STUDIES ON THE PHYSIOLOGY AND UTILIZATION OF VITAMIN B_1 IN SOME GARDEN CROPS

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With 59 Tables, 50 Textfigures and 3 Plates

CONTENTS

INTRODUCTION

Studies on the effect of thiamine upon the higher plants have been, for the most part, concerned with the growth of roots. In many reports, promoting effects of thiamine on the growth of excised roots were ascertained by tissue culture, but cases were also reported in which no effect could be recognized. (2, 12, 16, 17, 58, 127, 129)

With thiamine, NOGGLE and WIND (1943)(118) recognized an abnormal germination in orchids, while TSUGE (1944)(159) reported growth promotion in soy bean. Some promoting effects of thiamine on the germination of old seeds are recognized by the present author* and RUGE (1952). (153)

On the availability of thiamine to the cultivation practice, FUJII (1942)(43) reported an increase of sweet potato yield by applying "Oryzanin", an alcohol extract of rice-bran, containing vitamin B_1, to the soil or to the slips. KICH (1953)(91) also recognized an increasing rape-seed production by the soil application with thiamine solution. The same effect was obtained by HATTA and TOYOSATO (1955)(52) on the yield of rice plants.

ISHIKAWA (1956)(84) reported, that *Aralia cordata* produced softened and whitened shoots of good quality after the spraying of phytohormone (2, 4–dichlorophenoxyacetic acid or α–naphthaleneacetic acid), especially combined with thiamine. The injury caused by the former was considerably lessened by thiamine application. This was also recognized in the case of tomatoes when the fruit is set. He reported that the farmers who use the thiamine spray for growing garden crops are now increasing in number in the district near Tokyo.

The above reports are concerned with fragmentary phenomena and the fundamental explanations of thiamine action are not yet complete.

The present studies have been made with the purpose of ascertaining the effect of thiamine on higher plants and of making clear its action mechanism, as well as to establish the method of its application to the garden crops.

The main materials for the present experiments were sweet potatoes (*Ipomoea batatas* LAM.), potatoes (*Solanum tuberosum* LINN.) and kidney beans (*Phaseolus vulgaris* LINN.). The soakig method was adopted for seed germination; and the foliar spray method for the study in regard to the growth, yield, and the quality of the crops.

* Report at the meeting of the Horticultural Association of Japan in April, 1952.

CHAPTER I*

EFFECTS OF SEED SOAKING IN THIAMINE SOLUTION ON GERMINATION

To make clear the physiological role of thiamine in seed germination, changes of thiamine content and its forms in the process of seed germination under natural condition were investigated. At the same time, thiamine was applied to the seed by soaking and its effect on germination was observed.

1. Materials and Methods

The experiments were carried out in the winter of 1951. As the materials, Kentucky Wonder (products of 1947, 1948, 1949, 1950 and 1951) of Kidney beans, Burpee's Golden Bantam of Indian corn (product of 1951), a variety of radish Miyashige Aokubi (product of 1951) were used. Of these the radish seeds were obtained from the market (germination ratio 98 per cent), while all the rest came from the Farm of Shinshu University.

The seeds were soaked in 50 cc thiamine solution, prepared in an Erlenmeyer flask of 100 cc. After soaking they were spread on filter papers in Petri dishes, and the germination tests were performed in a thermostat. The pH value of thiamine solution was adjusted to 5.2 with hydrochloric acid. The thiamine used was vitamin B_1 hydrochloride of Wakō Pure Chemical Industries which is regarded as the free form in water solution (WATANABE 1942).

Thiamine analysis was made by the thiochrome reaction method (the permutite method, NISHIO 1946),(117) and each data was the average of more than four analyses. The fresh weight of seeds in every plot represents an average of 40 to 100 grains.

2. Results

(1) Promotion of seed germination by the application of thiamine.

A preliminary experiment was carried out to find the relation between the soaking period and the germination of kidney bean seeds. The greatest germination percentage was obtained by 24 hours of soaking at 9°C. Therefore, a soaking period of 24 hours was used in most of the experiments. Ten kinds of thiamine solution from 100 ppm to 10^{-7} ppm were prepared, and 20 seeds were soaked in 50 cc of each solution for 24 hours at 9°C, and were germinated in a thermostat at 30°C; 24 hours later, the germination percentage was determined. The results are shown in Table 1.

The optimum thiamine concentration for the germination of the seeds of both Kentucky Wonder and Masterpiece was found to be 0.01 ppm. The growth after germination was good, and no abnormal seedlings were observed.

* The argument of this chapter was reported at the meeting of the Horticultural Association of Japan, 1952.

Table 1. Effect of thiamine application on the germination of kidney beans.

B_1 concentration	Kentucky Wonder					Masterpiece				
	I	II	III	IV	Average	I	II	III	IV	Average
ppm	%	%	%	%	%	%	%	%	%	%
100	65	55	60	45	56±2.5	56	39	33	33	40±3.2
10	60	60	55	50	56±2.0	61	56	33	39	47±3.9
1	40	75	75	65	64±4.8	67	83	56	67	68±3.3
0.1	75	90	85	85	84±1.8	83	89	67	61	75±4.3
0.01	85	85	95	90	89±1.4	72	94	78	94	85±3.3
0.001	80	75	80	70	76±1.4	56	67	56	61	60±1.5
0.0001	60	45	60	50	54±2.2	48	56	33	39	44±3.0
0.00001	45	55	55	65	55±2.4	56	44	39	33	43±2.9
0.000001	40	55	55	45	49±2.2	56	33	28	33	37±3.7
0.0000001	40	60	50	40	48±2.8	39	39	22	39	35±2.5
0	25	40	35	35	34±1.8	17	28	33	22	25±2.0

Seeds were obtained in October of 1950; investigation: end of November, 1950. In each Petri dish, 20 seeds of Kentucky Wonder or 18 seeds of Masterpiece were put after soaking for 24 hours at 9°C; examination after 24 hours at 30°C.

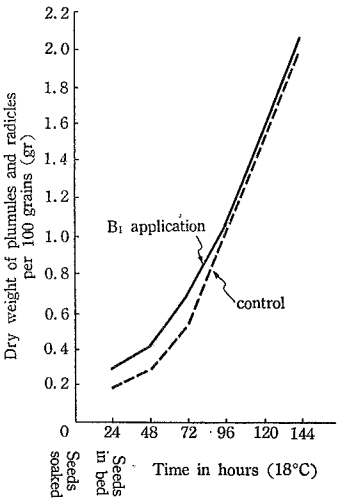

Fig. 1. Effect of thiamine on the growth of plumules and radicles of kidney beans.
Variety: Kentucky Wonder. The seeds were soaked in 0.01 ppm thiamine solution for 24 hours.

In order to observe the effect of thiamine on germination more in detail, seeds were soaked in thiamine solution of 0.01 ppm at 9°C for 24 hours, and were germinated on wet filter paper in a dark thermostat at 18°C. Every 24 hours, the dry weight of the plumule and radicle were determined. As shown in Fig. 1 the germination of soaked seed was accelerated especially in its earlier stage.

As given in Table 2, the duration of the soaking period had almost no influence upon the optimum concentration. Similar germination experiments with corn and radishes gave similar results (Tab. 3).

(2) Increase of the germination percentage of old seed of kidney beans by application of thiamine solution.

Thiamine solution was applied to the seeds of different ages, harvested in 1951, 1950, 1949 and 1948, and stored in the laboratory. The effect of thiamine (Tab. 4) was remarkable in the old seed stored for 2 years. It was promoted in germination percentage up to 33 per cent, while it did not germinate at all without thiamine supply.

Table 2. Relation between the hours of soaking and the B_1 concentration affecting the germination of kidney beans (variety Kentucky Wonder). Experiment December 1951.

Hours of soaking	3	12	24
Hours of germination at 30°C	60	24	24
B_1 concentration			
ppm	%	%	%
100	14±1.3	52±1.8	56±2.5
10	16±1.4	46±2.6	56±2.0
1	16±0.9	60±3.8	64±4.8
0.1	32±1.8	66±4.2	84±1.8
0.01	32±1.9	72±2.1	89±1.4
0.001	25±2.1	64±1.6	76±1.4
0.0001	15±1.2	46±1.2	54±2.2
0.00001	11±0.9	38±1.4	55±2.4
0.000001	13±0.8	39±2.1	49±2.2
0.0000001	5±0.7	27±2.2	48±2.8
0	3±0.6	22±1.1	34±1.8

The seeds were produced in 1951.

Table 3. Effect of thiamine application on the germination of Indian corns and radishes. Experiment November 1951.

Varieties	Indian corn (var. Burpee's Golden Bantam)					Radish (var. Miyashige Aokubi)				
No. of experiment	I	II	III	IV	Average	I	II	III	IV	Average
B_1 concentration										
ppm	%	%	%	%	%	%	%	%	%	%
100	10	15	20	10	13±1.4	40	27	20	30	29±2.4
10	35	30	25	35	31±1.4	20	30	30	40	30±2.4
1	50	40	45	40	44±1.4	47	43	27	40	39±2.5
0.1	45	55	60	70	58±3.1	80	60	87	73	75±3.4
0.01	40	65	80	60	61±4.8	83	73	90	80	82±2.1
0.001	35	40	45	50	43±1.9	67	60	43	63	58±3.1
0.0001	25	30	20	35	28±1.9	40	43	47	53	46±1.6
0.00001	30	25	20	15	23±1.9	43	47	23	43	39±3.2
0.000001	5	10	5	15	9±1.4	50	23	20	27	30±4.0
0.0000001	10	10	5	5	8±0.7	30	20	17	23	23±1.7
0	0	5	10	0	4±1.1	31	22	13	17	21±2.3

Indian corn : the seeds were produced in 1951 ; 20 seeds were put in each Petri dish. Radish : the seeds were produced in 1950 ; 60 seeds were put in each Petri dish.

(3) Content of thiamine and its forms during the germination process.

The content of thiamine and its forms in the cotyledons, plumules and radicles of the germ of kidney beans (product of 1951) were investigated (Figs. 2 and 3).

a. Germination in the dark.

The thiamine contents were compared on the dry weight basis. In both cotyledon

Table 4. Effect of thiamine application on the germination of old kidney beans (variety Kentucky Wonder). Experiment December 1951.

B_1 concentration	Seeds from the year :				
	1947	1948	1949	1950	1951
ppm	%	%	%	%	%
B_1 100	0	0	12±1.1	81±3.3	96±1.2
10	0	0	11±0.8	85±3.9	96±0.9
1	0	0	11±0.6	96±1.2	98±0.6
0.1	0	0	24±2.2	96±1.3	98±0.6
0.01	0	0	33±1.9	97±0.9	98±0.4
0.001	0	0	25±1.8	97±1.0	97±1.0
0.0001	0	0	10±0.6	90±2.7	96±1.3
0.00001	0	0	15±0.8	95±1.4	96±1.2
0.000001	0	0	6±0.3	84±3.2	97±0.9
0.0000001	0	0	0	89±4.6	96±1.1
0	0	0	0	82±2.3	90±1.2

Eighty seeds in each plot having 4 subplots, were soaked for 24 hours in each solution at 11°C ; then examined after 72 hours at 30°C.

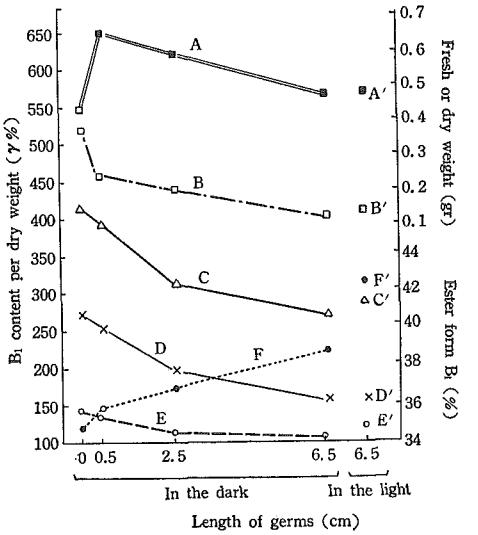

Fig. 2. Changes in thiamine content and its form in cotyledons in the process of germination of kidney beans (Kentucky Wonder).

A, B, C, D, E, F: in the dark and A′, B′, C′, D′, E′, F′ : in the light. A, A′ : fresh weight per grain. B, B′ : dry weight per grain. C, C′ : total B_1. D, D′ : free form B_1. E, E′ : ester form B_1. F, F′ : the ratio of ester form B_1. The seeds were produced in 1951, and analyzed in December, 1951.

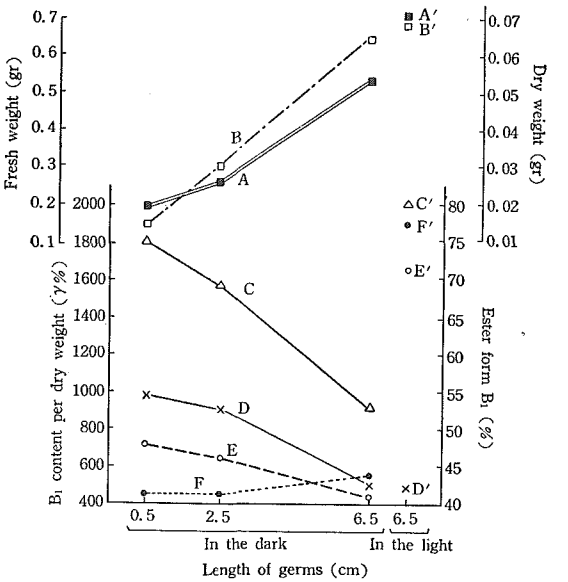

Fig. 3. Changes in thiamine content and its form in plumules + radicles in the process of germination of kidney beans.
Explanations see Fig. 2.

and axis, total, free and ester form thiamine diminished as the germination went on. But the relative amount of ester form B_1 compared with that of free form increased gradually. The total-vitamin B_1 per grain diminished from 1.41 to 0.81 γ when the seedlings were 6.5 cm long in the dark (Fig. 4).

b. Germination in the light.

When the germs were grown to the length of 6.5cm, those which germinated in the light and those in the dark were compared:

In cotyledons the fresh weight, the dry weight and the amount of thiamine in every form were a little larger in the light than in the dark. Especially the ester form vitamin B_1 increased to 42.5 per cent of total thiamine in the light against 39.2 per cent in the dark.

In plumules and radicles, the total thiamine content (C and C′ in Fig. 4), and especially, the content of ester form B_1 (F and F′) increased remarkably in the light.

In the light the absolute quantity of thiamine in plumule and radicle per grain amounted to 1.46 γ, while it was 0.47 γ in the dark.

(4) Duration of storage and the thiamine content in kidney bean seed.

Changes of thiamine content and its forms in the seeds stored 1–4 years under nearly the same conditions are shown in Fig. 5. With increasing age, the thiamine content, both in free and ester form, diminished remarkably.

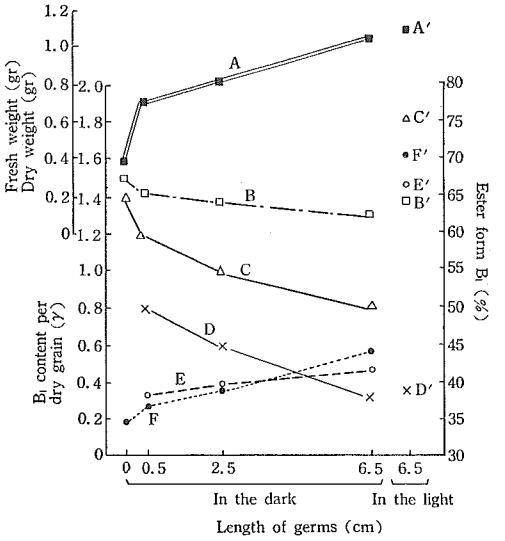

Fig. 4. Changes in thiamine content per grain with the process of germination of kidney beans (Kentucky Wonder).

A, B, C, D, E, F: in the dark and A′, B′, C′, D′, E′, F′: in the light. A, A′: fresh weight per grain. B, B′: dry weight per grain. C, C′: total B_1 content per grain. D, D′: total B_1 in cotyledons per grain. E, E′: total B_1 in plumules and radicles per grain. F, F′: the ratio of ester form B_1.

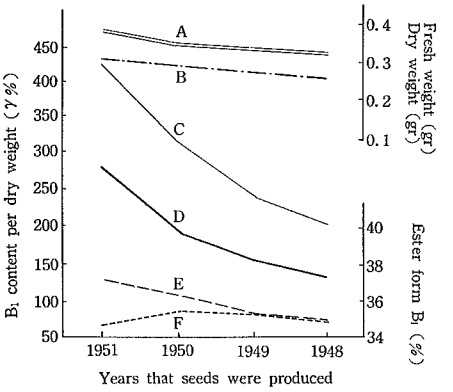

Fig. 5. Changes in thiamine content of kidney bean seeds (variety: Kentucky Wonder) of different ages.

A: fresh weight, B: dry weight per grain. C; total B_1 content per grain. D: free form B_1 content per grain. E: ester form B_1 content per grain. F: the ratio of ester form B_1. Analysis: January 1952.

(5) Content of thiamine and its forms in the germinating seed soaked in thiamine solution.

To make clear the change of thiamine in the seeds soaked in thiamine solution and in the seedlings obtained from them, some experiments were carried out. Seeds were soaked at 11°C for 24 hours and were germinated at 25°C in darkness. Thiamine determination was carried out immediately after soaking, and at the time when the seedlings reached 3 cm. Before the analysis the seeds were washed in water, deprived of their coats carefully, and washed again in distilled water. Seeds soaked in distilled water served as the control.

As shown in Table 5, the thiamine content of the experimental plot was far greater than that of the control one. In regard to total vitamin B_1, it was increased by 150~180 γ %.

Table 5. Effects of thiamine application on the changes of thiamine content and its form in the cotyledons of kidney beans (variety: Kentucky Wonder).

Length of germs		Water		B_1 application	
		Before germination, after soaking for 24 hours	Seedling 3 cm long, germinated in the dark	Before germination, after soaking for 24 hours	Seedling 3 cm long, germinated in the dark
Moisture		65.2%	58.4%	65.5%	60.0%
		γ %	γ %	γ %	γ %
Free form B_1	Per fresh matter	88.9	71.8	130.0	78.0
	per dry matter	255.5	172.6	376.7	195.1
	(Per cent)	(63.4)	(58.5)	(63.8)	(44.0)
Ester form B_1	Per fresh matter	51.3	51.1	83.7	99.4
	Per dry matter	147.5	122.8	213.2	248.6
	(Per cent)	(36.6)	(41.5)	(36.2)	(56.0)
Total B_1	Per fresh matter	140.2	122.9	213.7	177.4
	Per dry matter	403.0	295.4	589.9	443.7

Before analysis the seed coats were removed. The seeds were soaked at 11°C for 24 hours, applied with 0.01 ppm thiamine, and germinated in a 30°C dark thermostat. The seeds were produced in 1951, and analyzed in January 1952.

In the soaked seeds the increase in the free form vitamin B_1 was much greater than that of ester form. But, in the seedlings grown to the length of 3 cm, the ester form vitamin B_1 increased rapidly to a considerably high degree.

(6) Effect of thiamine application on the respiration of seeds.

Kidney beans produced in 1948, 1949, 1950, and 1951 were used as the materials. After having soaked in 0.01 ppm thiamine solution for 5 hours, the seeds were washed thoroughly. Two drops of phenol red was added to 50 cc of distilled water which has

been adjusted by 0.1 N NaOH to pH 8.4 in 100 cc flask.

Into this flask, 10 gr each of different aged seeds was soaked and germinated at 20°C in a thermostat. And the time of decolorization was determined (KOBAYASHI et al, 1951).(97)

From the results shown in Table 6, it will be inferred that the fresher the seeds the greater is the rate of respiration, and that the seeds treated with the thiamine solution respired more intensely than the control.

