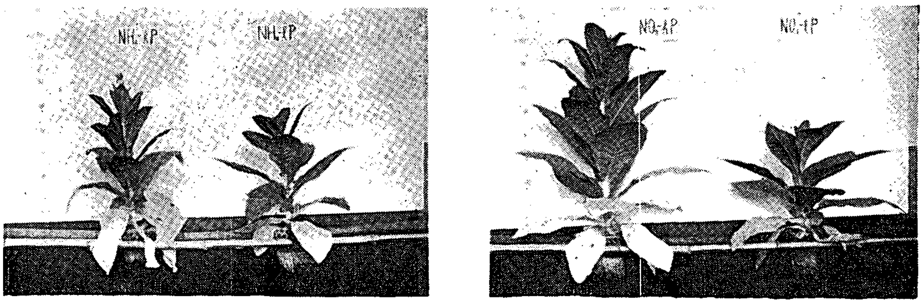

Plate 2· 74 days after the transplanting.

Analytical method

The samples were harvested on July 14, at the beginning of the maximum vegetative growth and on August 8, the time of change from vegetative to reproductive growth, then on August 20 at the time of flowering. The harvested samples were sorted as to lower leaf (containing the granulators and cutters), upper leaf (containing the red leaf and tips) and stalk. After killing the cells by heating to 70°C for 30 minutes, the samples were dried at 40∼50°C and pulverized.

Total nitrogen : Total nitrogen was determined according to Kjeldahl-Gunning-Arnold's method (1).

Protein nitrogen : Residue from the boiling extraction with 0.5 per cent acetic acid for five minutes was estimated according to Kjeldahl-Gunning-Arnold's method.

Nicotinic nitrogen : Nicotine in the filtrate and washings from boiling of the extraction with acetic acid was precipitated by the silicotungstate method (2).

Soluble nitrogen: Soluble nitrogen in the filtrate from the nicotinic nitrogen was estimated according to Kjeldahl-Gunning-Arnold's method.

Ammonia nitrogen: An adequate part of the filtrate from the nicotinic nitrogen was distilled by the aeration method by adding bicarbonate buffer. The volatilled ammonia was caught and determined colorimetrically by Nessler's method.

Amide nitrogen: An adequate part of the filtrate from nicotinic nitrogen, was hydrolysed by heating for two hours with the addition of sulphuric acid. Total ammonia was estimated by the aeration method and calculated from the difference between it and the ammonia nitrogen.

Nitrate nitrogen: Nitrate nitrogen in the filtrate from nicotinic nitrogen was determined by Harper's method (11).

Reducing sugar: Reducing sugar was estimated according to Somogy's method (18) in the filtrate from nicotinic nitrogen.

Crude starch: To the sample was added HCl to attain 2.5 per cent and hydrolysed by heating for two hours, then it was determined by Somogy's method and calculated from the difference.

The smoking test was made carefully on all samples, with special reference to the appearance of the biting taste.

Results and disscussion

Nitrogen

The results obtained are showed in Tables 2~8. The total nitrogen content increased gradually after the transplanting, and decreased since the beginning of the reproductive growth. The upper leaves were generally higher in nitrogen content than the lower leaves. Nitrogen content in the NH_4-N

Table 2 Total-N (% on dry basis) Seedling (^{5}/IV): 3.70

	Leaf and stalk			Leaf			Stalk		Root		
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	14/VII	8/VIII	20/VIII
NH_4-*l*P	4.33	4.83	4.18	Upper Lower Average	6.23 5.14 5.56	5.16 4.66 4.88	2.75	2.70	5.02	4.01	4.62
NH_4-*h*P	5.15	4.65	3.46	Upper Lower Average	5.98 4.51 5.28	4.97 3.43 4.26	2.60	2.33	5.00	4.40	4.19
NO_3-*l*P	4.00	4.21	3.59	Upper Lower Average	6.18 3.70 4.72	4.90 3.58 4.18	2.39	2.30	4.69	3.74	3.89
NO_3-*h*P	4.26	3.05	2.34	Upper Lower Average	4.73 2.89 3.49	3.39 2.26 2.78	1.95	1.48	4.81	3.78	3.03