Table 6. Effect of thiamine application on the respiration of kidney bean seeds.

	Seeds from the year :			
	1951	1950	1949	1948
	min.*	min.*	min.*	min.*
Control (Water)	19.0	28.0	32.0	36.5
B_1 application	17.0	25.0	27.0	35.0
L.S.D. (5 %)	2.7	2.2	3.8	3.2

* The time in the table shows that of decorization of alkaline water (pH 8.4) containing 2 drops of phenol red. The time shows the degree of CO_2 evolution. The fresh weight of the seeds at each plot was 10 gr. The flasks with the seeds were put into a 20°C double-glass thermostat.

3. Considerations

In kidney beans (two varieties), Indian corn, and radishes thiamine application promoted the germination. It was effective in increasing the germinative ratio of old seed. No abnormal germination has been recognized. In this point, the result is in accordance with the report of TSUGE (1947).(159)

The promoting effect of seed soaking was observed especially in the early period of germination, and the optimum concentration was 0.01 ppm or thereabouts. MURAKAMI (1943)(115) has reported the optimum concentration to be 0.01 ppm in rice plants, while TSUGE (1947)(159) reported it to be 0.0001 ppm in soy beans.

Concerning the change of thiamine content during germination, the decrease of the absolute quantity of thiamine in the dark went nearly parallel with that of the dry matter of seeds. But as the quantity of thiamine remarkably increased in the light, light seems to be indispensable for the synthesis of thiamine. It was shown that thiamine in the seed before germination was, for the most part, of its free form; and it was changed into ester form as the germination went on. Free form vitamin B_1, which was absorbed by the seedling in external application, was also converted to ester form as the germination proceeded.

KONDŌ and others(98) reported that in rice plants the ester form prevails in the leaves, and the free form in the ears.

From these facts it will be possible to infer that thiamine is synthesized in ester form, stored in seeds in its free form, and the latter is consumed by transfering again to ester form as the germination goes on.

The viability of seed seems also to have some connection with the content of thiamine. The older the seed is, the less is the content of thiamine, though the seed incapable of germinating is not entirely free from thiamine.

By the comparison of the respiration of seeds soaked in thiamine solution with that of control one, it has been recognized that the application of thiamine promotes the respiration of the seeds. (69)

Judging from the above mentioned facts, it will be concluded that thiamine is necessary for seed germination, that thiamine stored in the seeds is consumed till its formation begins in the light, and that the consumption of thiamine is accompanied by its change from the free form into the ester form. An artificial application of thiamine promotes the germination of seeds by augmenting the metabolic activity.

CHAPTER II*

EFFECTS OF FOLIAR THIAMINE SPRAY ON THE GROWTH, YIELD, AND QUALITY OF GARDEN CROPS

It will be expected that thiamine has some effect on the growth or on the yield of crops as on the germination of seeds and the growth of young roots. Foliar thiamine spray was sprayed on sweet potatoes, potatoes and kidney beans and the effective concentration of the solution was examined.

SECTION I
SWEET POTATOES

1. Materials and Methods

(1) Experiment on the growth and yield.

Sweet potatoes used for this experiment were: varieties Nōrin No. 1, Nōrin No. 4, Kantō No. 24, and Taihaku from the Farm of the Faculty of Agriculture, Shinshū University, Nōrin No. 1 from the Farm of Kyoto University, and Shijūnichiimo from the TAKII Nursery Company. Water culture, sand culture and field experiment were carried out.

For the water culture experiment, the KASUGAI's culture solution** for dry field crops was used. The solution in a 100 cc Erlenmeyer's flask wrapped with black cloth and placed in a thermostat of 20°C in the light, was changed every third day.

For the sand culture experiment, wooden boxes or pots were used. The quantity of fertilizers applied in solution to the unit area 3.3m^2 was as follows: ammonium

* A part of the argument of this chapter was reported at the meeting of the Horticultural Association of Japan in October, 1952.

** Composition: NH_4NO_3 57.3 mg, KCl 43.0 mg, $MgSO_4$ 120.0 mg, $Ca(NO_3)_2$ 117.0 mg, KH_2PO_4 38.3 mg, $MnCl_3$ 0.4 mg, 6 % $FeCl_3$ 2.5 *ml*, H_2O up to 1*l*.

sulphate 80 gr, superphosphate of lime 80 gr, potassium chloride 30 gr.

The field experiment was carried out by the random method, the details of which will be described in the paragraph concerning each experiment.

In most of the experiments, slips, each having 8 expanded leaves and weighing 9~10 gr, were used. They were put into the solution or the soil up to the 4th node.

Thiamine hydrochloride was dissolved in distilled water or in service water, and in the experiments of small scale, it was sprayed by a specially made sprayer of 10 cc which was graded to 1 cc. When close coating of leaf with thiamine solution was required, a spuirt of 2 cc which was graded to 0.1 cc was used.

The spray was made, as a rule, at 10 o'clock in the morning. The quantity, distance, and frequency of foliar thiamine spray will be described with each experiment; the controls were sprayed with distilled water or city water, pH values of which were adjusted with HCl equal to those of thiamine solution.

The sand culture experiment in 1954 was carried out at the Faculty of Agriculture, Kyoto University, while all other experiments were done at the Faculty of Agriculture, Shinshu University.

Results obtained were evaluated statistically.

(2) Experiment on the taste.

The method of the experiment will be described in the results.

2. Results

(1) Effect of foliar thiamine spray on the growth of the underground portion of sweet potatoes.

a. The effects on the initiation and development of roots.

Field experiment in 1952 (Fig. 6). The number of either the roots or the branch rootlets, the average length of roots, total length of roots, and the dry weight of roots were all increased by thiamine spray as compared with the control.

Water culture experiment in 1953. The results were similar to that of the foregoing experiment, as shown in Table 7 and Plate I–I.

Statistical inquiries showed the significant difference of the 1 per cent level in the number of the secondarily developed roots and that of the 5 per cent level in the rest.

Sand culture experiment in 1954. Using Shijūnichiimo as the material, a sand culture experiment similar to the above was carried out at Kyoto University, and nearly the same results were obtained (Tab. 8 and Pl. I–II).

From the experiments mentioned above, it may certainly be said that the spraying of thiamine solution upon the leaves of sweet potatoes significantly promotes the initiation and the development of roots.

b. Effects of foliar thiamine spray on the formation of tubers.

Field experiment in 1952. The results are shown in Fig. 7. The number, the total fresh weight and the average weight of tubers were increased as compared with those of the control.

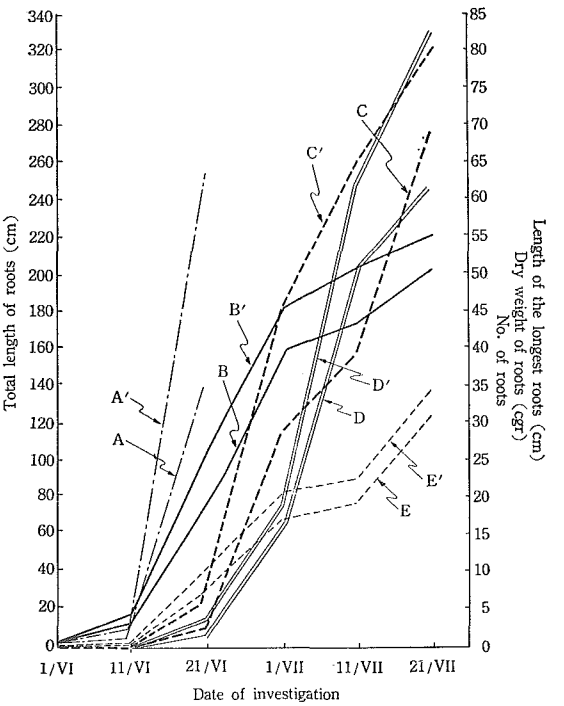

Fig. 6. Effects of foliar thiamine spray on the root initiation and root development of young sweet potato plants.

Field experiment at Ina (Shinshu Univ.) in 1952. The variety used was Nōrin No. 1. From mother tubers (imbedded on April 25) slips with 8 expanded leaves were set out in the farm on June 1. Every 10th day, 2cc of 1 ppm thiamine solution per plant was sprayed on the leaves, and every 10th day until July 21, 10 plants each were taken from the sprayed and the control plot, and investigated. A, B, C, D: the control plots, A′, B′, C′, D′: the B_1 sprayed plots. A, A′: total length of roots. B, B′: no. of the primarily developed roots. C, C′: no. of the secondarily developed roots. D, D′: dry weight of roots. E, E′: length ofthe longest roots.

Table 7. Effect of foliar thiamine spray on the growth of sweet potato young plants in water culture (results per plant). Experiment at Ina (Shinshu Univ.) in 1953.

	Control			B_1 spray			Difference and its significance	L.S.D.
	Starting time	12 days after starting	difference	Starting time	12 days after starting	Difference		
Length of stems (cm)	25.1	28.5	+3.4	25.7	29.9	+4.2	+0.8	1.4(5%)
Diameter of stems (mm)	41.5	41.8	+0.3	41.0	48.9	+7.9	+7.6**	7.1(1%)
Fresh weight of plant (gr)	18.2	12.2	−6.0	18.6	15.3	−3.3	+2.7	3.2(5%)
No. of primarily developed roots	0	7.5	+7.5	0	19.0	+19.0	+11.5**	8.6(5%)
No. of secondarily developed roots	0	34.8	+34.8	0	70.9	+70.9	+36.1*	21.2(1%)
Length of the longest roots (cm)	0	6.7	+6.7	0	9.1	+9.1	+2.4	4.1(5%)
Total length of roots (cm)	0	40.5	+40.5	0	71.5	+71.5	+31.0*	29.2(5%)
Fresh weight of roots (gr)	0	0.26	+0.26	0	0.33	+0.33	+0.07*	0.06(5%)
Dry weight of roots (gr)	0	0.021	+0.021	0	0.038	+0.038	+0.017	0.028(5%)

The variety was Nōrin No.1. The experiment was carried out in a double glass thermostat at 20°C. Two cc of 1 ppm thiamine solution per plant was sprayed on the leaves each day, 9 times in all. The investigation was made 12 days after the slips were set out. The number of blocks was 8, and each plot had 1 plant. L.S.D.: the level of significant difference.

* 5 per cent level, ** 1 per cent level.

Table 8. Effect of foliar thiamine spray on the growth of young sweet potato plants (results per plant.). Sand culture experiment at Kyoto in 1954.

	Control	B_1 spray	Difference and its significance	L.S.D.
No. of primarily developed roots	32.0	50.0	18.0**	6.9 (1%)
No. of secondarily developed roots	183.6	270.0	86.4*	78.8 (5%)
Total length of primarily developed roots (cm)	241.1	372.1	131.0**	90.3 (1%)
Fresh weight of roots (gr)	2.66	4.25	1.59**	1.11(1%)
Fresh weight of stems and roots (gr)	9.49	10.63	1.14	1.78(5%)

The variety was Shijūnichiimo. Two cc of 1 ppm thiamine solution per plant was sprayed 3 times on the leaves. Every pot had 1 plant and the number of blocks was 8. Observations 12 days after planting.

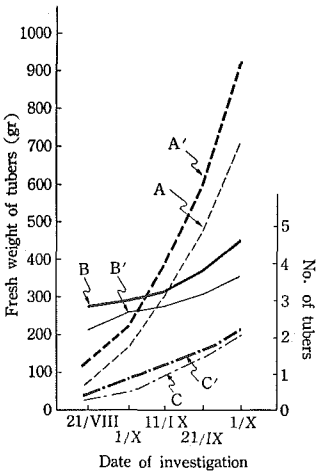

Fig. 7. Effects of foliar thiamine spray on the formation and growth in thicnness of sweet potato tubers.

Field experiment at Ina in 1952. The variety used was Nōrin No. 1 and its slips were set out at the farm on June 1. Every 10th day, 2 cc of 1 ppm thiamine solution per plant was sprayed on the leaves, and every 10th day, the tubers were investigated. A, B, C: the control plots, A′, B′, C′: the B_1 sprayed plots. A, A′: fresh weight of tubers per plant. B, B′: no. of tubers per plant. C, C′: mean fresh weight of tuber.

In another experiment, thiamine solution was sprayed 6 times on the young plants during the 60 days afrer planting. The result shown in Plate III–XIX indicates a promoting effect of thiamine upon the tuber formation.

Table 9 shows the result at the harvest time, on October 20. No significant difference was observed in the number of tubers, but the weight of tubers in the experimental plot was significantly increased as compared with that in the control.

Field experiments in 1953. In two field experiments similar results to the above-mentioned experiment in 1952, were obtained (Tabs. 10, 11 and Pl. I–III).

Sand culture experiment in 1954. A similar sand culture experiment was carried out and similar results were obtained (Tab. 12 and Pl. I–IV).

Field experiment in 1954. By spraying 10 times during the whole growing period, the total weight of tubers and the weight of larger tubers were significantly increased as compared with the control. The weight of smaller tubers was somewhat decreased but showed no satististical significance (Tab. 13).

From these results, it is clear that the foliar spray of thiamine has promoting effects on the formation and growth of tubers, resulting in the increase of the number and the weight of larger tubers.

(2) Relation between foliar thiamine spray and the growth of the shoots of sweet potatoes.

According to the results shown in Tables 7, 9, 10, 11, 12 and 13, it may be recognized that foliar thiamine spray increases the weight of stems and leaves, though no significant difference was ascertained in the length of the stems.

(3) Relation between the mode of foliar thiamine spray and the growth of sweet potatoes.

Comparison btween 3 plots i. e. the plot sprayed 5 times in the first half, the plot sprayed 5 times in the latter half of the growing period, and the plot sprayed 10 times during the whole period (Tab. 13). The effects was most evident on the plot sprayed throughout the whole period, next to it, in the plot sprayed in the first half of the

Table 9. Fffects of foliar thiamine spray on the growth [and yield of sweet potasoes (var. Nōrin No. 1). Field experiment at Ina in 1952.

	Control	B_1 spray	Difference and its significance	L.S.D.	Percentage of difference
No. of stems per plant	5.6	5.9	+0.3	1.5 (5%)	+6.5
Lengh of stems in cm per plant	42.7	43.3	+0.6	9.4 (5%)	+1.3
Diameter of stems in mm per plant	12.8	14.2	+1.4*	1.3 (5%)	+11.7
Fresh weight of stems and leaves in kg per 0.1 ha	1692.4	1914.4	+222.0**	180.8 (1%)	+13.1
No. of tubers per 0.1 ha	16800.0	18700.0	+1900.0	2971.0 (5%)	+14.4
Total fresh weight of tubers in kg per 0.1 ha	1840.5	2112.0	+271.5**	101.7 (1%)	+14.8

Mother tubers for this test were imbedded on April 25, 1952 ; slips planted on June 1, and harvested and investigated on October 20. The number of blocks was 6, and each plot was 1.65m^2 in area. The distance for planting was 0.9 × 0.3m. Three hundred gr of lime, 100 gr of ammonium sulphate, 100 gr of superphosphate of lime, and 30 gr of potasium chloride per 3.3m^2 were applied to the field as the basic fertilizer. Five cc of 1 ppm thiamine solution per plant was sprayed on the leaves 6 times at intervals of 10 days in the first half of the growing period.

Table 10. Effect of foliar thiamine spray on the yield of sweet potatoes (kg per 0.1 ha). Field experiment at Ina in 1953.

	Control	B_1 spray	Difference and its significance	L.S.D.	Percentage of increase or decrease
Fresh weight of stems and leaves	1382.6	1699.9	+317.3*	296.6 (5%)	+23.0
Fresh weight of tubers larger than 112 gr	955.1	1118.3	+163.2**	102.0 (1%)	+17.1
Fresh weight of smaller tubers	223.9	207.4	−16.5	42.4 (5%)	−7.4
Total fresh weight of tubers	1178.6	1325.6	+147.0**	74.3 (1%)	+12.5

The variety used was Nōrin No. 1. Mother tubers were imbeded on April 9, 1953; slips were planted on May 30, and harvested and investigated on October 16. The number of blocks prepared for the test was 6, and cach plot was 49.5m^2 in area. The distance for planting was 0.9 × 0.3m. Five cc (in the first half of the growing period) or ten cc (in the latter half) of 1 ppm thiamine solution per plant was sprayed 6 times on the leaves.

Table 11. Effects of foliar thiamine spray on the sweet potato yields (kg per 0.1 ha) of 4 varieties. Field experiment at Ina in 1953.

Varieties	Fresh weight of stems and leaves		Fresh weight of tubers					
			Larger tubers		smaller tubers		Total	
	Control	B_1 spray	Control	B_1 spray	Control	B_1 spray	Control	B_1 spray
Nōrin No. 1	1387.5	2208.8	907.5	1053.8	273.8	251.3	1181.3	1305.1
Nōrin No. 4	2021.3	2317.5	997.5	1275.0	596.3	476.3	1593.8	1751.3
Kantō No. 24	1380.0	1868.0	1222.5	1481.3	281.3	225.0	1503.8	1706.3
Taihaku	1702.5	1905.0	900.0	1065.0	592.5	461.3	1492.5	1526.3
Average	1622.8	2062.5	1006.8	1218.8	436.0	353.5	1442.8	1572.3
Difference and its significance	−439.7**		212.0**		−82.5		−192.5	
L.S.D.	514.1		199.1		491.6		129.4	
Percentage of increase or decrease	−34.4		−21.0		−18.9		−8.9	

Explanations and remarks see Table 10.

Table 12. Effect of foliar thiamine spray on the earlier growth of sweet potato plants (results per plant). Sand culture experiment at Ina in 1954.

	Control	B_1 spray	Difference and its significance	L.S.D
No. of tubers	4.9	9.1	+1.2*	1.2(5%)
Fresh weight of tubers (gr)	14.1	18.9	+4.5**	3.1(1%)
Fresh weight of tubers and small roots. (gr)	18.9	24.4	+5.5**	5.1(1%)
Diameter of stems (mm)	40.0	44.9	+4.9*	4.0(5%)
Length of stems (cm)	59.3	57.1	+0.8	9.1(5%)
Fresh weight of stems and leaves (gr)	19.8	23.9	+3.8**	1.4(1%)

The variety used was Nōrin No. 1. Two cc of 1 ppm thiamine solution per plant was sprayed on the leaves 4 times. Investigation 49 days after planting.

Table 13. Relations between the yield of sweet potato (var. Nōrin. No. 1) and the period of foliar thiamine spray (results per 0.1 ha).

1. B_1 spray (5 times) in the first half of growing period.

	Control	B_1 spray	Differenee to the control and its significance	Percentage of difference
Fresh weight of stems and leaves (kg)	2696.6	2920.5	+223.9	+8.3
No. of larger tubers	3337.5	3837.5	+500.0	+15.0
No. of smaller tubers	8425.0	8393.8	−31.2	−3.7
Total no. of tubers	11792.5	12231.3	+498.8	+4.0
Fresh weight of larger tubers (kg)	675.0	805.5	+130.5	+19.3
Fresh weight of smaller tubers (kg)	369.0	355.9	−13.1	−3.6
Total fresh weight of tubers (kg)	1044.0	1160.3	+116.3*	+11.2

2. B_1 spray (5 times) in the latter half of growing period.

	B_1 spray	Difference to the control and its significance	Percentage
Fresh weight of stems and leaves (kg)	2665.9	−30.7	−0.1
No. of larger tubers	3675.0	+337.5	+10.1
No. of smaller tubers	9281.3	+856.3	+10.2
Total no. of tubers	12959.3	+1193.8	+10.1
Fresh weight of larger tubers (kg)	733.9	−58.9	+8.7
Fresh weight of smaller tubers (kg)	367.9	−1.1	−0.3
Total fresh weight of tubers (kg)	1101.8	+57.8	+5.5

3. B_1 spray (10 times) in the whole growing period.

	B_1 spray	Difference to the control and its significance	Percentage of difference	L.S.D.
Fresh weight of stems and leaves (kg)	3055.1	+358.5*	+13.3	292.9 (5%)
No. of larger tubers	4175.0	+837.5**	+25.1	596.5 (5%) 803.6 (1%)
No. of smaller tubers	8393.8	−31.5	−3.7	1809.2 (5%)
Total no. of tubers	12568.8	+806.3	+6.9	1693.6 (5%)
Fresh weight of larger tubers (kg)	840.0	+165.0**	+24.4	111.4 (5%) 149.6 (1%)
Fresh weight of smaller tubers (kg)	374.3	+5.3	+1.4	67.9 (5%)
Total fresh weight of tubers (kg)	1214.3	+170.3**	−16.3	116.6 (5%) 157.1 (1%)

The mother tubers for the test were imbedded on April 25, 1954; slips were planted on June 7, harvested and investigated on October 13. The number of blocks was 8, and each plot was 19.8m² in area. The distance for planting was 0.9×0.3m. 21.6 kg of ammonium sulphate, 27.6 kg of superphosphate of lime and 12.0 kg of potassium chloride were applied to the field per 0.1 ha as the basic fertilizer, and 16.9 kg of urea was applied as the supplemental fertilizer. Five cc of thiamine solution per plant was sprayed on the leaves at an interval of 14 days. Every large tuber was above 112.5 gr.

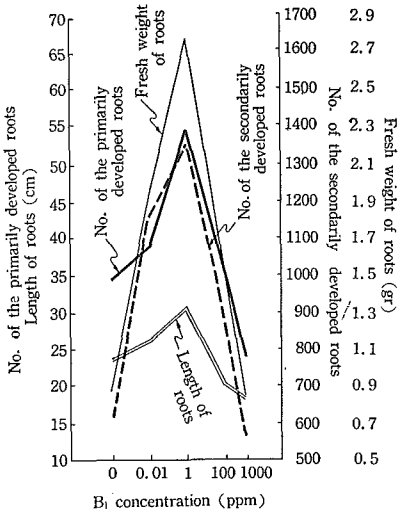

Fig. 8. Relation between the concentration of thiamine solution sprayed and the root development of sweet potato young plants.

Sand culture experiment at Ina in 1952. Data obtained per plant 30 days after planting. The variety used was Nōrin No. 1. Two cc of thiamine solution per plant was sprayed on the leaves 3 times in all.

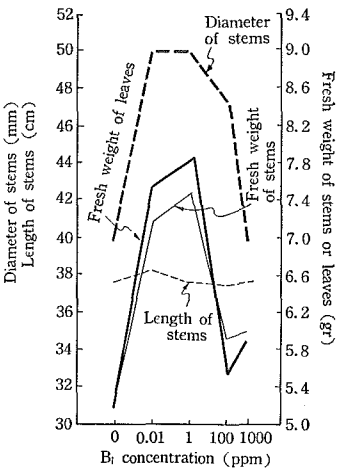

Fig. 9. Relation between the thiamine concentration for foliar spray and the growth of young sweet potato shoots.

Sand culture experiment at Ina in 1952. Data 30 days after planting. The remarks are the same as in Fig. 8.

period, and then in the latter half of the growing period.

(4) Relation between the concentration of thiamine solution for spray and the growth of sweet potatoes.

Sand culture experiment in 1952 (Figs. 8, 9 and Pl. I–V). For the growth of roots 1 ppm was the optimum concentration. The number of the primarily and secondarily developed roots, and the length and weight of roots were increased. For the shoot growth, 1 ppm was also optimum. The diameter and the number of stems, and the weight of both stems and leaves were increased.

Field experiment in 1952. The results shown in Figs. 10, 11 and Plate I–VI indicate that for the total fresh weight of the underground portion, fresh weight of total tubers and for the average fresh weight per tuber, 1 ppm was the optimum concentration, next to it, 0.01 ppm. For the diameter and number of stems and the fresh weight of shoots, 1 ppm was the optimum, but somewhat lower for the length of stems.

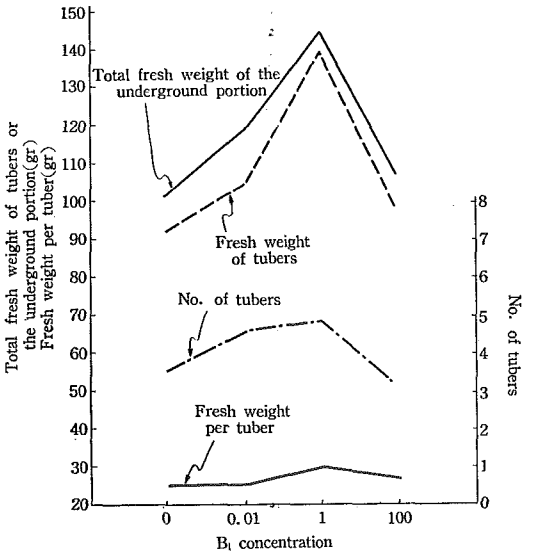

Fig. 10. Relation between thiamine concentration for foliar spray and the formation and growth in thickness of sweet potato tubers.

Field experiment at Ina in 1952. Data (per plant) 70 days after planting. The variety used was Nōrin No. 1, the slips were set out at the farm on July 3. Two cc of thiamine solution per plant was sprayed on the leaves 7 times in all.

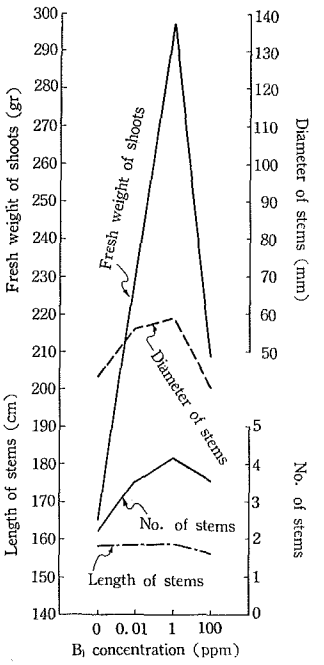

Fig. 11. Relation between thiamine concentration for foliar spray and the growth of sweet potato shoots.

Field experiment at Ina 1952. Data (per plant) 70 days after planting. The remarks are the same as in Fig. 10.

From these results, it may be infered that the optimum concentration of thiamine solution for the foliar spray on sweet potatoes is 1 ppm or thereabouts.

(5) Relation between foliar thiamine spray and the taste of tubers.

The materials for the test were the tubers cultivated in pots with soil or sand in 1954. The method of the cultivation and treatment of materials were shown in the remarks of Table 14.

Table 14. Relations between foliar thiamine spray and the taste of sweet potato tubers.

Sampling day (in 1954)	Soil culture Oct. 19	Sand culture Oct. 21
Persons who tasted	31	25
Persons voted for the tubers from non-sprayed plot	6	8
Percentage	19.4	32.0
Persons voted for the tubers from B_1 sprayed plot	22	17
Percentage	71.0	68.0
Persons voted for both of the tubers	3	0
Percentage	9.6	0

The variety was Nōrin No. 1. In the soil culture, unglazed pots of 27 cm diameter were used. Basic fertilizer applied to each pot was as follows : ammonium sulphate 5 gr, superphosphate of lime 5 gr, potasium chloride 2 gr. In the sand culture, the WAGNER-pots with sand were used, and as a fertilizer, a thrice concentrated hydroponic solution of KASUGAI for dry field crops was applied 2 times in a week. In each experiment, the slips with 8 expanded leaves were planted on May 20, and the tubers were harvested on October 12. During this period 1 ppm thiamine solution was sprayed fully on the leaves 6 times.

The havested tubers, including both larger and smaller ones from the control and B_1 sprayed plots, were cut into similar forms and were tested according to the random sampling method by the members of the Faculty of Agriculture, Shinshu University.

The results (Tab. 14) show that, 68～74 per cent of the persons judging the tubers, voted that the plots applied with thiamine were more delicious than the control tubers.

Therefore, it may safely be said that foliar thiamine spray is effective in improving the taste of sweet potato.

3. Considerations

From the results mentioned above, it will be inferred that the thiamine, when sprayed in 1 ppm solution, is very effective to increase the yield, and improves the quality of sweet potatoes. Some investigators[77,86,95] believe that in the early stage of development, every sweet potato root has the potentiality to grow in thickness, and within the period of 10～20 days, or a little earliar, after the setting out of the slips, the fate of the young roots as to whether or not they become converted into tubers, is determined. At this period, ITŌ et al (1948)[86] recognized an occurrence of cambium in the xylem parenchyma of the roots of 3 mm in diameter. The determination is controlled by various circumstances at this period.

The increase of yield, especially by the earlier thiamine application may be, in the first place, attributable to the promotion of root initiation and its development in that stage.

The thiamine was also effective in increasing the diameter but not the length of the

stem. As will be described later (CHAP. IV, SECT. I), thiamine spray increases the C–N ratio in sweet potatoes, or it promotes flowering and podding of kidney beans, and thus shortens the growth period. These may also be connected in some way with the increase of the yield, for in the cold districts of high altitude as Ina, (ca. 760 m above sea) where the field experiments were performed, the shortening of the growth period may also have non-negligible influence upon the yield of the sweet potato.

Thiamine is an indispensable factor for the development of excised root.[127~129, 168] A thiamine deficiency even in the intact field plants may be concluded in some parts of plant, or in some period of plant growth.*

SECTION II
POTATOES

1. Material and Method

The material for the present experiments was the "Danshaku" variety obtained from the Nobeyama farm of the Shinshu University. The experiments were carried out at the farm of the Faculty of Agriculture, Shinshu University, where the soil was infertile and especially acid.

The quantity of fertilizers applied to 3.3 m^2 was as follows: lime 400 gr, ammonium sulphate 200 gr, superphosphate of lime 200 gr, potassium chloride 100 gr. They were applied at the same time as the basic fertilizers, and no compost was added. The details of the cultivation and treatment will be described later.

The number and fresh weight of the tubers were investigated at harvest time; they were classified into two growps, namely, the larger tubers, each above 60 gr, and the smaller ones.

2. Results and Considerations

(1) Relation between foliar thiamine spray and the yield of potatoes.

Field experiment in 1952 (Tab. 15). With the thiamine spray, the number of the larger tubers increased by 14.5 per cent at the 5 per cent level with a significant difference, that of the smaller tubers decreased by 1.2 per cent without any significant difference, the fresh weight of the larger tubers was increased by 20.4 per cent at the 1 per cent level, and the total number, and the total fresh weight of tubers were increased at the 5 per cent level.

Field experiment in 1953. The number of blocks in this experiment was 8, and 1 plot had 9.9 m^2. Details of the cultivation and treatment were similar to those of the experiment in 1952. The larger tubers increased and the smaller ones decreased (Tab. 16).

Field experiment in 1954 (Tab. 17). The experiment was carried out in a similar

* By the farmers in the Kantō district in Japan, it has been said that rice bran is remarkably effective on the yield of sweet potatoes, and FUJII's experiment[43] on "Oryzanin" solution from rice bran was also effective.

Table 15. Effect of foliar thiamine spray on the yield of potatoes per plant. Field experiment at Ina in 1952.

		Control	B_1 spray	Difference and its significance	L.S.D.	Percentage of difference
No. of tubers	Larger tubers	7682	8813	+1131*	882 (5%)	+14.5
	Smaller tubers	1430	1413	−17	312 (5%)	−1.2
	Total	9112	10226	+1114*	923 (5%)	+12.2
Fresh weight of tubers (kg)	Larger tubers	631.9	760.5	+128.6**	119.6 (1%)	+20.6
	Smaller tubers	417.0	445.9	+28.9	60.8 (5%)	+6.9
	Total	1048.9	1206.4	+157.5*	144.4 (5%)	+15.0

The variety used was "Danshaku". Twenty plots, each being 3.3 m^2 in area. The distance for planting was 0.9×0.36 m. The mother tubers were sown on May 11, and harvested on July 29. During the growing period 5 cc of 1 ppm thiamine solution per plant was sprayed 4 times on the leaves at one week intervals. The Weight of "larger" tubers was above 60 gr.

Table 16. Effect of foliar thiamine spray on the yield of potatoes (kg per 0.1 ha). Field experiment at Ina in 1953.

		Control	B_1 spray	Difference and its significance	L.S.D.	Percentage of difference
No. of tubers	Larger tubers	5600	6567	+967*	816 (5%)	+17.3
	Smaller tubers	14050	12800	−1250*	982 (5%)	−8.9
	Total	19650	19367	−283	412 (5%)	−1.4
Fresh weight of tubers	Larger tubers	549.4	671.3	+121.9*	111.8 (5%)	+22.2
	Smaller tubers	382.5	416.3	+33.8	88.1 (5%)	+8.8
	Total	931.9	1087.6	+155.7*	143.6 (5%)	+16.7

The variety used was Danshaku. The number of blocks in field for the test was 8, and every plot was 9.9 m^2 in area. Five cc (in the first half of the growing period) or ten cc (in the latter half) of 1 ppm thiamine solution per plant was sprayed 6 times on the leaves. The weight of each larger tuber was above 60 gr.

way to that in 1953 and the results obtained were also similar.

From these results it is evident that foliar thiamine spray is effective in increasing the number and fresh weight of the larger tubers, and the total yield of potatoes, especially the percentage of the larger tubers.

(2) Relation between foliar thiamine spray and the taste of tubers.

The same experiment as that of sweet potatoes (CHAP. II, SECT. I) was carried out. The results shown in Table 18 indicates that the percentage of the persons who judged the tubers from the plots applied with thiamine to be more delicious than those

from the control plots, was 77.8 or 85.0 per cent.

Therefore, it may safely be said that foliar thiamine spray is effective in improving the taste of potato tubers.

Table 17. Effect of foliar thiamine spray on the yield of potatoes (kg per 0.1 ha). Field experiment at Ina in 1954.

		Control	B_1 spray	Difference and its significance	L.S.D.	Percentage of difference
No. of tubers	Larger tubers	9938	11413	+1475**	688 (1%)	+14.8
	Smaller tubers	15050	15950	+900	2926 (5%)	+5.9
	Total	24988	27363	+2375	1885 (5%)	+9.5
Fresh weight of tubers	Larger tubers	612.0	772.9	+160.9*	154.5 (5%)	+26.3
	Smaller tubers	423.8	460.9	+37.1	89.6 (5%)	+8.8
	Total	1035.8	1233.8	+198.0	160.1 (1%)	+19.1

The remarks are the same as those in Table 16.

Table 18. Relation between foliar thiamine spray and the taste of potato tubers.

Sampling day (in 1954)	Soil culture Oct. 22	Sand culture Oct. 24
Persons who tasted	20	18
Persons voted for the tubers from non-sprayed plot	1	2
Percentage	5.0	11.2
Persons voted for the B_1 sprayed plot	17	14
Percentage	85.0	77.8
Persons voted for both of the tubers	2	2
Percentage	10.0	11.0

The variety used was Danshaku. The other remarks are the same as those in Table 14.

SECTION III*
KIDNEY BEANS

1. Material and Methods

The materials for the present experiments were kidney beans, of Kentucky Wonder, Burpee's Stringless Green Pods and Masterpiece varieties. For water culture, KAUSGAI's solution** was put into the 500 cc Erlenmeyer's flask wrapped with black cloth, and

* The argument of this section was reported at the meeting of the Horticultural Association of Japan in October, 1952.

** See page 203.

the solution was renewed every third day. For sand culture, unglazed pots with a diameter of 27 cm filled with washed sand were used. As the fertilizer, the three times concentrated KASUGAI's culture solution was applied at one week intervals. For soil culture in pots, the same unglazed pots as mentioned above, with uniformalized soil were used.

The quantity of fertilizers per 0.1 ha, applied to the soil culture in pots and in the field experiment, was as follows: ammonium sulphate 52.5 kg, superphosphate of lime 58.4 kg, potassium chloride 26.25, lime 112.5 kg. One pot had 1 plant as a rule, but in the sand culture experiment in 1954, 1 pot had 2 plants throughout the entire growing season. The distance for planting in the field was 0.9×0.3m.

Most of the experiments were carried out at the Agricultural Faculty of Shinshu University, but some were carried out at the Agricultural Faculty of Kyoto University. Therefore, the conditions of environment in which the experiments were carried out, were very different.

The thiamine sprays were generally done at 10 o'clock in the morning. The control plot in each experiment was sprayed with distilled or city water which had the pH adjusted with HCl to that of the thiamine solution. In the case of the water culture experiment, the flasks were enclosed by cardbord and stopped with absorbent cotton impregnated with vaseline, and the thiamine solution was coated carefully on the leaves to prevent contaminating the culture solution. But in the other experiment some of the sprayed thiamine solution might have flowed into the sand or soil.

2. Results

(1) Effect of foliar thiamine spray on the growth of kidney bean roots.

Water culture experiment in 1951 (Pl. I–VII). The thiamine application on the leaves of kidney beans was effective in promoting the development of roots.

Sand culture experiment in 1951. A similar sand culture experiment was performed and similar results were obtained (Pl. III–XX).

Soil culture experiment in pots in 1952. With the Masterpiece variety, a similar soil culture experiment in pots was performed and similar results were obtained (Tab. 19).

Field experiments in 1953 a. The varieties were Masterpiece (Fig. 12) and Kentucky Wonder (Fig. 13). In both of the varieties the foliar thiamine spray was found to be effective on the length, fresh weight, and dry weight of the roots.

Field experiment in 1953 b. With the Masterpiece variety, a similar field experiment was performed and similar results were obtained (Tab. 20).

Sand culture experiment in 1954. Using Masterpiece as the material, a similar sand culture experiment was performed and similar results were obtained (Tab. 21).

From the results shown above, it is clear that the foliar thiamine spray had promoting effect on the growth of kidney bean roots.

(2) Effects of foliar thiamine spray on the growth of shoots and the yield of kidney beans.

Soil culture experiment in pots in 1952 a. From the results shown in Table 19,

Table 19. Effects of foliar thiamine spray on the growth and yield of kidney beans (results per plant at the harvest time). Soil culture experiment in pots at Ina in 1952.

	Control	B_1 spray	Difference and its significance	L.S.D.	Percentage of difference
Length of stems (cm)	31.62	28.62	+3.00	5.86(5%)	+9.5
Diameter of stems (mm)	62.10	66.21	+4.11	5.60(5%)	+6.6
Fresh weight of stems (gr)	38.32	49.83	+11.51**	9.28(5%)	+30.0
Fresh weight of roots (gr)	1.29	2.19	+0.90**	0.62(1%)	+69.7
Length of roots (cm)	32.42	34.40	+1.98**	1.87(5%)	+6.1
No. of pods	9.00	12.06	+3.06*	2.10(5%)	+11.8
Fresh weight of pods (gr)	38.32	49.83	+11.51**	9.28(1%)	+30.0

The variety used was Masterpiece. The number of blocks prepared was 8, and each plot had 1 plant. The seeds were sown on May 10, and the grains were harvested on September 6. During the growing period, 3 times in the first half 2 cc and 3 times in the latter half 5 cc of 1 ppm thiamine solution per plant were sprayed on the leaves.