Table 3. Protein–N (% on dry basis) Seedling (^{5}/IV) : 2.95

	Leaf and stalk			Leaf			Stalk		Root	
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	8/VIII	20/VIII
NH_4–*l*P	2.97	3.30	2.52	Upper Lower Average	4.42 3.41 3.79	3.46 2.58 2.96	1.81	1.17	3.85	3.74
NH_4– .P	3.66	3.39	1.98	Upper Lower Average	4.95 3.73 3.98	3.58 1.67 2.72	1.49	1.25	3.50	3.32
NO_3–*l*P	2.68	2.97	2.24	Upper Lower Average	4.66 2.44 3.36	3.17 2.27 2.68	1.61	1.20	2.75	3.34
NO_3–*h*P	3.06	1.85	1.29	Upper Lower Average	3.69 2.03 2.69	1.62 2.44 0.96	1.07	0.88	3.39	2.67

Table 4. Soluble–N (% on dry basis) Seedling (^{5}/IV) : 0.59

	Leaf and stalk			Leaf			Stalk		Root	
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	8/VIII	20/VIII
NH_4–'P	1.02	1.11	1.26	Upper Lower Average	1.32 1.18 1.25	1.25 1.54 1.42	0.73	1.35	0.20	0.62
NH_4–*h*P	1.26	0.91	1.14	Upper Lower Average	1.02 0.78 0.91	1.04 1.17 1.12	0.93	0.97	0.71	0.71
NO_3–*l*P	1.01	0.92	1.10	Upper Lower Average	1.15 0.90 1.00	1.38 1.00 1.17	0.63	0.94	0.86	0.44
NO_3–*h*P	0.83	1.00	0.71	Upper Lower Average	0.76 0.60 0.66	0.62 0.99 0.83	0.77	0.51	0.29	0.21

Table 5. Nicotine–N (% on dry basis) Seedling (^{5}/IV)

	Leaf and stalk			Leaf			Stalk		Root	
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	8/VIII	20/VIII
NH_4–*l*P	0.34	0.43	0.40	Upper Lower Average	0.48 0.55 0.51	0.46 0.54 0.50	0.21	0.19	0.25	0.26
NH_4–*h*P	0.24	0.34	0.34	Upper Lower Average	0.40 0.38 0.39	0.34 0.59 0.42	0.18	0.12	0.26	0.16
NO_3–*l*P	0.31	0.32	0.26	Upper Lower Average	0.37 0.37 0.37	0.36 0.32 0.33	0.15	0.16	0.20	0.11
NO_3–*h*P	0.30	0.21	0.24	Upper Lower Average	0.28 0.26 0.27	0.32 0.34 0.33	0.11	0.10	0.15	0.15

Table 6. NO_3–N (% on dry basis) Seedling (^{5}/IV) : 0.010

	Leaf and stalk			Leaf			Stalk		Root	
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	8/VIII	20/VIII
NH_4–*l*P	0.007	0.011	0.018	Upper Lower Average	0.015 0.006 0.009	0.021 0.022 0.022	0.018	0.008	0.001	0.001
NH_4–*h*P	0.014	0.006	—	Upper Lower Average	0.006 0.006 0.006	— — —	0.008	—	—	—
NO_3–*l*P	0.162	0.112	0.065	Upper Lower Average	0.106 0.158 0.136	0.200 0.221 0.211	0.027	0.065	0.031	0.031
NO_3–*h*P	0.027	0.011	—	Upper Lower Average	0.013 — 0.005	— — —	0.025	—	—	—

Table 7. Amide–N (% on dry basis) Seedling (^{5}/IV) : 0.028

	Leaf and stalk			Leaf			Stalk		Root	
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	8/VIII	20/VIII
NH_4–*l*P	0.149	0.422	0.391	Upper Lower Average	0.297 0.485 0.420	0.360 0.551 0.470	0.470	0.223	0.043	0.359
NH_4–*h*P	0.095	0.213	0.118	Upper Lower Average	0.055 0.354 0.196	0.041 0.211 0.144	0.270	0.084	0.043	0.352
NO_3–*l*P	0.096	0.099	0.186	Upper Lower Average	0.169 0.035 0.091	0.251 0.218 0.222	0.130	0.109	0.045	0.094
NO_3–*h*P	0.068	0.147	0.095	Upper Lower Average	0.167 0.091 0.122	0.206 0.102 0.147	0.200	0.018	0.024	0.040