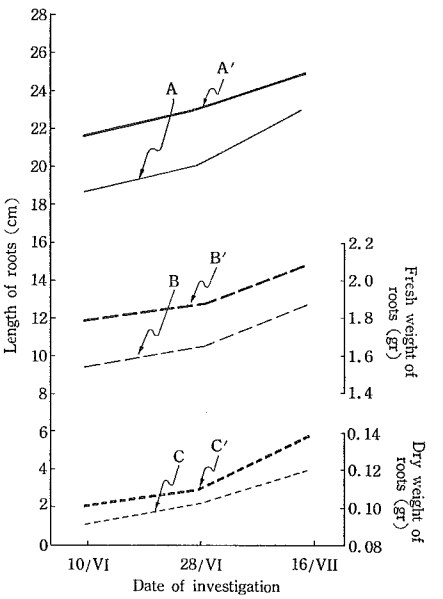

Fig. 12. Effect of foliar thiamine spray on the growth (per plant) of Masterpiece roots.

Field experiment at Ina in 1953. The seeds were sown on May 12. Every 10th day, 2 cc of 1 ppm thiamine solution per plant was sprayed on the leaves 3 times in all. A, B, C: the control plots, A′, B′, C′: the B_1 sprayed plots. A, A′: length of the longest root. B, B′: fresh weight of roots. C, C′: dry weight of roots.

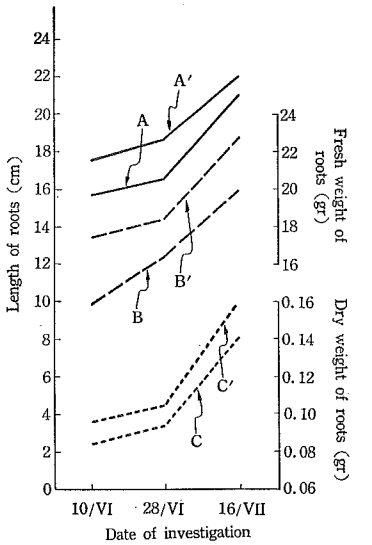

Fig. 13. Effect of foliar thiamine spray on the growth (per plant) of Kentucky Wonder roots.

Field experimens at Ina in 1953. The remarks are the same as in Fig. 12.

Table 20. Effects of foliar thiamine spray on the growth and yield of kidney beans (results per air dry plant after harvest). Field experiment at Ina in 1953.

	Control	B_1 spray	Difference and its significance	L.S.D.	Percentage of difference
Length of stems (cm)	23.4	23.8	+0.4	4.5(5%)	+1.7
Diameter of stems (mm)	33.1	35.9	+2.7	3.4(5%)	+8.2
Weight of stems (gr)	11.9	17.1	+5.2*	4.3(5%)	+43.7
Weight of roots (gr)	2.9	4.1	+1.2*	1.0(5%)	+41.4
No. of pods	7.4	9.8	+2.4*	2.2(5%)	+32.4
Weight of pods (gr)	14.4	20.0	+6.0**	5.2(1%)	+41.7

The variety used was Masterpiece. The number of blocks was 10, and every plot was 1.65 m^2 in area. The seeds were sown on May 8, and the grains were harvested on September 1. During the growing period, 2 cc (in the first half of the growing period) and 5 cc (in the latter half) of 1 ppm thiamine solution per plant was sprayed 6 times on the leaves.

Table 21. Effects of foliar thiamine spray on the growth and yield of kidney beans (results per air dry plant after harvest). Sand culture experiment at Kyoto in 1954.

	Control	B_1 spray	Difference and its significance	L.S.D.	Percentage of difference
Length of stems (cm)	22.3	23.3	+1.0	2.3(5%)	+4.5
Diameter of stems (mm)	40.6	42.0	+1.4	3.0(5%)	+3.5
Weight of stems (gr)	1.7	2.1	+0.4**	0.38(1%)	+23.5
Wieght of roots (gr)	0.8	1.2	+0.4**	0.35(1%)	+50.0
No. of pods	4.3	5.5	+1.2*	1.0(5%)	+27.9
Weight of pods (gr)	6.1	8.2	+2.1*	1.6(5%)	+34.4

The variety used was Masterpiece. The number of blocks was 10, and every plot had 1 plant. The seeds were sown on May 26, and the grains were harvested on August 11. During the growing period, 2 cc of 1 ppm thiamine solution per plant was sprayed 6 times on the leaves.

it was recognized that the length and diameter of kidney bean stems at the harvest time were not significantly increased in the treated plots as compared with those of the control; but the fresh weight of both stems and pods was increased at the 5 per cent significant level.

Soil culture experiment in pots in 1952 b. The varieties used were Masterpiece and Kentucky Wonder. The number of flowers 50 days after sowing were examined (Tab. 22).

The foliar thiamine spray had a good effect on increasing the number of flowers of the two varieties.

The surviving green leaves of these two varieties were counted at the harvest time

Table 22. Effect of thiamine spray on the flowering of kidney beans. Soil culture experiment in pots in 1952.

Varieties	Treatment	No. of flowers per plant	Difference and its significance	L.S.D.
Kentucky Wonder	Control	0.30	0.80*	0.68 (5%)
	B_1 spray	1.10		
Masterpiece	Control	0.85	1.70**	1.53 (5%)
	B_1 spray	2.55		

The seeds were sown on May 10, and the investigation was practised on June 29. During the growing period, 2 cc of 1 ppm thiamine solution per plant was sprayed 4 times. The number of blocks for the test was 10, and every plot had 1 plant.

Table 23. Relation between the defoliation of kidney beans and foliar thiamine spray. Soil culture experiment in pots at Ina in 1952.

Varieties	Treatment	Survived green leaves per plant	Difference and its significance	L.S.D. (5%)
Kentucky Wonder	Control	11.50	5.25*	5.02
	B_1 spray	6.25		
Masterpiece	Control	4.10	3.40*	3.23
	B_1 spray	0.70		

Thiamine solution was sprayed on the leaves 10 times in all. The investigation was practised on September 7 (the harvest time). The other remarks are the same as those in Table 22.

on September 7, after 10 foliar thiamine sprayings during a 100 day period. The results shown in Table 23 indicate that the sprayed plot had fewer surviving green leaves as compared with those of the control. Thus the thiamine application seems to bring about an early senescence.

Soil culture experiment in pots in 1952 c. Two cc of 0, 0.01, 1 or 100 ppm thiamine solution per plant were sprayed on Masterpiece leaves 10 times during a 100 day period. The observation made on the vegetative stage (Fig. 14) indicates that the number of the surviving leaves decreased as the concentration of thiamine sprayed on the leaves was increased.

Field experiment in 1953 a. The results are shown in Figs. 15 and 16. The length, diameter, and the fresh weight of the stems, the number and fresh weight of the leaves, the number of flowers, and the total fresh weight of shoots were increased by thiamine spray.

Field experiment in 1953 b. A similar field experiment on Masterpiece undertaken

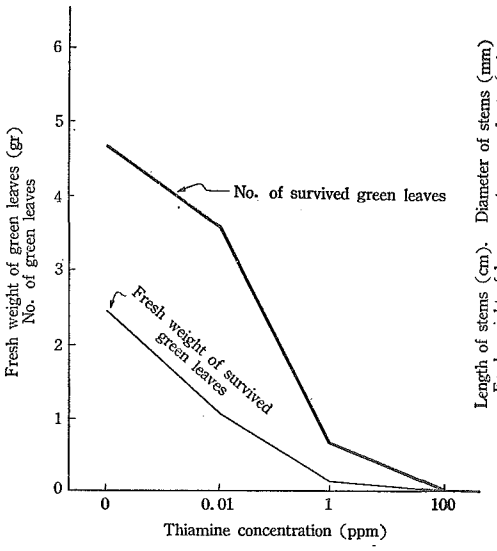

Fig. 14. Relation between the defoliation of kidney beans and the concentration of thiamine solution sprayed on the leaves.

Soil culture experiment in pots at Ina in 1952. The variety used was Masterpiece. The blocks prepared were 8, and each plot had I plant. The seeds were sown on May 10 and the investigation was carried out on August 29. Thiamine solution was sprayed on the leaves 10 times in all.

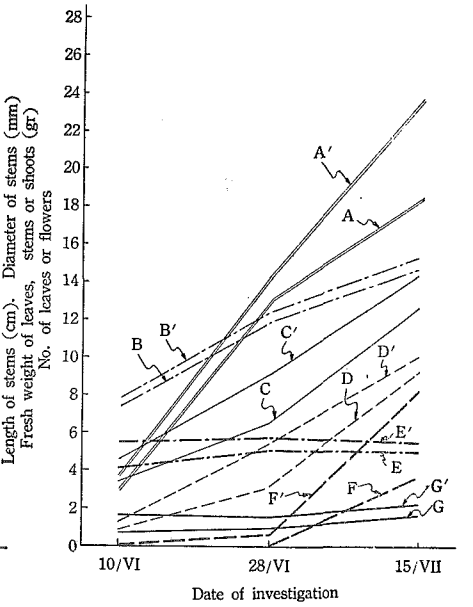

Fig. 15. Relations between foliar thiamine spray and the growth and development (per plant) of stems, leaves and flowers of Masterpiece.

Field experiment at Ina in 1953. A, B, C, D: the control plots, A′, B′, C′, D′: the B_1 sprayed plots. A, A′: no. of leaves. B, B′: length of stems. C, C′: fresh weight of shoots. D, D′: fresh weight of leaves. E, E′: diameter of stems. F, F′: no, of flowers. G, G′: fresh weight of stems.

for yield comparison in the same year had similar results (Tab. 20).

Sand culture experiment in 1954 a. A similar sand culture experiment on Masterpiece in 1954 had again similar results (Tab. 21 and Pl. II–VIII).

Sand culture experiment in 1954 b. The experiment with Masterpiece sown on August 18 consisted of following 4 plots; namely plot 1 sprayed at one week intervals, 5 times in all in the first half of the growing period; plot 2 sprayed at one week intervals, 7 times in all in the latter half of the period; plot 3 sprayed at one week intervals, 10 times during the whole growing period, and the control (non sprayed plot).

Once a week from September 22 to November 4, the flower buds, flowers, and pods of the kidney beans were investigated. The results shown in Fig. 17 indicate that on the flower buds, flowers, and pods, the foliar thiamine spray influenced most strikingly the plot sprayed throughout the whole growing period, next to it the plot sprayed in the first half, and then the plot sprayed in the latter half of the growing period. Moreover thiamine spray advanced the time of blooming and the harvest to a slight

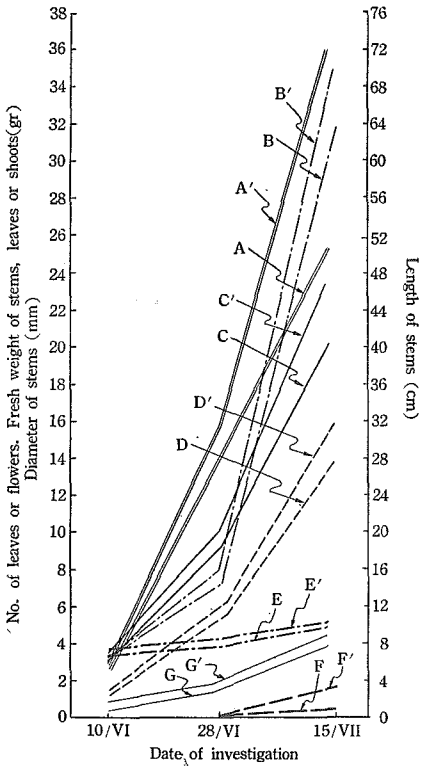

Fig. 16. Effects of foliar thiamine spray on the growth and development (per plant) of stems, leaves and flowers of kidney beans.

Field experiment at Ina in 1953. Variety: Kentucky Wonder. The other remarks are the same as in Fig. 15.

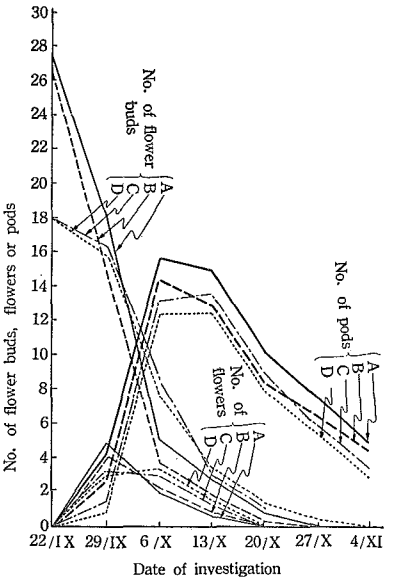

Fig. 17. Relations between the period of foliar thiamine spray and the formation and growth of flower buds, flowers, and pods of kidney beans.

Sand culture experiment at Kyoto in 1954. A: B_1 spray through the whole growing period (10 times). B: B_1 spray in the first half of the period (5 times). C: B_1 spray in the latter half of the period (7 times). D: control. Variety: Masterpiece. The seeds were sown on August 18, and 5 cc of 1 ppm tiamine solution per plant was sprayed on the leaves each time.

extent.

Field experiment in 1954. The results shown in Table 24 indicate that the increase in the number of pods by thiamine spray was significant at the 1 per cent level, and that of the fresh weight of pods, bean grains, stems and roots, and the perfect bean grains was significant at the 5 per cent level.

The effects of foliar thiamine spray on the aerial part of kidney beans are summarized as follows:

Foliar spray of 1 ppm thiamine solution increases the number of leaves, flower buds, and pods, the fresh weight of total pods. Average fresh weight of pods and grains, and the percentage of perfect bean grains are also increased. The treatments replenish the seeds and pods, promote the growth, and shorten the growing period.

Table 24. Effects of foliar thiamine spray on the growth and yield of kidney beans at the harvest time. Field experiment at Ina in 1954.

	Control	B_1 spray	Difference and its significance	L.S.D.	Percentage of difference
Length of stems (cm, per plant)	30.6	30.5	−0.1	1.2(5%)	−0.3
Diameter of stems (mm, per plant)	49.8	52.5	+3.4	5.2(5%)	+6.8
No. of pods (per 0.1 ha)	29370	34350	+4980**	2364(1%)	+16.9
Fresh weight of pods (kg, per 0.1 ha)	94.8	114.4	+19.6*	12.9(5%)	+20.8
Percentage of perfect bean grains	74.4	86.9	+12.5*	10.1(5%)	+16.5
Fresh weight of 1 thousand bean grains (gr)	486.3	511.9	+25.6*	18.8(5%)	+5.3
Fresh weight of roots and stems (kg, per 0.1 ha)	105.1	123.1	+18.0*	14.9(5%)	+17.1

The variety used was Masterpiece. The seeds were sown on May 19, and the grains were harvested on August 27. During this period, 5 cc of 1 ppm thiamine solution per plant was sprayed on the leaves 6 times in all. The number of blocks prepared was 6, and the area of 1 plot was 9.9 m^2

(3) Relation between the concentration of thiamine solution for foliar spray and the growth of kidney beans.

Soil culture experiment in pots in 1952. Two cc of 0.01, 1, or 100 ppm thiamine solution per plant were sprayed 10 times at an interval of 10 days during the period from May 20 to August 29 upon the leaves of Masterpiece sown May 10. The plants were harvested on September 10 and investigated on October 10 after air drying. The number of blocks in this experiment was 8.

The results obtained are represented in Figs. 18, 19 and Plate II–IX. One ppm concentration of thiamine solution was the optimum; it was especially effective on the fresh weight and length of the roots, on the diameter of stems, the air dry weight of stems and pods, the number of pods and on the ramification of stems. But more dilute solution was effective for the stem length.

From the above experiments, it may be inferred that the optimum concentration of thiamine solution for the foliar spray on kidney beans is 1 ppm or thereabouts.

(4) Relation between the time of foliar thiamine spray and the growth of kidney beans.

The details of this experiment were described in the paragraph of the sand culture experiment in 1954. The effect was greatest in the plot sprayed throughout the growing period, next to it, in the plot sprayed in the first half, and lastly in the plot sprayed in the latter half of the growing period (Fig. 17).

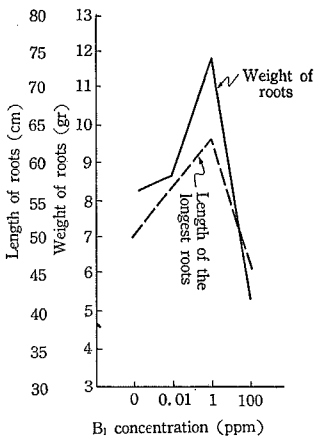

Fig. 18. Relation between the concentration of thiamine solution sprayed on leaves and the growth of kidney bean roots (results per air dry weight of plant).

Soil culture experiment in pots at Ina in 1952. Variety : Masterpiece. The other remarks are the same as in Fig. 14.

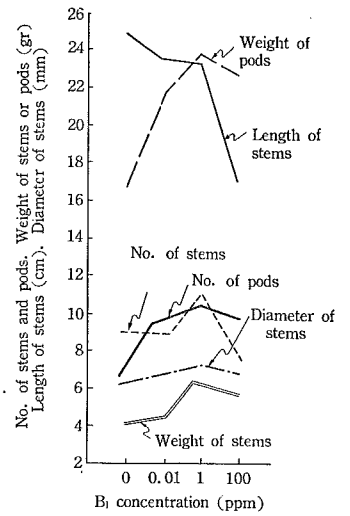

Fig. 19. Relation between the concentration of thiamine solution sprayed on leaves and the growth of stems and pods of kidney beans (results per air dry weight of plant).

Soil culture experiment in pots at Ina in 1952. Variety : Masterpiece. The other remarks are the same as in Fig. 14.

The dry weight of the harvested pods from each plot was compared one with another. The result obtained (Tab. 25) was similar to that mentioned above. In this case, a certain significant difference was recognized between the first two plots and the control, but no significant difference was discerned between the third plot and the control.

Table 25. Relation between the yield (result at the harvest time per plant) of kidney beans and the period of foliar thiamine spray. Sand culture experiment in Kyoto in 1954.

	Dry weight of pods	Index
	gr	
Control (Water)	5.5	100
B_1 spray in the first half of the growing period	8.8	160
B_1 spray in the latter half of the growing period	6.6	120
B_1 spray through the whole growing period	11.5	209
L.S.D. (5%)	3.1	

The remarks are the same as those in Fig. 17.

(5) Relation between the part of the plant sprayed and the growth of kidney bean roots.

The effects of thiamine application to the different parts of the plant were examined in an experiment carried out in 1951, using "Burpee's Stringless Green Pods." The number of blocks was 10. The experiment was started on August 27 and terminated on October 6. During this period 1 cc of 1 ppm thiamine solution per plant was coated 6 times on the different parts of plants.

In four experimental plots thiamine was given to the following parts. Plot 1: both leaf surfaces. Plot 2: upper leaf surface. Plot 3: lower leaf surface. Plot 4: stems. The control plants received no treatment.

The results are shown in Fig. 20. The growth of roots decreased in the order of plots: 1>3>2>4> control.

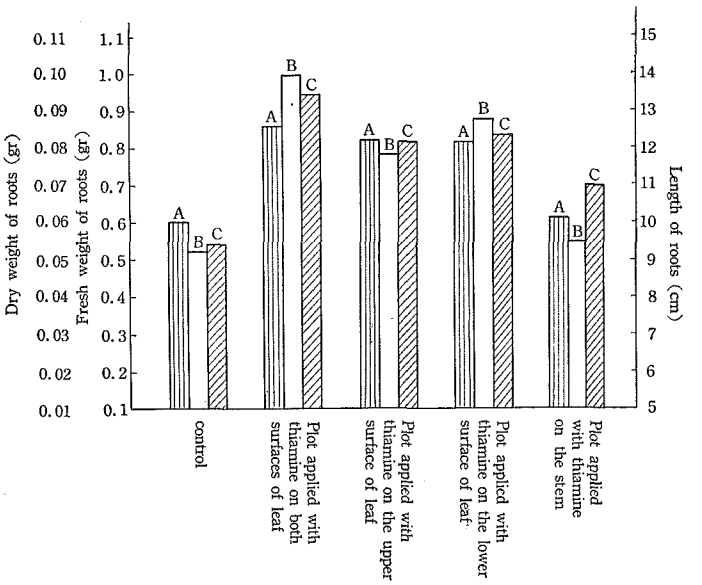

Fig. 20. Relation between the portion of plant applied with thiamine and the growth of kidney bean roots per plant.