Table 8. NH_4–N (% on dry dasis) Seedling (^{5}/IV) : 0.038

	Leaf and stalk			Leaf			Stalk		Root	
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	8/VIII	20/VIII
NH_4–*l*P	0.048	0.123	0.138	Upper Lower Average	0.122 0.119 0.120	0.146 0.163 0.155	0.130	0.102	0.083	0.058
NH_4–*h*P	0.121	0.125	0.199	Upper Lower Average	0.103 0.195 0.146	0.105 0.230 0.181	0.054	0.039	0.059	0.039
NO_3–*l*P	0.066	0.040	0.091	Upper Lower Average	0.036 0.048 0.043	0.150 0.092 0.113	0.029	0.042	0.026	0.066
NO_3–*h*P	0.045	0.025	0.024	Upper Lower Average	0.027 0.031 0.030	0.036 0.020 0.027	0.015	0.021	0.049	0.049

plants was always higher than that of NO_3-N plants, these results were in accord with those obtained by Evans, H. I. *et al* (8) and Vickery, H. B. *et al* (20).

The heavier application of phosphate produced vigorous growth accompanying the higher absorption of nitrogen at the earlier vegetative growth, thus resulting in the higher content of nitrogen. Thereafter vigorous photosynthesis followed, and then the nitrogen content decreased, namely, the plants aquired an earlier change from stage of the vegetative growth to that of the reproductive growth.

Protein nitrogen content of the NH_4-N plants was appreciably higher than that of the NO_3-N plants in the earlier stage, but in the later stage those of both plants decreased similarly. So in general, NH_4-N plants contained a higher amount of protein nitrogen as compared with the NO_3-N plants. In the earlier growth stage, the heavy application of phosphate produced a higher content of protein nitrogen but this content decreased earlier and faster than the phosphorus poor plants. Nicotinic nitrogen increased gradually after transplanting and decreased a little, as the plant approached maturity, which showed a marked difference between the status of the total and protein nitrogen in the plant. Such results may be explained as follows. The physiological theory of the nicotine formation is well known from many reports (3, 4, 15, 16).

The nicotine is synthesized exclusively in the roots, transported upward in the transpiration stream (5) and accumulated in the leaves. Accumulated nicotine in the leaves seems to be quite stable and is difficult to move to the other parts of the plants (19). As nicotine has no direct relation concerning the protein nitrogen metabolism. The NH_4-N plants contained a higher amount of nicotinic nitrogen than the NO_3-N plants, which would suggest that the NH_4-N is suitable for the nicotine synthesis, as NH_4-N is the reduced form. The nicotine content decreased according to the increase of the phosphate supply in the culture solution. Thus, the addition of a heavy supply of phosphate favored the accumulation of carbohydrate, and the depression of nicotine content. This result differs from that of Emde, H. (7), which indicated a relationship between the carbon assimilation and the amount of the nicotine synthesis. Komatu (13) pointed out that the addition of a heavier amount of phosphate caused no decrease in the nicotine content.

The soluble nirogen content had no definite tendency as to the amount of phosphate and nitrogen sources. Only the nitrate nitrogen accumulated abundantly in the plants of *l*P plots, and also nitrate nitrogen was found to accumulate in the NH_4-N plants, nevertheless ammonia nitrogen is unable to become oxidized to the nitrate nitrogen in the plant tissue (6), because in the NH_4-N plants a small amount of NO_3-N was added. In the plant supplied with the lower concertration of phosphate, the nitrate nitrogen was found to accumulate, being especially high in NO_3-*l*P plant, owing to the insufficient reduction of the nitrate. Therefore phosphate seeme to have an important effect on the nitrate reduction. High nitrate accumulation took place in the leaves rather than in the roots. In general, the contents of ammonia and amide nitrogen in the NO_3-N plants were higher than in the NH_4-N plants. Amide nitrogen increased according to the development of plant growth, particularly in the NH_4-*l*P plant.