Water culture experiment at Ina in 1951. A: fresh weight of roots. B: dry weight of roots. C: length of roots. Variety: Burpee's Stringless Green pod. The seeds were sown on August 27, and the investigation was carried out on October 6. The leaves or stems were coated with 1 cc of 1 ppm thiamine solution per plant 6 times in all.

(6) Relation between the frequency of foliar thiamine spray and the growth of kidney bean roots.

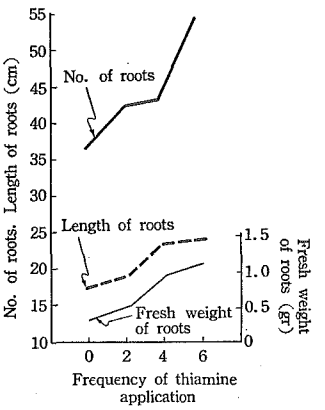

Fig. 21. Relation between the frequcncy of foliar thiamine application and the growth of kidney bean roots per plant.

Water culture experiment at Ina in 1951. Variety: Burpee's Stringless Green Pod. The seeds were sown on May 2 and the investigation was carried out on June 25. The leaves were coated with 2 cc of 1 ppm thiamine solution per plant each time.

The experiment was carried out, using "Burpee's Stlingless Green Pods" as the material. The experiment was started on May 2, and closed on June 25. During the period from May 12 to June 22, 2 cc of 1 ppm thiamine solution per plant was coated on both upper and lower leaf surfaces in the following frequencies: 0, 2, 4 and 6 times.

From the results (Fig. 21) it was recognized that the greater the frequency of thiamine spray the greater the number, length, and the fresh weight of the roots.

3. Considerations

From the experiments above mentioned, it was recognized that the foliar thiamine spray increased the weight of total pods, average weight of pod and grain, the percentage of perfect grains, and thus increased the total yield and improved the quality of kidney beans.

The promoting effect of the root growth by foliar thiamine spray favours the absorption of water and nutrient, or the applied thiamine promotes the growth of stems and leaves, and increases the assimilation area; these may be responsible for the increase of yield and improvement of the quality of crops, especially in the plot sprayed at the first half of the growing period.

But it is not evident in the present experiment, which of the organs, root or shoot, is initially influenced by the thiamine spray to promote the growth of the other and to favour the yield.

It is also not evident whether or not the increase in the flower bud formation is the direct effect of thiamine on the differentiation. An improvement of C–N ratio due to the increase of leaf area, in cooperation with the increased absorption of water and nutrient by enlarged root system, may have some bearings on these problems (cf. CHAP. IV, SECT. I). The growing period is also shortend by thiamine application. In the cold districts this latter may have a benificial effect on the yield of crops.

But the effect may differ according to the concentration, quantity of thiamine applied, the interval of application, the growing period of the plant and other factors. These will be dealt with in the following chapter.

CHAPTER III

FACTORS AFFECTING THE ACTIVITY OF PLANTS SPRAYED WITH THIAMINE ON THE LEAVES

SECTION I*

ENVIRONMENTAL CONDITIONS

The present section deals with the effect of light, temperature, exogenous supply of sugar and fertilizers on the early growth of sweet potatoes sprayed with thiamine.

1. Material and Methods

The varieties of sweet potatoes used for the tests were Nōrin No. 1 nursed at the Kyoto University Farm, and Shijūnichiimo at the Takii Farm, Kyoto. These plants were cultivated in boxes or pots with well washed sand.

The fertilizers, ammonium sulphate, superphosphate of lime and potassium chloride containing each 11.25 kg of N, P_2O_5, K_2O_5 per 0.1 ha were applied in solution, usually at an interval of 5 days. In the long term experiment, 20 cc of Knop solution** was applied per plant at an interval of 15 days.

For the experiment on the surplus effect of fertilizers, the above mentioned fertilizers containing each 22.5 kg of N, P_2O_5, K_2O per 0.1 ha, were applied in dust form. In this case, the moderate or surplus quantity was judged by the good or bad effect on the growth of the plants.

In the tests on the effect of light, large dark boxes or dark glass houses were used for the exclusion of light, however, a little light entered the boxes or rooms during the irrigation, fertilization and foliar spray of thiamine.

For the experiment on the effect of temperature, the dark and the light rooms of 20° and 30°C in the conditioned greenhouse at the Faculty of Agriculture, Kyoto University were used.

Thiamine used was vitamin bydrochloride dissolved in distilled water or survice water. In the spraying procedures a small quantity of solution dropped into the sand might be absorbed by the roots. To the control plants, distilled or service water, of which the pH was adjusted to that of the thiamine solution, was sprayed.

The slips with 7 or 8 fully expanded leaves were used for the tests, choosing those with uniform fresh weights for each experimental plot.

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* The argument of this section was reported at the meeting of the Horticultural Association of Japan in October, 1954.

** Composition : KNO_3 0.5 gr, $CaNO_3$ 2.0 gr, $MgSO_4 \cdot 5H_2O$ 0.5 gr, KH_2PO_4 0.5 gr, $FeCl_3$ 2 drops of 5% solution, H_2O 1*l*.

2. Results

A. Light and thiamine.

(1) *Light in combination with sugar.* Young plants of sweet potato (var. Shijunichiimo) cultivated in sand fertilized moderately were sprayed in various ways during the 20 days after planting.

The results (Tab. 26) indicate that the percentages of increase in the root and in the shoot development, by thiamine spraying, were both greater in the dark than in the light.

Table 26. Relation between light and thiamine for the growth of young sweet potato plants (results per plant 20 days after planting).

Treatment	In the light					In the dark			
	No. of primarily developed roots	No. of secondarily developed roots	Total length of primarily developed roots	Fresh weight of roots	Fresh weight of stems and leaves	No. of primarily developed roots	Total length of roots	Fresh weight of roots	Fresh weight of stems and leaves
			cm	gr	gr		cm	gr	gr
O	32.0	183.6	241.1	2.66	9.49	7.5	14.2	0.04	6.04
S	33.8	164.5	287.7	3.08	7.81	9.4	15.3	0.07	4.46
B_1	50.0	270.0	372.1	4.25	10.63	12.0	26.0	0.14	6.90
SB_1	51.5	380.3	387.3	4.42	10.89	13.1	28.1	0.18	6.88
L.S.D. (5%)	5.0	78.8	67.0	0.82	1.78	3.5	24.1	0.059	0.49

Comparative percentage of increase or decrease to the control.

		S	B_1	SB_1
Fresh weight of roots	In the light	+15.8	+ 60.0	+ 71.3
	In the dark	+71.9	+200.8	+369.3
Fresh weight of stems and leaves	In the light	−17.8	+ 12.0	+ 14.7
	In the dark	−21.1	+ 14.7	+ 14.0

Sand culture experiment at Kyoto in 1954. O: no sucrose, no thiamine. S: 3×20 ppm sucrose per plant (foliar spray). B_1: 3×1 ppm thiamine per plant (foliar spray). The average fresh weights of stems and leaves per plant at the beginning of this experiment were 6.98 gr in the light and 6.97 gr in the dark.

(2) *Light in combination with surplus nitrogenous fertilizer.* Young plants of sweet potatoes (var. Nōrin No. 1) cultivated in sand were investigated 10 days after planting.

The result (Tab. 27 and Pl. II–X) shows again that the effect of thiamine is greater in the dark than in the light.

Table 27. Relations between light, surplus nitrogenous fertilizer and thiamine for the growth of young sweet potato plants (results per plant, 10 days after planting).

		O	OB_1	N	NB_1	L.S.D. (5%)
In the light	Fresh weight of roots (gr)	1.12	1.25	0.36	0.66	0.19
	Fresh weight of stems and leaves (gr)	16.8	17.4	9.6	13.5	3.20
In the dark	Fresh weight of roots (gr)	0.15	0.20	0.07	0.15	0.05
	Fresh weight of stems and leaves (gr)	9.7	13.2	6.5	9.1	1.64

Comparative percentage of increase or decrease to the control.

		N	B_1	NB_1
Fresh weight of roots	In the light	−68.4	+11.7	−41.4
	In the dark	−53.3	+33.4	0
Fresh weight of stems and leaves	In the light	−42.8	+ 3.6	−19.6
	In the dark	−33.2	+36.2	−6.1

Sand culture experiment at Kyoto in 1954. O: no fertilizer, no thiamine. B_1: 2×1 ppm thiamine per plant (foliar spray). N: application of ammonium sulphate 22.5 kg per 0.1 ha in sand. The average fresh weight of stems and leaves per plant at the beginning of this experiment was 11.2 gr.

Table 28. Relations between temperature, light and thiamine for the growth of young sweet potato plants (results per plant 15 days after planting).

	Fresh weight of roots				Fresh weight of stems and leaves			
	Real weight	Difference and its significance	L.S.D.	Percentage of difference	Real weight	Difference and its sighificance	L.S.D	Percentage of difference
	gr	gr	gr	gr	gr	gr	gr	%
30°DO	0.065	+0.053**	0.046	+83	4.2	+1.4**	1.2	+33
30°DB_1	0.118		(1%)		5.6		(1%)	
20°DO	0.182	+0.087**	0.031	+47	5.1	+1.2**	1.1	+24
20°DB_1	0.269		(1%)		6.3		(5%)	
30°LO	2.050	+0.588	0.640	+29	11.7	+0.5	0.7	+4
30°LB_1	2.638		(5%)		12.2		(5%)	
20°LO	1.763	+0.717	0.680	+40	9.1	+1.1*	0.9	+12
20°LB_1	2.480		(5%)		10.2		(5%)	

Sand culture experiment at Kyoto in 1954. L: in the light. D: in the dark. O: no thiamine. B_1 : 3×1 ppm thiamine per plant (foliar spray). The average fresh weight of stems and leaves per plant at the beginning of this experiment was 7.6 gr.

(3) *Light in combination with various temperatures.* Young plants of sweet

potatoes cultivated in sand in the air-conditioned green house were investigated 15 days after planting.

The results obtained (Table 28 and Pl. II–XI) show that in both of the temperature, 20° and 30°C, the effect of thiamine was greater in the dark than in the light.

B. Temperature and thiamine.

The experiments (Tab. 28 and Pl. II–XI) showed following results:

(1) *In the dark*, the thiamine spray was more effective at 30° than at 20° C: for the increase of root fresh weight 83 (30°): 47 (20°) and for that of leaves and stems 33:24.

(2) *In the light*, the effect is converted; the thiamine spray increased the fresh weight of both root and shoot at 20° more than at 30°C.

In the light, the growth is more favorable at high temperature than at low temperature and in the dark the low temperature is more favorable than high temperature. Therefore it seems that thiamine is more effective under temperatures unfavorable for the growth of plants.

C. Sugar and thiamine.

(1) *Sugar in combination with light.* For the growth of roots the combined application of thiamine and sugar was more effective than the application of thiamine or sugar alone (Tab. 26, Pl. II–XII and Pl. II–XIII). The growth of the shoots was retarded by the spray of sugar alone, and the combining effect whith thiamine was distinct.

(2) *Sugar in combination with surplus nitrogenous fertilizer.* This experiment was carried out in a similar way as described in Table 27 and Pl. II–X. But thiamine and sugar were sprayed on the leaves, and the fertilizer was applied in sand. Two cc of 5 ppm glucose, and 2 cc of 1 ppm thiamine were applied on each plant 2 times during a 10 day period.

The results shown in Table 29 and Pl. III–XIV indicate that the application of sugar and fertilizer in combination with thiamine was more favorable for the growth of plants than the single application of these substances. The application of thiamine with sugar was especially effective for the reduction of injury caused by the application of surplus nitrogenous fertilizer.

(3) *Sugar in combination with α-naphthalene acetic acid.* The effect of thiamine and that of α-naphthalene acetic acid (NAA) on the reduction of injury caused by the application of surplus sucrose were compared one with the other.

After soaking in each solution described in Table 30 for 72 hours, the young plants of sweet potato (var. Nōrin No. 1) were cultivated in boxes with sand.

The growth of the plants soaked in 2 per cent sucrose solution alone was very reduced, and this was remedied to some extent in the sucrose solution combined with 1 ppm thiamine, but not in the solution combined with 0.1 ppm NAA. A single application of thiamine or NAA, especially of the latter, promoted the early growth of sweet potato plant, probably because of the good root development.

Table 29. Relations between sugar, surplus nitrogenous fertilizer and the thiamine spray for the growth of young sweet potato plants (results 10 days after planting).

	No glucose		Glucose spray	
	Fresh weight of roots	Fresh weight of stems and leaves	Fresh weight of roots	Fresh weight of stems and leaves
	gr	gr	gr	gr
O	1.12	16.8	1.14	14.3
OB_1	1.25	17.4	1.29	15.2
N	0.36	9.9	0.81	12.9
NB_1	0.66	13.5	1.19	15.5
L.S.D (5%)	0.19	3.2	0.24	2.4

Comparative percentage of increase or decrease to the control plot with no glucose and no nitrogenous fertilizer.

	N	G	B_1	NG	GB_1	NB_1	NB_1G
Fresh weight of roots	−68.4	+1.8	+11.7	−27.7	+15.3	−41.4	+6.3
Fresh weight of stems and leaves	−42.8	−14.1	+3.6	−23.2	−9.5	−19.6	−8.9

Sand culture experiment at Kyoto in 1954. O: no thiamine and no glucose. B_1: 1 ppm thiamine spray. N: application of ammonium sulphate (as N 22.5 kg per 0.1 ha) in sand. G: 5 ppm glucose foliar spray. The average fresh weight of stems and leaves per plant at the biginning of this experiment was 11.2 gr.

Table 30. Relations between surplus sugar, α-naphthalene acetic acid and thiamine for the growth of young sweet potato plants (result per plant 7 days after planting).

	No. of primarily developed roots	Total length of roots	Fresh weight of roots	Fresh weight of stems and leaves
		cm	gr	gr
O	19.8	72.7	0.21	6.51
B_1	24.6	100.8	0.36	6.99
NAA	29.6	113.9	0.41	7.07
S	3.6	12.8	0.06	5.11
SB_1	14.6	48.3	0.30	6.64
SNAA	3.0	15.6	0.14	6.13
SB_1NAA	16.2	48.1	0.34	6.42
$SB_{100}NAA$	5.0	18.9	0.18	6.27
L.S.D. (5%)	10.01	29.88	0.22	1.33

Sand culture experiment at Kyoto in 1954. O: control. B_1: 1 ppm, and B_{100}: 100 ppm thiamine application. NAA: 0.1 ppm α-naphthalene acetic acid application. S: 2 per cent sucrose application. The average fresh weight of stems and leaves per plant at the beginning of this experiment was 6.41 gr.

(4) *Sugar in combination with fertilizer elements.* Moderate quantities of fertilizers were applied. Two cc each of 1 ppm thiamine and 5 ppm suclose solution was sprayed 4 times during a 49 day period. The result shown in Table 31 indicates that the effects of sucrose combined with thiamine were plus upon the growth of roots with nitrogen or potassium, but minus upon that of shoots with the same elements.

Table 31. Relations between sugar, fertilizer elements applied moderately and thiamine for the growth of young sweet potato plants (results per plant 49 days after planting).

	OB_1	NB_1	PB_1	KB_1	OSB_1	NSB_1	PSB_1	KSB_1	L.S.D.(5%)
Number of tubers	2.5	3.7	5.2	4.3	3.2	4.5	4.3	5.5	0.81
Fresh weight of tubers (gr)	6.7	9.7	14.0	13.0	9.1	12.0	11.9	15.4	2.37
Fresh weight of stems and leaves (gr)	21.1	28.8	26.1	27.6	20.7	25.4	20.7	21.4	4.58

Comparative percentage of increase or decrease to the plot sprayed with thiamine solution alone (no fertilizers).

		NB_1	PB_1	KB_1
Fresh weight of roots	No sucrose	−44.8	+108.1	+ 94.0
	Sucrose spray	+79.0	+ 77.6	+129.8
	Difference	+34.2	− 30.5	+ 35.8
Fresh weight of stems and leaves	No sucrose	+36.4	+ 23.7	+ 30.7
	Sucrose spray	+20.4	− 1.9	+ 1.4
	Difference	−16.0	− 25.6	− 29.4

Sand culture experiment at Kyoto in 1954. O: no fertilizer. N: ammonium sulphate application. P: superphosphate of lime application. K: potassium chloride application. These fertilizers (as N, P_2O_5 and K_2O each 3.75 kg per 0.1 ha) were applied three times in sand as solution. B_1: 1 ppm thiamine foliar spray. S: 5 ppm sucrose foliar spray. The average fresh weight of stems and leaves per plant at the beginning of this experiment was 8.6 gr.

(5) *Kind of sugar combined with thiamine.* Sucrose, levelose or glucose of 20 ppm concentration and thiamine of 1 ppm concentration were used. The results shown in Table 32 and Plate III–XV indicate that for the growth of young sweet potato plants (var. Nōrin No. 1), the application of all kinds of sugar combined with thiamine was more effective than the sugar application alone, but no significant difference was discerned among the three kinds of sugar with thiamine.

(6) *Concentration of sugar combined with thiamine.* Effect of 0～20 ppm sucrose sprays on the growth of young sweet potato plants (var. Nōrin No. 1) was investigated. The sucrose solutions mixed with 1 ppm thiamine were sprayed 4 times during a 40 day period.

The results shown in Fig. 22 indicate that the fresh weight of stems and leaves, and the length of stems decreased according to the increasing concentrations of sugar,

Table 32. Kind of sugar and thiamine for the growth of young sweet potato plants (results per plant 49 days after planting).

	No. of tubers	Fresh weight of tubers	Total fresh weight of roots	Diameter of stems	Length of stems	Fresh weight of stems and leaves
		gr	gr	mm	cm	gr
O	4.9	14.1	18.9	40.0	56.3	19.8
B_1	6.1	18.6	24.4	44.9	57.1	23.6
S	5.8	13.7	20.8	42.0	45.4	18.6
SB_1	6.1	19.0	24.8	46.7	55.2	21.1
L	5.2	14.5	22.3	41.1	41.1	15.6
LB_1	6.0	19.3	26.0	25.6	48.5	20.0
G	5.4	15.5	22.2	42.2	45.8	18.7
GB_1	6.5	20.2	27.9	46.0	54.2	21.4
L.S.D.(5%)	1.06	2.46	3.78	4.00	6.09	1.05

Sand culture experiment at Kyoto in 1954. O: no thiamine, no sugar. B_1: 1 ppm thiamine foliar spray. S: 20 ppm sucrose foliar spray. L: 20 ppm levulose foliar spray. G: 20 ppm glucose foliar spray. The average fresh weight of stems and leaves per plant at the beginning of this experiment was 8.6 gr.

whereas the number and the fresh weight of tubers increased up to the 10 ppm solution and then decreased, perhaps because of the decrease of assimilation area by the spraying of high concentration sugar.