Carbohydrate

The results of the analysis are shown in Tables 9~12. The total carbohydrate content in the plants decreased since the period of seedling to the vegetative growth, which signifies vigorous protein synthesis. In the later or reproductive growth stage, the carbohydrate accumulated highly due to the

Table 9. Total carbohydrate (% on dry basis) Seedling (5/IV) : 17.03

	Leaf and stalk			Leaf			Stalk		Root		
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	14/VIII	8/VIII	20/VIII
NH_4-*l*P	24.67	16.45	25.98	Upper Lower Average	12.41 15.24 14.12	26.21 24.94 25.49	23.21	26.99	14.12	12.97	19.22
NH_4-*h*P	12.08	13.11	22.49	Upper Lower Average	10.45 10.16 10.32	19.48 15.44 17.06	22.44	30.10	12.39	15.57	18.02
NO_3-*l*P	14.79	16.82	21.37	Upper Lower Average	14.34 15.47 14.49	20.16 17.31 18.62	23.31	27.33	10.92	17.10	19.42
NO_3-*h*P	11.54	18.51	27.11	Upper Lower Average	15.67 14.62 15.06	27.12 24.18 25.46	27.16	29.57	10.46	17.48	19.79

Table 10. Starch (% on dry basis) Seedling (5/IV) : 11.97

	Leaf and stalk			Leaf			Stalk		Root	
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	8/VIII	20/VIII
NH_4-*l*P	17.13	10.06	18.54	Upper Lower Average	7.36 9.73 8.79	20.88 19.07 19.85	13.72	14.24	12.28	16.95
NH_4-*h*P	8.46	7.56	12.72	Upper Lower Average	5.04 5.24 5.13	11.00 8.39 9.43	15.79	17.29	14.68	16.33
NO_3-*l*P	9.87	12.00	14.55	Upper Lower Average	9.92 10.04 9.98	14.74 12.79 13.69	19.19	16.29	15.94	17.61
NO_3-*h*P	7.49	10.29	17.65	Upper Lower Average	7.02 7.28 7.20	20.99 17.45 18.87	17.14	15.84	16.27	17.18

Table 11. Soluble sugar (% on dry basis) Seelding (^{5}/IV) : 5.26

	Leaf and stalk			Leaf			Stalk		Root	
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	8/VIII	20/VIII
NH_4-*l*P	7.54	6.39	7.44	Upper Lower Average	5.05 5.51 5.33	5.33 5.87 5.64	9.49	12.66	0.69	2.27
NH_4-*h*P	3.62	5.55	9.77	Upper Lower Average	5.41 4.92 5.19	8.48 7.05 7.63	6.68	12.81	0.89	1.69
NO_3-*l*P	4.92	4.82	6.82	Upper Lower Average	4.42 5.43 5.01	5.42 4.52 4.93	4.12	11.04	1.16	1.81
NO_3-*h*P	3.05	8.22	9.46	Upper Lower Average	8.65 7.34 7.86	6.13 6.93 6.59	9.98	13.73	1.21	2.61

Table 12. Reducing sugap (% on dry basis) Seedlieg (^{5}/IV) : 3.72

	Leaf and stalk			Leaf			Stalk		Root	
	14/VII	8/VIII	20/VIII		8/VIII	20/VIII	8/VIII	20/VIII	8/VIII	20/VIII
NH_4-*l*P	4.28	4.42	4.39	Upper Lower Average	3.03 2.83 2.99	3.29 4.10 3.76	6.16	5.70	0.28	1.28
NH_4-*h*P	2.14	3.11	5.75	Upper Lower Average	2.91 2.32 2.64	6.35 4.79 5.41	4.62	6.22	0.68	1.10
NO_3-*l*P	2.53	2.83	3.91	Upper Lower Average	2.79 2.55 2.65	3.87 2.76 3.26	3.51	5.34	0.74	1.51
NO_3-*h*P	1.59	4.64	5.36	Upper Lower Average	5.47 3.06 4.01	3.92 4.46 4.24	6.20	7.04	1.05	1.83

effect of vigorous photosynthesis. This shows that the total carbohydrate content of the NH_4-*l*P plant alone was abnormal because that of the plants of other plots were almost equal.