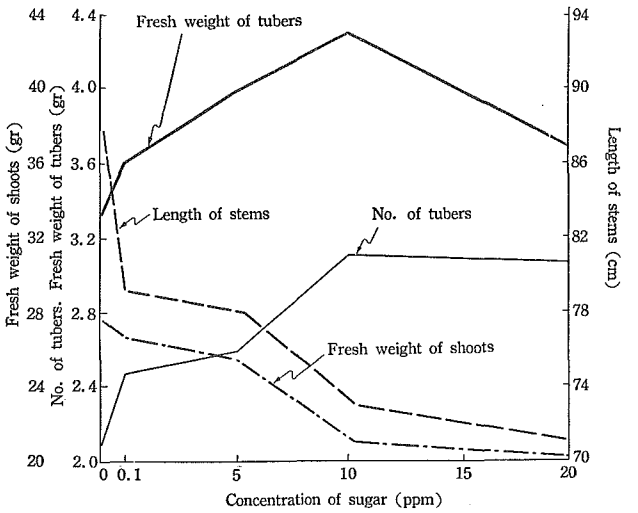

Fig. 22. Effect of sugar concentration combined with thiamine on the growth of sweet potatoes. Results per plant 40 days after planting.

The spraying of sugar with thiamine is therefore effective for the root development and tuber differentiation, but for the growth in thickness of tubers, it is not always

useful without further consideration upon the growth of stems and leaves (Pl. III–XVI).

From all the above-mentioned facts, the relation between sugar and the effect of thiamine on plants, was recognized as follows.

Spraying of sugar combined with thiamine promotes the root development and tuber differentiation, but for the growth in thickness of tubers, it is not effective excepting the sugar treatment at a low concentration. The growth of shoots is retarded by the spraying of sugar alone but this retarding influence is diminshed by combining with thiamine.

D. Fertilizer elements and thiamine.

(1) *Fertilizer elements in moderate quantity in combination with thiamine.* Young plants of sweet potato (var. Nōrin No. 1) cultivated on sand were fertilized moderately and sprayed with 2 cc of 1 ppm thiamine on each plant 3 times during a 15 day period.

The results shown in Table 33 and Plate III–XXI indicate that the ratio of the growth increase by the application of phosphate fertilizer in combination with thiamine was the greatest.

Table 33. Relation between fertilizer elements applied moderately and thiamine for the growth of young sweet potato plants (results per plant 15 days after planting).

	O	OB_1	N	NB_1	P	PB_1	K	KB_1	L.S.D.(5%)
Fresh weight of roots (gr)	3.7	4.5	4.9	5.6	4.1	6.1	5.5	5.6	0.54
Fresh weight of stems and leaves (gr)	14.0	15.0	17.8	18.5	16.7	18.1	17.0	17.4	0.86

Comparative percentage of increase or decrease to the control(O).

	OB_1	N	NB_1	P	PB_1	K	KB_1
Fresh weight of roots	+21.6	+32.4	+51.3	+10.8	+64.9	+48.7	+51.3
Fresh weight of stems and leaves	+ 7.1	+27.1	+32.1	+19.6	+29.2	+21.4	+24.3

Sand culture experiment at Kyoto in 1954. O: no fertilizer, no thiamine. N: ammonium sulphate application. P: super phosphate of lime application. K: potassium chloride application. These fertilizers (as N, P_2O_5, K_2O each 3.75 kg per 0.1 ha) were applied 3 times in sand as solution. B_1: 1 ppm thiamine foliar spray. The average fresh weight of stems and leaves per plant at the beginning of this experiment was 9.8 gr.

(2) *Fertilizer elements in moderate quantity in combination with thiamine and sugar.* The results (Tab. 31) indicate that the number and the fresh weight of tubers in the plot applied with phosphate fertilizer were largest (Pl. III–XXI), but the fresh

weight of shoots was largest in the plot applied with nitrogenous fertilizer.

(3) *Fertilizer elements in surplus quantity in combination with thiamine.* Ammonium sulphate, superphosphate of lime or potassium chloride, each 22.5 kg of N, P_2O_5, K_2O per 0.1 ha, were applied in sand, and 2 cc of 1 ppm thiamine was sprayed 2 times during a 10 day period.

The results shown in Table 34 and Plate III–XVII indicate that the application of surplus ammonium sulphate had the worst influence upon the growth of young sweet potato plants, but the spraying of thiamine was most effective at this plot.

Table 34. Relation between fertilizer elements applied surplusly and thiamine for the growth of young sweet potato plants (results per plant 10 days after planting).

	O	OB_1	N	NB_1	P	PB_1	K	KB_1	L.S.D.(5%)
Fresh weight of roots (gr)	1.12	1.25	0.36	0.61	0.81	1.11	0.86	1.04	0.24
Fresh weight of stems and leaves (gr)	16.8	17.4	9.6	13.5	16.4	17.4	17.0	17.1	3.48

Comparative percentage of increase or decrease to the control.

	OB_1	N	NB_1	P	PB_1	K	KB_1
Fresh weight of roots	+11.6	−67.8	−40.5	−27.6	−0.9	−23.2	−7.1
Fresh weight of stems and leaves	+ 4.0	−47.5	−21.8	− 2.6	+4.0	+ 1.3	+2.0

Sand culture experiment at Kyoto in 1954. O: no fertilizer, no thiamine. N: ammonium sulphate application. P: superphosphate of lime application. K: potassium chloride application. The fertilizers (as N, P_2O_5, K_2O each 22.5kg per 0.1 ha) were applied all together in sand as dust. B_1: 1 ppm thiamine foliar spray. The average fresh weight of stems and leaves per plant at the beginning of this experiment was 9.6 gr.

Therefore, when fertilizers were applied in moderate quantity in the soil, the thiamine spray was most effective in combination with phosphatic fertilizer, but when fertilizers were applied in surplus quantity it was most effective in combination with the fertilizer of worst influence.

3. Considerations

From the results obtained above, it seems to be proved that thiamine is highly effective for the plants grown under unfavorable conditions, such as darkness (especially accompanied with high temperature), surplus sugars, surplus fertilizers, etc. The effect of the spraying of thiamine combined with sugar is greater for the growth than the sum of the effects of each constituent, and by the thiamine application the growth ratio of plants having moderate phosphate supply was highly increased in comparison with that

supplied with other elements, viz. nitrogenous or potash fertilizer. The application of thiamine combined with sugar was most effective for the reduction of the injury caused by surplus nitrogenous fertilizer.

These facts suggest that generally in abnormal conditions and also in the germination or in the starting period, the thiamine production or its supply is inclined to be insufficient and the plant growth must reluctantly run after the deficiencies. By the application of thiamine, the thiamine content is increased, and the metabolism, not only of carbohydrate but also of other substances, will be carried out smoothly and excellently. Hence it seems that thiamine is effective for the exclusion of physiological obstacles.

SECTION II*

AGRICULTURAL CHEMICALS

The influence of various agricultural chemicals on the effect of thiamine was studied for the practical purposes of the thiamine spray.

1. Materials and Methods

Sweet potato (var. Nōrin No. 1), Kidney bean (var. Masterpiece) were used for the experiments with spreader, and potato (var. Danshaku) for the experiment with Bordeaux mixture. The plants were grown in pots with soil fertilized with 5 gr each of ammonium sulphate, superphosphate of lime and potasium chloride per pot.

In the experiments with spreader, 0.02 per cent "Gramin", a spreader produced by Sankyō Pharmaceutical Co. and thiamine hydrochloride solution of 1 ppm were used. During the experimental period, spraying of thiamine, with and without "Gramin", was performed 6 times at one week intervals and the yields were compared.

In the experiment with Bordeaux mixture, plot 1 was sprayed by Bordeaux mixture, and then on the next day by thiamine spray. The composition of the Bordeaux mixture used was 6 gr–6 gr–1000 cc, and the final thiamine concentration was 1 ppm. An equal quantity of "Gramin" was added to every suspension or solution.

Experiments on the thiamine stability in relation to the chemicals mixed were also performed. The decrease in the quantity of thiamine with the time was determined after 500γ% solution of thiamine was mixed with the solution, suspensions or emulsions of various agricultural chemicals.

2. Results and Considerations

(1) Effect of spreader.

The number and the fresh weight of the sweet potato larger tubers were increased and those of the smaller ones were decreased by the application of thiamine with the spreader as compared with the control (Tab. 35).

* The argument of this section was reported at the meeting of the Horticultural Association of Japan in April, 1957.

Table 35. Effects of foliar thiamine spray mixed with "Gramin", a spreader, on the yield and quality of sweet potatoes (results per plant).

		Spray of thiamine alone	Spray of thiamine mixed with "Gramin"	Difference and its significance	L.S.D. (5%)	Percentage of difference
No. of tubers	Larger tubers	1.15	2.00	+0.45*	0.41	+25.8
	Smaller tubers	1.33	1.00	−0.33	0.49	−24.8
	Total	2.88	3.00	+0.12	0.28	+ 4.2
Fresh weight of tubers		gr	gr	gr	gr	
	Larger tubers	1409	1843	+434*	382	+30.0
	Smaller tubers	523	399	−124	98	−23.6
	Total	1932	2242	+310	344	+16.0

Experiment in pots with soil at Ina in 1956. The variety used was Nōrin No. 1. The number of blocks was 8, each having 2 plots and 1 plant per plot. The weight of every "larger tuber" was above 60 gr. Thiamine: 1 ppm solution. Spreader: 0.2cc/*l* "Gramin." Thiamine and Gramin were sprayed fully on the leaves 6 times.

Result of a similar experiment with kidney beans (Tab. 36) shows that the fully ripened pods of kidney beans were increased, while the unripened pods were decreased by the addition of spreader to the thiamine solution.

Table 36. Effects of foliar thiamine spray mixed with "Gramin" on the yield and quality of kidney beans (results per air dried plant).

		Spray of thiamine alone	Spray of thiamine mixed with "Gramin"	Difference and its significance	L.S.D. (5%)	Percentage of difference
No. of pods	Ripe pods	3.3	3.6	+0.3	0.62	+ 9.1
	Unripe pods	0.9	0.7	−0.2*	0.18	−22.2
	Total	4.2	4.3	+0.1	0.12	+ 2.4
Weight of pods		gr	gr	gr	gr	
	Ripe pods	6.4	7.1	+0.7	0.91	+ 9.4
	Unripe pods	0.3	0.1	−0.2*	0.18	−66.7
	Total	6.7	7.2	+0.5	0.78	+ 7.4

The variety used was Masterpiece. The other remarks are the same as those in Table 35.

It seems that thiamine spray mixed with "Gramin" is more effective than the thiamine spray alone.

(2) Effect of Bordeaux mixture.

The results shown in Table 37 and Plate III–XVIII indicate that the effect of a separate application of Bordeaux mixture and thiamine is significantly great.

(3) Stability of thiamine in mixture with various agricultural chemicals.

The results shown in Table 38 and Fig. 23 indicate that the thiamine mixtured

Table 37. Effects of foliar thiamine spray mixed with Bordeaux mixture on the yield and quality of potatoes (results per plant).

		Spray of Bordeaux mixture alone	Spray of thiamine mixed with Bordeaux mixture	Separate sprays of thiamine and Bordeaux mixture	L.S.D. (5%)
No. of tubers	Larger tubers	2.3	4.0	4.6	1.4
	Smaller tubers	3.3	2.8	2.5	0.9
	Total	6.0	6.8	7.1	1.4
		gr	gr	gr	gr
Fresh weight of tubers	Larger tubers	146.3	192.8	220.8	42.6
	Smaller tubers	40.7	28.3	29.3	9.3
	Total	187.0	221.1	250.1	43.4

The variety used was Danshaku. The weight of each larger tuber was above 40 gr. The Bordeaux mixture used was the 6gr-6gr-1000cc one, and the Gramin was mixed into each solution. The other treatments were the same as those in Table 35.

with chemicals of acidic nature such as "Gramin," "Improved Rino" (a spreader) or nicotine sulphate was relatively stable, but in a mixture with alkaline chemicals such as Bordeaux mixture or sulfur lime was unstable.

These facts coincide with the results obtained in the above-mentioned experiments on the relation between thiamine spray mixed with Gramin or Bordeaux mixture and the yield of crops.

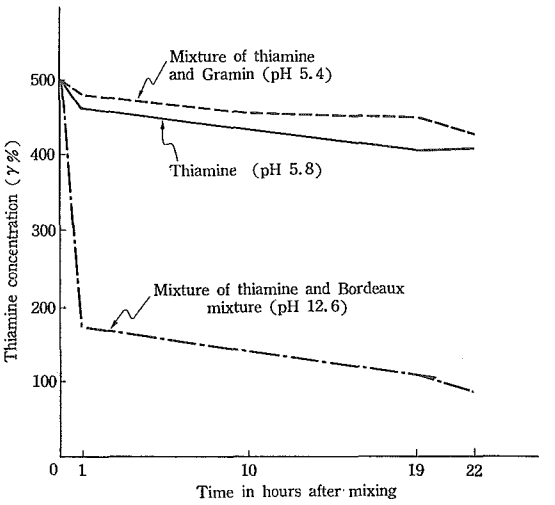

Fig. 23. Thiamine stability when mixed with "Gramin" or Bordeaux mixture.

The concentration of Gramin used was 0.22 cc/*l* and that of copper sulphate or quicklime in Bordeaux mixture was each 6.25 gr/*l*. This experiment was carried out at 30°C.

Table 38. Thiamine stability when mixed with various agricultural chemicals.

Kinds of chemicals	Survived rates of thiamine (500γ% at the starting time)				Average pH	Concentrations of chemicals per *l*	Makers of chemicals
	2 hours after mixing	Index*	45 hours after mixing	Index*			
	γ%		γ%				
Distilled water	479.5	100	382.5	80	5.8	0	
Improved Rino spreader[1]**	480.1	100	421.0	88	5.6	0.4cc	Nihon Nōyaku.
Liquid fish oil soap	90.0	19	58.5	12	8.2	3.0cc	Tomono Nōyaku.
Calcium caseinate	195.0	41	127.5	27	9.2	1.4gr	Tomono Nōyaku.
Nicotine sulphate Ishiguro 40	478.2	100	433.0	90	5.4	1.1cc	Ishigro Seiyaku.
Sankyo DN emulsion[2]	240.0	50	228.2	48	5.8	0.7cc	Sankyō Seiyaku
Derris emulsion	307.5	64	258.2	54	5.8	2.0cc	Ishihara Seiyaku.
DDT emulsion 20	230.0	48	140.5	29	6.0	1.7cc	King Jochūkiku Kōgyo.
BHC wettable power 5	216.0	45	82.5	17	7.4	5.4cc	Sankyō Seiyaku.
DDT wettable powder 20	142.5	30	75.0	16	7.2	3.0gr	Sankyō Seiyaku.
Lead arsenate	397.5	83	367.5	77	5.6	3.0gr	Nihon Nōyaku.
Soid wettable sulfur[3]	82.5	17	60.0	13	7.0	3.3gr	Sankyō Seiyaku.
Dithene wettable powder[4]	477.5	100	400.0	83	5.8	2.2gr	Sankyō Seiyaku.
Dilute liquid lime-sulfur	95.0	20	58.0	12	11.2	10.0gr	King Jochūkiku Kogyō.
Sankyō Bordeaux[5]	247.5	52	137.5	29	6.8	2.6gr	Sankyō Seiyaku.
Urea	465.0	97	397.5	83	5.8	5.0gr	Tōyō Kōatsu.
Yōgen[6]	452.0	94	420.0	88	5.6	5.0gr	Tōyō Kōatsu.

* The concentration of thiamine in distilled water 2 hours after mixing was indicated as 100.

** Chief ingredients I) Ethyl ester of coconut oil fatty acid, coconut oil monoglyceride and coconut oil fatty acid. 2) 2, 4-Dinirto-6-cyclohexylphenol. 3) Colloid sulfur. 4) Zinc ethylen bis-dithiocarbamate. 5) Basic copper sulfate and phenyl mercuric acetate. 6) Urea.

Generally speaking, the application of thiamine mixed with alkaline chemicals is undesirable; and only seperate applications will assure hopeful results But the thiamine was relatively stable in a mixture with fertilizers for foliar spray such as urea or "Yōgen."*

CHAPTER IV

EFFECTS OF THE FOLIAR APPLICATION OF THIAMINE ON THE PHYSIOLOGICAL ACTION OF PLANTS

SECTION I**
CHEMICAL COMPOSITION

1. Materials and Methods

(1) General chemical composition.

The tubers of sweet potatoes and potatoes, and the grains of kidney beans harvested in the field experiments at Ina in 1955 were analyzed.

As to the methods of culture and treatment, they were already described in the preceding chapter (CHAP. II, Tabs. 13, 17 and 21). The methods of analysis are as follows:

Crude protein: total nitrogen was determined by the Semimicro-Kjeldahl method, the obtained value was multiplied by 6.25 and this was regarded as crude protein; crude fat: by the Soxhlet extraction; crude fiber: by the A.O.A.C method; caloric value: calculated by the RUBNER coefficient. Moisture and ash were determined by the usual methods.

(2) Carbohydrate.

The tubers of sweet potatoes and potatoes, and the grains of kidney beans harvested in the soil and sand culture experiments in pots in 1955 (CHAP. II, Tabs. 14 and 18) were analyzed. The analysis was practised by the Bertrand method. The total carbohydrate is shown as the sum of total sugar and starch after being converted into glucose.

(3) C–N ratio.

The C–N ratio of potato tubers and bean grains.

The C–N ratio of potato, sweet potato tubers, and of kidney bean grains was calculated from the results on the general chemical composition described above.

The C–N ratio of stems, leaves and roots.

* Chief ingredient: Urea.

** The argument of this section was reported at the meeting of the Horticultural Association of Japan in April, 1956.

The plants of sweet potatoes and kidney beans described in the above-mentioned (2) were dried immediatly after harvest and analyzed in December, 1955. The determination of nitrogen was made by the Semimicro-Kjeldahl method, and that of carbohydrate by the Bertand method.

(4) Thiamine.

Sweet potato tubers (var. Nōrin No. 1, Nōrin No. 4, Kantō No. 31 and Taihaku) cultivated and harvested in the field experiment in 1953 already described (CHAP. II, SECT. I, Tab. 11), potato tubers (var. Danshaku) harvested in the field experiment in 1952 (CHAP. II, SECT. II, Tab. 15), and kidney bean grains (var. Masterpiece) harvested in the soil culture experiment in pots in 1952 (CHAP. II, SECT. III, Fig. 14) were analyzed. The determination of thiamine was made according to the method already described (CHAP. I).

2. Results

(1) Effects of foliar thiamine spray on the general composition of bean grains and tubers.

Foliar thiamine spray seems to be effective, though only slightly, in increasing the content of nitrogen free extract and calorific value (Tab. 39), and the C-N ratio in tubers and beans (Tab. 40).

Table 39. Relations between foliar thiamine spray and the general chemical composition of bean grains and sweet potato and potato tubers. (Wet basis.)

		H_2O	Crude protein	Crude fat	Nitrogen free extract	Crude fiber	Ash	Calorie /100gr
		%	%	%	%	%	%	cal
Sweet potato tubers	Control	71.21	1.34	0.20	25.05	1.03	1.17	110
	B_1 spray	68.73	1.31	0.20	27.77	0.95	1.04	121
Potato tubers	Control	79.02	2.01	0.12	17.41	0.62	0.82	81
	B_1 spray	78.36	1.90	0.12	18.11	0.64	0.87	83
Kidney bean grains	Control	14.87	20.97	6.10	49.12	3.31	5.63	344
	B_1 spray	13.73	20.71	6.04	50.90	3.20	5.42	350

The materials harvested in the field experiment at Ina in 1955 (see Tables 13, 17 and 21) were analyzed.

Table 40. Relations between foliar thiamine spray and the C-N ratio of tubers and bean grains. (Wet basis.)

	Sweet potato tubers	Potato tubers	Kidney bean grains
Control	116.8	54.1	14.6
B_1 spray	132.4	59.6	15.4

The C-N ratio in this table was calculated from the results of experiment in Table 39.