In general, the carbohydrate content increased suddenly when the plants reach the stage of reproductive growth, especially in the leaves, and also its content of the upper leaves was higher than that of the lower leaves. The carbohydrate content of the stalks and the roots increased gradually in accord with the development of the plant, as compared with the leaves.

As to the nitrogen sources, generally speaking, the NO_3-N plants contained higher amount of the carbohydrate than the NH_4-N plants, except NH_4-*l*P plot.

The influence of the heavy supply of phosphorus brough about strong augmentation of the carbohydrate content after the middle of July, and in particular, thereafter the carbohydrate content of the NO_3-*h*P plant increased continuously until the harvest. The tendency of the status of the starch, soluble sugar and reducing sugar content was to agree with that of the total carbohydrate content. The soluble sugar amount in the roots was less than that in the leaves and stalks, and the greater part of the carbohydrate in the roots consisted crude starch.

Although Sommer, A. L. (17) showed that if the plants receive an abundant supply of nitrogen, phosphorus retards maturity, in our experiments it was found that the heavy addition of phosphate promotes maturity, that is, an earlier depression of the total and protein nitrogen and a large accumulation of the carbohydrate even though the nitrogen supply is ample in the culture solution. When the burley tobacco plants absorbe a large amount of ammonia nitrogen, the status of the nutrition becomes abnormal since the earlier stage of the growth, even though the phosphate supply may be rich, so the accumulation if the carbohydrate at the time of the reproductive growth is remarkably delayed and the plant growth is inferior. In the plants cultured by the nitrate nitrogen and lower concentration of phosphate, the nitrogen was stored in the tissue and as the reduction capacity of nitrate was low, oxidation-reduction potential rose, the negative growth was inhibited and the nitrogen accimilation or carbohydrate accumulation was insufficient. The NO_3-*h*P plant only showed profitable results for all the conditions.

The ratio of soluble sugar to total nitrogen

The ratio of the soluble sugar to total nitrogen in the leaves harvested on August 20 is calculated in Table 13.

Table 13. Soluble sugar/Total-N

	Leaf $\left(\frac{20}{VIII}\right)$	
NH_4-*l*P	Upper	1.04
	Lower	1.26
NH_4-*h*P	Upper	1.70
	Lower	2.05
NO_3-*l*P	Upper	1.02
	Lower	1.26
NO_3-*h*P	Upper	1.81
	Lower	3.07

This ratio in the upper leaves of the *l*P plants were smallest, consequently the quality was most inferior and particularly the biting taste was severe.

In the lower leaves of *l*P plants, the ratio was significantly higher and the quality was more excellent than in the upper leaves. The difference in the ratio between the NH_4-N plants and NO_3-N plants were not clear in the *l*P plants, so in the weak phosphorus deficiency, the supplied nitrogen forms appeared to have no significant effect on the quality. If the plants are supplied with abundant phosphate, this ratio becomes exceedingly wide, the quality is improved highly, and the biting taste almost disappeared. The NO_3-N plants showed a wider ratio, and aquality better than the NH_4-N plants. The supply of NO_3-*h*P seems to be one of the best materials to obtain excellent burley tobacco.

The ratio shown in this paper in general were wider than those of the previous pot culture (10). In the pot culture, the amount of the phosphate supply was limited as compared with the water culture. The heavy application of phosphate should be given consideration as a means for the improvement of the quality.

By the water culture test, the writers could confirm that the promotion of maturity is most important to obtain excellent quality and to eliminate the biting taste in northeastern Japan. Therefore in the burley tobacco culture, an earlier crop and heavy application of phophate are most important from the practical agricultural point of view.

Summary

1. Water culture experiments of the burley tobacco were carried on, concerning the concentration of phosphorus and the kinds of nitrogen resources.

2. The nitrate nitrogen was far better than the ammonia nitrogen for the burley tobacco, since nitrogen content in the plants supplied with nitrate nitrogen declined earlier and faster, and the carbohydrate content increased remarkaly in the later growth stage.

3. The ratio of the soluble sugar to the total nitrogen was wider in the plant cultured with higher concentration of phosphate, nitrate nitrogen than that in the plant raised respectively with lower concentration of phosphate, ammonia nitrogen. In general the lower leaves have wider ratio than the upper leaves.

4. The heavy application of phosphate brought on excellent harvest with high quailty.

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