(2) Effects of foliar thiamine spray on the composition of carbohydrate in tubers and bean grains.

The results shown in Tables 41~43 show that foliar thiamine spray is effective, though only slightly, in increasing the contents of sugar and starch in tubers and beans.

Table 41. Relation between foliar thiamine spray and the composition of carbohydrate in sweet potato tubers. (Wet basis.)

		Reducing sugar	Non-reducing sugar	Total sugar	Starch	Total carbohydrate
		%	%	%	%	%
Soil culture	Control	0.94	3.26	4.20	19.22	25.56
	B_1 spray	0.96	4.84	5.80	20.72	28.82
	L.S.D. (5%)	0.08	1.21	1.48	1.76	3.18
Sand culture	Control	1.15	4.38	5.53	19.22	26.89
	B_1 spray	1.27	4.39	5.66	21.74	29.82
	L.S.D. (5%)	0.11	0.08	0.17	2.22	2.62

The same materials as shown in Table 14 were analyzed.

Table 42. Relation between foliar thiamine spray and the composition of carbohydrate in potato tubers. (Wet basis.)

		Reducing sugar	Non-reducing sugar	Total sugar	Starch	Total carbohydrate
		%	%	%	%	%
Soil culture	Control	0.22	0.45	0.67	15.43	17.81
	B_1 spray	0.30	0.62	0.92	16.80	19.59
	L.S.D. (5%)	0.07	0.14	0.21	1.48	1.69
Sand culture	Control	0.12	0.79	0.91	14.51	17.03
	B_1 spray	0.14	0.97	1.11	15.89	18.78
	L.S.D. (5%)	0.04	0.15	0.17	1.32	1.48

The remarks are the same as those in Tables 18 and 41.

Table 43. Relation between foliar thiamine spray and the composition of carbohydrate in kidney bean grains. (Wet basis.)

		Reducing sugar	Non-reducing sugar	Total sugar	Starch	Total carbohydrate
		%	%	%	%	%
Soil culture	Control	1.79	2.43	4.22	38.48	46.98
	B_1 spray	1.88	2.98	4.86	40.22	49.49
	L.S.D. (5%)	0.13	0.42	0.54	2.11	2.84
Sand culture	Control	1.76	2.78	4.54	37.71	46.44
	B_1 spray	2.12	3.22	5.34	39.48	49.21
	L.S.D. (5%)	0.31	0.36	0.68	2.12	2.98

The materials harvested after practising the same cultivation and treatment as those described in Tables 14 and 18 were analyzed.

(3) Effects of foliar thiamine spray on the carbohydrate, and nitrogen contents, as well as the C–N ratioes of stems, leaves and roots.

The results (Tab. 44) show that foliar thiamine spray is effective in increasing the content of carbohydrtae but not in increasing the content of nitrogen; thus the spray increases the C–N ratio of plants.

Table 44. Relations between foliar thiamine spray and the carbohydrate, nitrogen contents as well as the C–N ratio of stems, leaves and roots. (Dry basis at the harvest time.)

		Sweet potatoes			Kidney beans		
		Stems	Leaves	Roots	Stems	Leaves	Roots
		%	%	%	%	%	%
Carbo-hydrate	Control	32.98	22.82	29.15	33.42	18.86	28.40
	B_1 spray	36.20	24.09	29.96	35.68	22.12	29.01
	L.S.D. (5%)	2.21	1.03	1.08	2.04	1.22	0.98
Nitro-gen	Control	2.28	4.21	2.04	2.16	4.12	2.24
	B_1 spray	2.40	4.18	1.92	2.26	3.98	2.20
	L.S.D. (5%)	0.17	0.13	0.21	0.14	0.31	0.06
C/N	Control	14.46	5.42	14.29	15.47	4.58	12.70
	B_1 spray	15.08	5.76	15.60	15.78	5.56	13.18
	L.S.D. (5%)	0.56	0.29	0.80	0.54	0.62	0.36

The stems, leaves and small roots of plants used as the materials for the experiment in Tables 41 and 43 were analyzed.

(4) Effects of foliar thiamine spray on the thiamine content of tubers and bean grains.

a. *Sweet potatoes.* The results shown in Table 45 show that foliar thiamine spray is effective, though only slightly, in increasing the thiamine content of tubers.

Table 45. Relation between foliar thiamine spray and the thiamine contents of sweet potato tubers. (Wet basis.)

Varieties	Nōrin No. 1	Nōrin No. 4	Kantō No. 24	Kantō No. 31	Taihaku	Average	L.S.D.
	γ%	γ%	γ%	γ%	γ%	γ%	γ%
Control	125.6	129.2	122.4	129.2	95.2	120.3	20.46 (5%)
B_1 spray	149.2	132.8	163.2	142.8	136.0	144.8	33.33 (1%)

The materials harvested in the field experiment at Ina in 1953 (Table II) were analyzed.

b. *Potatoes.* Similare results are shown in Table 46.

c. *Kidney beans.* The total, ester and free form B_1 in the grains harvested in the experiment shown in Fig. 14 were determined. The results shown in Fig. 24 show that foliar thiamine spray is effective in increasing the free form thiamine content of the bean grains.

Table 46. Relations between foliar thiamine spray and the thiamine contents of potato tubers. (Wet basis.)

	Larger tubers	L.S.D. (5%)	Smaller tubers	L.S.D. (5%)
	γ%	γ%	γ%	γ%
Control	114.4	13.9	105.6	15.3
B_1 spray	128.6		122.6	

The materials harvested in the field experiment at Ina in 1952 (Table 15) were analyzed. The average fresh weight of the larger tubers was 140 gr and that of the smaller tubers was 30 gr.

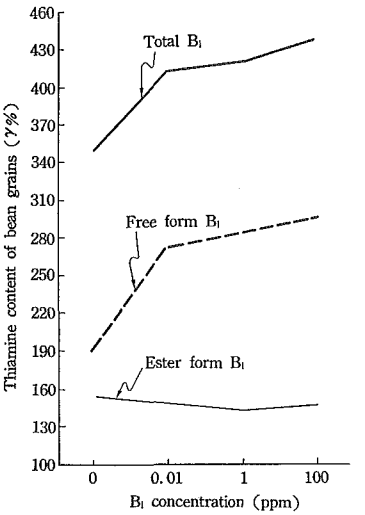

Fig. 24. Relations between foliar thiamine spray and the thiamine contents of kidney bean grains as well as the forms of thiamine in them. (Thiamine contents per fresh matter.)
The bean grains of Masterpiece, harvested in the soil culture experiment in pots (Fig. 14) were analyzed on October 28 in 1952.

3. Considerations

Firstly, it was recognized that foliar thiamine spray modified, though only slightly, the composition of carbohydrate; the sugar and starch contents in tubers and bean grains increased. This seems to have certain influence on the taste of crops or tubers (CHAP. II).

Secondly, the foliar spray was effective in increasing the C–N ratio in plants. It is already reported that C–N ratio at a high level promotes the root initiation (HICKS 1928),[57] tuber formation (KAMIBAYASHI 1938, KOBAYASHI 1945)[89,96] and flower-bud differentiation (YOSHII 1951).[175] Thus the thiamine is to be concerned, as co-

carboxylase in carbohydrate metabolism, to the fullest for the causes of these or other related phenomena.

SECTION II*

CHANGE OF THIAMINE IN PLANT BODY

1. Materials and Methods

Uniformly grown slips of sweet potato taken from the nursery on the previous day to the experiment and put in city water, were tested. In the case of roots and young tubers of sweet potato, these were carefully dug out from the field and put in city water, and on the next day they were tested; kidney beans were tested after being cultivated in water.

The method of thiamine application will be later described in the paragraph of results. The control plants were supplied with distilled water, the pH value of which was adjusted with hydrochloride acid to be equal to the thiamine solution. The method of thiamine determination is described in CHAP. I.

2. Results

(1) Effect of foliar thiamine spray on the thiamine content of sweet potato plants.

a. *Thiamine content in stems and leaves at various parts of growing plants.* Slips of Nōrin No. 1 (average weight 12.5 gr) with 9 expanded leaves were put in water, and three expanded leaves (B_5~ B_7)** were coated with 1 cc of 10 ppm thiamine solution with a squirt on both upper and lower surfaces of leaves. Twenty-four hours after that treatment, the total B_1 was determined (Tab. 47.)

Table 47. Effects of foliar thiamine application on the thiamine contents of stems and leaves at various parts of sweet potato plants (γ% unit, wet basis).

		Control	B_1 application	Difference and its significance	L.S.D.
Stems	Upper part (U)	22.7	34.7	12.0*	8.3 (5%)
	Middle part (M)	17.5	26.4	8.9**	3.8 (1%)
	Lower part (L)	20.7	34.8	14.1**	6.1 (1%)
Leaves	B_2~T	86.1	111.4	25.3**	24.7 (1%)
	B_3, B_4	106.6	131.4	24.8**	14.9 (1%)
	B_8, B_9	160.8	195.4	34.6*	29.0 (5%)

Experiment at Ina in July, 1952. T, A_1, A_2, B_1~B_9 see Fig. 25. Leaves B_5~B_7 at the middle part of stems were coated with 1 cc of 10 ppm thiamine solution per plant and after 24 hours each part was analyzed.

* The argument of this section was reported at the meeting of the Horticultural Association of Japan in April, 1953.

** The youngest expanded leaf of the sweet potato is marked with B_1 and the oldest unexpanded leaf with A_1; the marks of expanded leaves go basipetally as B_2, B_3, B_4, ----, and those of unexpanded leaves acropetally as A_1, A_2, ---- and T (shoot apex) (see Fig. 25).

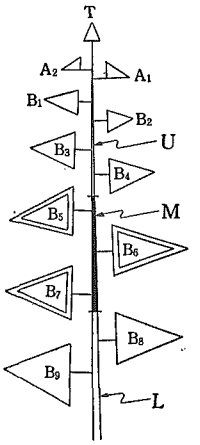

Fig. 25. Situation of leaves coated with thiamine solution and the part of sweet potato plant taken as the material for analysis in the experiment of Table 47.
B_1~B_9: number of expanded leaves from the top.

The thiamine content in the stems increased remakably in the growing point and its vicinity (U) ; next to it, in the lower part (L) which is the part for water absorption or root initiation ; the same tendency was observed in the young leaves growing vigorously.

b. *Thiamine content in the part of root initiation.* The slips (average weight 20.5 gr) of sweet potato (var. Nōrin No. 1, Nōrin No. 4, Kantō No. 24, Kantō No. 31 and Taihaku) with 9 expanded leaves were treated as in experiment **a**, and after 24 hours total B_1 in the basal part of the stems was determined (Tab. 48).

The results indicate that in all the varieties used, the thiamine content of the lower part of the stem from which the roots initiate was increased by thiamine application.

c. *Thiamine content of small roots.* One month after the planting of 5 varieties of sweet potato, each plant was coated with 5 cc of 10 ppm

Table 48. Effect of foliar thiamine application on the thiamine contents of the root producing part of sweet potato stems (γ% unit, wet basis).

Varieties	Nōrin No. 1	Nōrin No. 4	Kantō No. 24	Kantō No. 31	Taihaku	Average	Difference and its significance	L.S.D. (5%)
Control	19.0	20.2	17.0	21.3	28.5	21.2	8.4*	5.7
B_1 application	32.5	28.2	24.2	28.3	35.1	29.6		

Experiment at Ina in July, 1952. Leaves were coated with 1 cc of 10 ppm thiamine solution per plant and after 24 hours the stem parts (each 2 cm) at the nodes B_7~B_9 were analyzed.

thiamine solution, and 24 hours later, the total B_1 in the small roots was determined (Tab. 49). The content in the small roots of sweet potato was increased by thiamine application.

d. *Thiamine content of young tubers.* Young tubers from the materials described in the above experiment **c**, were used. The fresh weight of each tuber was less than 15 gr. The results shown in Table 50 indicate that the thiamine content of young tubers was increased within 24 hours by foliar thiamine spray.

Judging from the results mentioned above, the thiamine absorbed by the leaves is transferred to all the plant organs in a short time, and accumulated in the parts of vigorously metabolic activity.

Table 49. Effect of foliar thiamine application on the thiamine contents of sweet potato roots (γ% unit, wet basis).

Varieties	Nōrin No. 1	Nōrin No. 4	Kantō No. 24	Kantō No. 31	Taihaku	Average	Difference and its significance	L. S. D.
Control	68.0	76.5	34.0	68.0	57.0	60.7	19.4**	18.6(1%)
B_1-application	88.2	90.6	68.5	85.0	68.4	80.1		

Experiment at Ina in July, 1952. Two cc of 1 ppm thiamine solution per plant was sprayed on the leaves. Twenty-four hours after that treatment, the small roots were analyzed.

Table 50. Effect of thiamine application on the thiamine contents of young sweet potato tubers (γ% unit, wet basis).

Varieties	Nōrin No. 1	Nōrin No. 4	Kantō No. 24	Kantō No. 31	Taihaku	Average	Difference and its significance	L. S. D.
Control	27.2	40.4	29.8	30.6	32.5	32.0	11.0*	9.6 (5%)
B_1-application	44.2	57.8	38.3	34.0	40.8	43.0		

Experiment at Ina in July, 1952. Twenty-four hours after thiamine spray, the tubers obtained from the experiment described in Table 49 were analyzed.

(2) Form of thiamine absorbed through the leaf surface and its transformation.

Slips (average weight 23.9 gr) of sweet potato (var. Nōrin No. 1) with 9 expanded leaves were coated on the leaves with 1 cc of 10 ppm thiamine solution per plant with a squirt. Twenty-four hours and again at 76 hours after treatment, free or ester form thiamine in the lower part of the stems between the nodes B_7~B_9 was determined (Tab. 51): **i)** Thiamine increasing in the lower part of the stems 24 hours after the application is mostly that of free form. **ii)** Thiamine increasing after 76 hours is that of both free form and ester form. The ratio of ester form is thus increased with the time elapsing after the application.

Table 51. Relation between the transformation of thiamine in the lower part of sweet potato stems and the time elapsed (γ% unit, wet besis).

Time elapsed	Control		B_1 application		L. S. D.	
	24hr	76hr	24hr	76hr	5%	1%
Free form B_1	16.7	13.9	27.1	20.6	4.2	6.5
Ester form B_1	5.5	5.6	6.2	10.6	1.6	2.3
Total B_1	22.2	19.5	33.3	31.2	3.8	5.5

Experiment at Ina in July, 1952. Leaves were coated with 1 cc of 10 ppm thiamine sloution per plant. After 24 hours the stems at the nodes B_7~B_9 were analyzed.

(3) Parts of leaf and the rate of thiamine absorption.

Slips (average weight 26.5 gr) of sweet potato (var. Nōrin No. 1) with 9 expanded leaves were put in water, and on the next day coated with 1 cc of 10 ppm thiamine solution per plant. Twenty-four hours after treatment, the total B_1 in the lower part of the stems at the nodes $B_7 \sim B_9$, as well as in the petioles at the nodes $B_3 \sim B_9$, was determined.

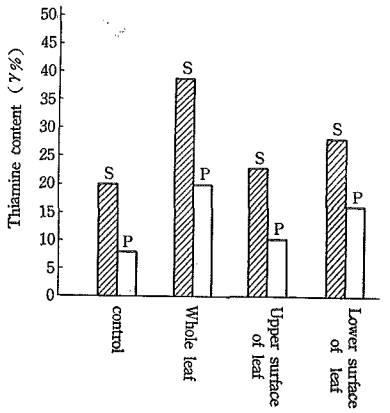

Fig. 26. Relation between the part of leaf applied with thiamine and thiamine contents of stems and petioles (results per fresh matter).
Experiment at Ina in August, 1952. S: stems. P: petioles. The leaves were coated with 2cc of 10 ppm thiamine per plant and after 24 hours the thiamine contents were determined.

These results (Fig. 26) indicate that the rate of thiamine absorption by the lower surface of the leaf is greater than that by the upper surface of leaf.

(4) Thiamine absorption by kidney bean leaf.

Masterpiece variety cultivated in water was tested. During a 10 day period in the early growing period of the plant, 1 cc of 10 ppm thiamine solution per plant was coated 5 times on both of the leaf surfaces.

The results (Table 52) indicate that the thiamine absorbed is accumulated mostly at the growing point of the stem and its vicinity, or at the part of root development, that is, at the parts of vigorous activity of plants.

Table 52. Relations between foliar thiamine application and the thiamine contents of kidney bean stems (γ% unit, wet basis).

		Control	B_1 application	Difference and its significance	L.S.D.
Stems	Upper part	57.7	118.9	61.2**	48.3 (1%)
	Middle part	31.3	65.1	33.8*	28.5 (5%)
	Lower part	42.1	99.1	57.0**	49.2 (1%)

Experiment at Ina in June, 1952. Leaves were coated with 1 cc of 10 ppm thiamine solution per plant 5 times in a 10 day period, and thereafter the stems were analyzed.

4. Considerations

From the experiments of present and foregoing sections, it was recognized that the thiamine absorbed by the leaves was transferred to all the organs of plants in a short time, and accumulated in the reserve organs or in the parts of high metabolic activity,

as this has already been reported by many researchers. [22, 42, 63, 67, 134, 140]

Thiamine reserved in the dormant seeds (Tab. 5) is mainly of free form, and with the increase in metabolic activity in tissues and organs the percentage of ester form, i. e. the active form, of thiamine is also increased. This would indicate the natural course of thiamine produced by the leaves.

SECTION III

WATER ABSORPTION, TRANSPIRATION AND OSMOTIC PRESSURE

1. Material and Methods

Young plants of sweet potato (var. Nōrin No. 1) cultivated in boxes with sand were used. Twenty potometer were applied for determining the rate of water absorption and a balance for determining the rate of transpiration. Osmotic pressure was determined by the cryoscopic method. The determination of the rate of water absorption and transpiration was carried out in a glass house. Other details will be described in the paragraph of results.

2. Results and Consideration

(1) Relation between thiamine spray and the water absorption of young sweet potato plants.

The first experiment. Slips weighing on the average 12.2 gr were planted on August 13, 1954 and cultivated in sand with KASUGAI's water culture solution for dry field crops* in decuple concentration.

After planting thiamine solution was sprayed 4 times, and the water absorption of these plants was measured on August 27, 1954, the day after the last thiamine spraying.

On the morning of experimental day, the plants were dug out and the roots were washed in water. Each plant was then put into potometer, and one hour before measuring the rate of water absorption, the total weight was measured for determining the rate of transpiration. The equipment was arranged in a glass house and the absorbed water was measured every hour during an 8 hour period from 9.31 a. m. to 5.30 p. m..

At the end of the experiment, the total weight of the potometer with water and plant, the fresh weight of young plants and that of the leaves were measured.

The results (Tab. 53 and Fig. 27) indicate that the rate of water absorption of plants sprayed with thiamine was increased per plant (at the 1 per cent level) or per gr of fresh plant and fresh leaf (at the 5 per cent level).

The second experiment. The young plants of sweet potato were tested on August 28, 1954 by the same method as described in the first experiment. The results shown in Table 54 and Fig. 28 indicate that by the moderate foliar thiamine spray, the rate of water absorption was increased at the 5 per cent level of significant difference per plant and per gr of fresh leaf.

* See page 203.

Table 53. Relation between foliar thiamine spray and the water absorption of young sweet potato plants. (Experiment 1.)

	Average fresh weight per plant	Average fresh weight of leaf per plant	Water absorbed per plant			Water absorbed per gr fresh plant			Water absorbed per gr fresh leaf		
			Quantity	Difference and its significance	L.S.D. (1%)	Quantity	Difference and its significance	L.S.D. (5%)	Quantity	Difference and its significance	L.S.D. (5%)
	gr	gr	cc	cc	cc	cc	cc	cc	cc	cc	cc
Control	18.5	5.7	37.64			2.03			6.60		
				12.99**	11.20		0.41*	0.36		1.45*	1.39
B_1 spray	20.5	6.2	49.98			2.44			8.05		

Data show the total quantities of absorbed water for 8 hours. The number of blocks for the test was 6, having 1 plant in each plot. Measurment 15 days after planting. B_1 spray: 4 times 5 cc 1 ppm thiamine solution per plant on the leaves.

Table 54. Relation between thiamine spray and the water absorption of young sweet potato plants. (Experiment 2.)

	Average fresh weight per plant	Average fresh weight of leaf per plant	Water absorbed per plant			Water absorbed per gr fresh plant			Water absorbed per gr fresh leaf		
			Quantity	Difference and its significance	L.S.D. (5%)	Quantity	Difference and its significance	L.S.D. (5%)	Quantity	Difference and its significance	L.S.D. (5%)
	gr	gr	cc	cc	cc	cc	cc	cc	cc	cc	cc
Control	21.4	6.7	37.49			1.75			5.59		
				5.10*	4.82		0.20	0.24		0.67*	0.48
B_1 spray	21.8	6.8	42.59			1.95			6.26		

Measurment 16 days after planting. Other remarks are the same as those in Table 53.

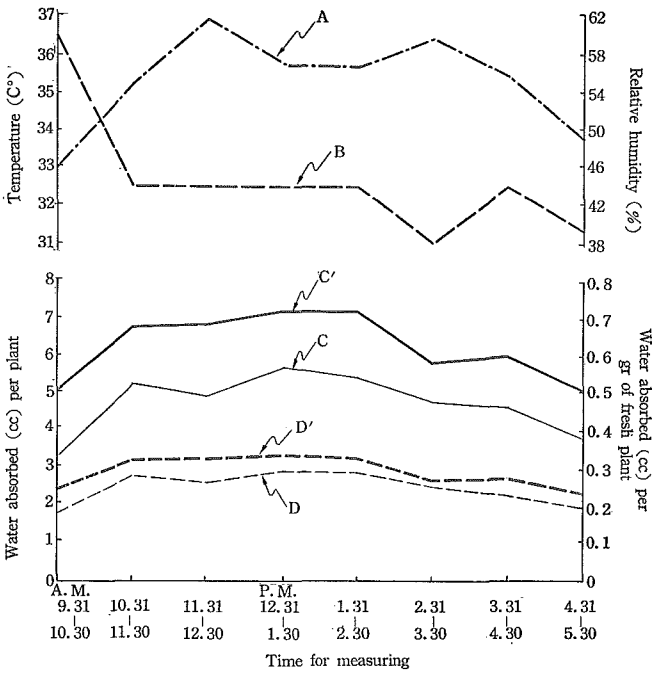

Fig. 27. Relation between thiamine spray and the water absorption of young sweet potato plant. (Experiment 1.)

A: temperature. B: relative humidity. C, C′: water absorbed per plant. D, D′: water absorbed per gr. of fresh plant. C, D: the control plots. C′, D′: the sprayed plots.

The third experiment. Young plants were tested on September 1, 1954. During 20 days after planting, 5cc of 1 ppm thiamine solution per plant was sprayed on the leaves 5 times ; after the last spraying the plants with the roots were dug out and immersed in water in the laboratory. The water absorption was measured on the 3rd day after immersion (the 4th day after the last spraying).

The results shown in Table 55 and Fig. 29 show that by the moderate foliar thiamine spray, the rate of water absorption in plants was increased at 1 per cent level of significant difference per plant and at 5 per cent level per gr of fresh plant or fresh leaf.

From these results, it is evident that the rate of water absorption in plants is increased by the moderate foliar thiamine spray and the effect is comparatively durable.

(2) Relation between thiamine spray and the transpiration of yong sweet potato plants.

The first experiment. The same materials used for the first experiment mentioned above on water absorption were used for this experiment, and the rate of transpiration during a 10 hour period was determined (Tab. 56). By the moderate foliar thiamine

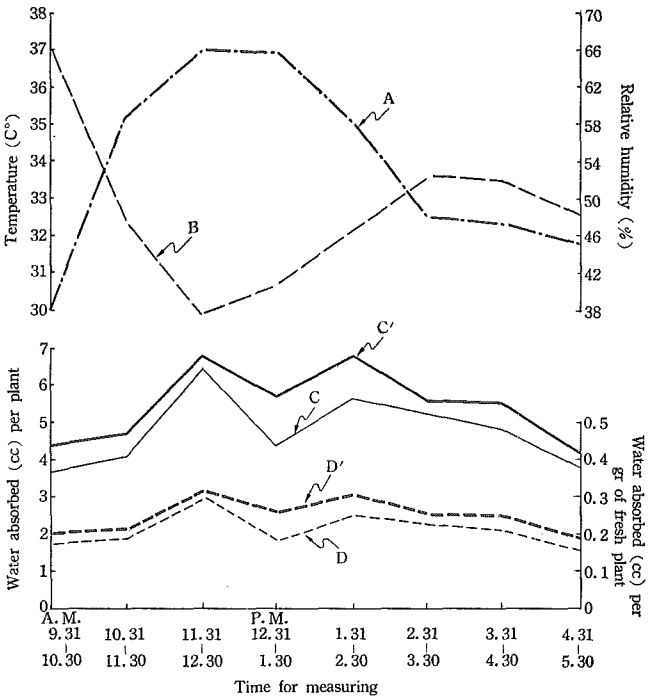

Fig. 28. Relation between thiamine spray and the water absorption of young sweet potato plants. (Experiment 2.)
The marks are the same as in Fig. 27 and the other explanation is the same as in Table 54.

spray, the transpiration of plants was promoted at the 5 per cent level of significant difference per plant and per gr of fresh leaf.

The second experiment. The same materials used for the second experiment on water absorption were used for this experiment, and the rate of transpiration during a 10 hour period was determined. The result obtained were similar to those of the first experiment (Tab. 57).

From these facts, it is evident that the transpiration of plants is promoted by foliar thiamine spray of a moderate concentration.

As will be described later (CHAP. V), the respiration of plants was promoted by thiamine application. This may have some relation to promoting the water absorption and transpiration of plants.

(3) Relation between thiamine spray and the osmotic pressure of sweet potato roots.

The osmotic pressure of small sweet potato roots was determined cryoscopically. The young plants for the test were planted on June 2, 1954. 5 cc of 1 ppm thiamine solution was sprayed on each plant 4 times during the period from June 2 to June 30,

Table 55. Relation between foliar thiamine spray and the water absorption of young sweet potato plants. (Experiment 3.)

	Average fresh weight per plant	Average fresh weight of leaf per plant	Water absorbed per plant			Water absorbed per gr fresh plant			Water absorbed per gr fresh leaf		
			Quantity	Difference and its significance	L.S.D. (5%)	Quantity	Difference and its significance	L.S.D. (5%)	Quantity	Difference and its significance	L.S.D. (5%)
	gr	gr	cc	cc	cc	cc	cc	cc	cc	cc	cc
Control	19.7	5.8	8.25			0.42			1.42		
				7.06*	4.89		0.20*	0.17		0.62*	0.46
B_1 spray	24.6	7.5	15.31			0.62			2.04		

The data show the total quantities of absorbed water for 7 hours. Measurment 20 days after planting. B_1 spray: 5 times 5 cc 1 ppm thiamine solution per plant on the leaves. Other remarks are the same as those described in Table 53.

Table 56. Relation between foliar thiamine spray and the transpiration of young sweet potato plants. (Experiment 1.)

	Average fresh weight per plant	Average fresh weight of leaf per plant	Transpiration per plant			Transpiration per gr fresh plant			Transpiration per gr fresh leaf		
			Quantity	Difference and its significance	L.S.D. (5%)	Quantity	Difference and its significance	L.S.D. (5%)	Quantity	Difference and its significance	L.S.D. (5%)
	gr	gr	cc	cc	cc	cc	cc	cc	cc	cc	cc
Control	18.5	5.7	43.57			2.36			7.64		
				9.96*	9.16		0.25	0.31		0.99	0.68
B_1 spray	20.5	6.2	53.53			2.61			8.63		

The data show the total quantities of transpiration for 10 hours. The other remarks are the same as those in Table 53.

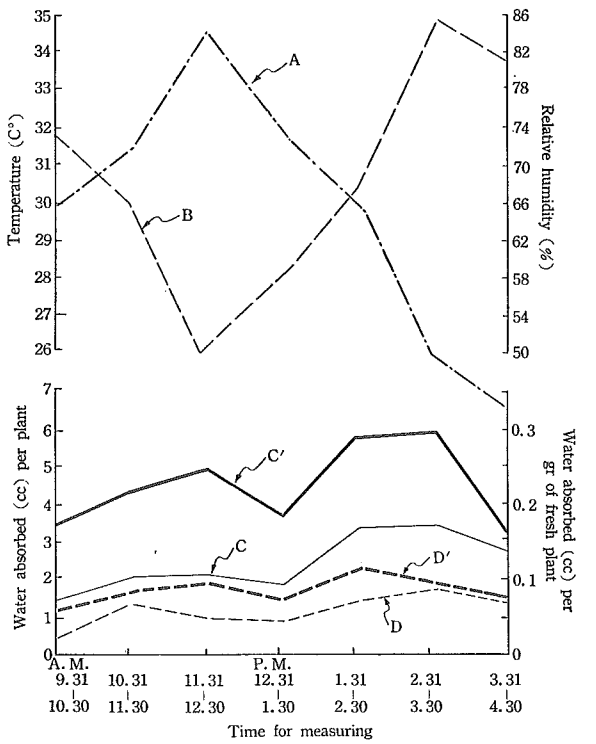

Fig. 29. Relation between thiamine spray and the water absorption of young sweet potato plants. (Experiment 3.)

The remarks are the same as in Table 55 and Fig. 27.

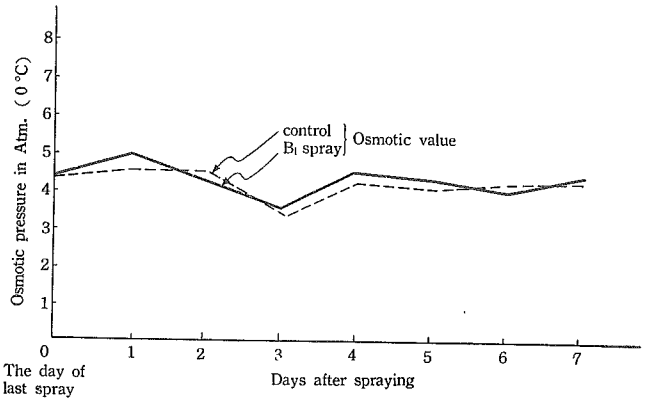

Fig. 30. Relation between foliar thiamine spray and the osmotic pressure of sweet potato roots.

The determination of osmotic value was started after the foliar spray with 5 cc 1 ppm thiamine solution per plant 4 times in all.

Table 57. Relation between foliar thiamine spray and the transpiration of young sweet potato plants. (Experiment 2.)

	Average fresh weight per plant	Average fresh weight of leaf per plant	Transpiration per plant			Transpiration per gr fresh plant			Transpiration per gr fresh leaf		
			Quantity	Difference and its significance	L.S.D. (5%)	Quantity	Difference and its significance	L.S.D. (5%)	Quantity	Difference and its significance	L.S.D. (5%)
	gr	gr	cc	cc	cc	cc	cc	cc	cc	cc	cc
Control	21.4	6.7	38.65			1.81			5.77		
				4.85	4.78*		0.19	0.38		0.63*	0.42
B_1 spray	21.8	6.8	43.50			2.00			6.40		

The data show the total quantities of transpiration for 10 hours. The other remarks are the same as those in Table 54.

June 30 being the last day of the spraying. From July 1 till July 7, every day at 10 a. m., the osmotic value was measured.

The results shown in Fig. 30 show that thiamine spray is not significantly effective on the osmotic pressure of the roots.

SECTION IV*
PHOTOSYNTHESIS

1. Materials and Methods

(1) Measurment of CO_2-absorption of leaves under natural conditions.

The experiments were carried out in the glass house of Kyoto University. The temperature was 12～14°C and the intensity of natural illumination was 3000～4000 luxes in the glass house.

All the plants for the test were cultivated in pots with sand, and their leaves were used under natural conditions. The CO_2 absorbed was obtained by determining the difference between the quantities of CO_2 in the air that were flowed through cellophane chambers with and without the leaf (TAKANO1955).(155)

In the case of sweet potatoes (var. Nōrin No. 1), a full quantity of 1 ppm thiamine solution was sprayed on the leaves 4 times at 5 day intervals, and the sixth expanded young leaf from the top (B_6**) was used for the examination. In the case of kidney beans, after 3 times 1 ppm thiamine foliar spray at 5 day intervals in full quantity, the lowest simple leaves just above the cotyledons in each young plant were used.

(2) Measurement by manometry.

The experiments were carried out by the WARBURG manometry (ISAWA 1955).(81) One cc each of 1) SÖRENSEN's mixed buffer solution of citrate and hydrochloric acid, 2) WARBURG's bicarbonate and carbonate mixture as the source of CO_2, and 3) thiamine solutions were put in the main chamber of manometer, and by adjusting with HCl, the final pH value of the solution was kept at 8.6. The last concentration of thiamine applied were 0, 1, 100 and 1000 ppm.

Eight 1 cm^2 punched pieces of leaf were floated on the solution, keeping their lower surfaces in touch with the surface of the solution. Measurement was carried out at 30°+0.1°C. The O_2-uptake during 30 minutes was determined in the dark, and the apparent evolved O_2 during 30 minutes was determined in the light illuminated by a 200-watt incandescent electric bulb. After repeating the measurments, the rate of photosynthesis was determined.

Materials for the test were the leaves of sweet potato (var. Nōrin No. 1), potato (var. Danshaku), kidney beans (var. Masterpiece), and cabbage (var. Nagaokakōhai).

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* The argument of this section was reported at the meeting of the Horticultural Association of Japan in April, 1956.

** See page 245.

2. Results and Considerations

(1) Experiment under natural conditions.

The results shown in Tables 58 and 59 indicate that 1 ppm thiamine foliar spray had significant promoting effects on the photosynthesis of sweet potato leaves per unit area at the 5 per cent level and on that of kidney bean leaves at the 1 per cent level.

Table 58. Relation between foliar thiamine spray and the assimilation of sweet potato leaves under natural conditions. (mg CO_2/gr·cal light/cm²/min × 100 basis.)

	I	II	III	IV	V	VI	Average	L.S.D.
Control	3.11	3.94	4.95	3.60	4.15	3.90	3.94	0.853 (5%)
B_1 spray*	5.58	4.61	5.01	4.68	4.81	5.16	4.98	1.343 (1%)

Experiment at Kyoto in November, 1954. * One ppm thiamine solution was sprayed fully on the leaves of materials 4 times at intervals of 5 days after planting. After this treatment, the "B_0"-leaves in each plant were tested.

Table 59. Relation between foliar thiamine spray and the assimilation of kidney bean leaves under natural conditions. (mg CO^2/gr·cal light/cm²/min × 100 basis.)

	I	II	III	IV	V	VI	Average	L.S.D.
Control	1.55	1.83	1.53	1.65	1.33	1.62	1.59	0.33 (5%)
B_1 spray*	2.42	2.65	1.98	1.98	2.21	1.82	2.18	0.51 (1%)

Experiment at Kyoto in November, 1954.* One ppm thiamine solution was sprayed fully on the leaves of materials 4 times at intervals of 5 days after the germination. After this treatment, the simple leaves just above the cotyledons tested.

(2) Experiment by manometry (Figs. 31~34).

One ppm and 100 ppm thiamine application generally promoted the assimilation, while 1000 ppm application exerted retarding effects on the sweet potatoes and kidney beans.

From these results it may safely be said that one of the causes of moderate foliar thiamine sprays increasing the yield and improving the quality of crops, lies in the promotion of the assimilation.

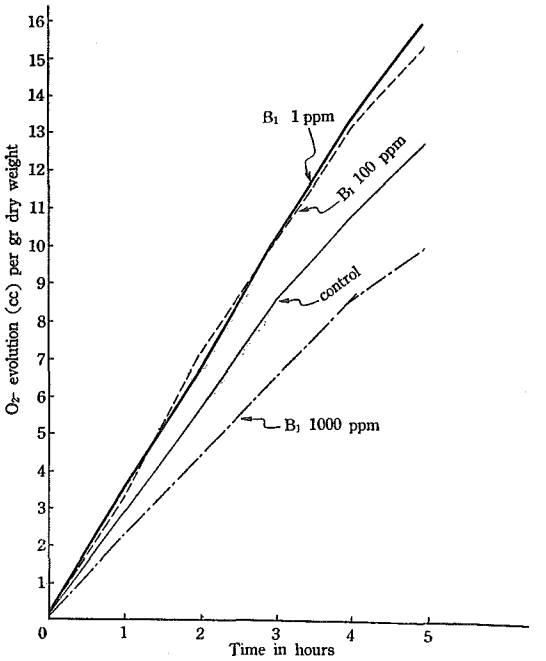

Fig. 31. Relation between thiamine application and the assimilation of sweet potato leaves determined by manometry. (Cumulative curves.)

Experiment at Ina in June, 1955. As the material of this experiment, the B_5 or B_6 leaves of young plants having 6.2 gr average fresh weight and 6 expanded leaves were used.

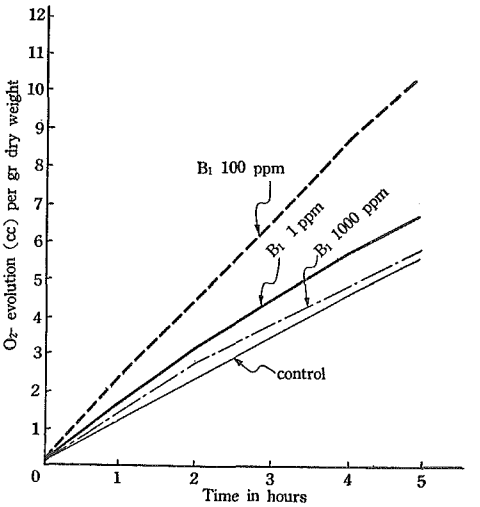

Fig. 32. Relation between thiamine application and the assimilation of potato leaves determined by manometry. (Cumulative curves.)

Experiment at Ina in June, 1955. As the material of this experiment, the largest leaf in each young plant 10 days after germination was used.

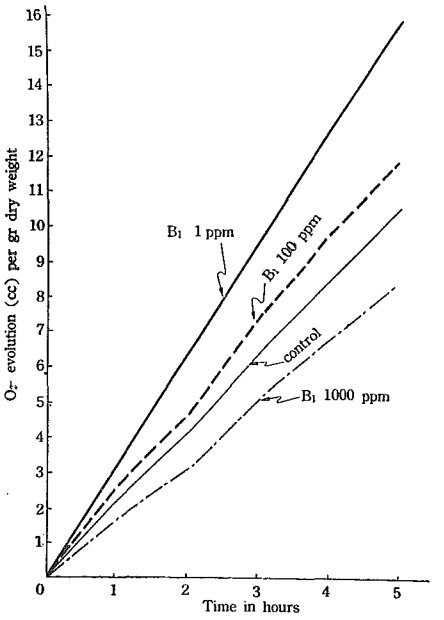

Fig. 33. Relation between thiamine application and the assimilation of kidney bean leaves determined by manometry. (Cumulative curves.)

Experiment at Ina in March, 1955. As the material of this experiment, the simple leaves in each seedling having attained 2 expanded leaves in a glass-covered thermostat at 20°C were used.

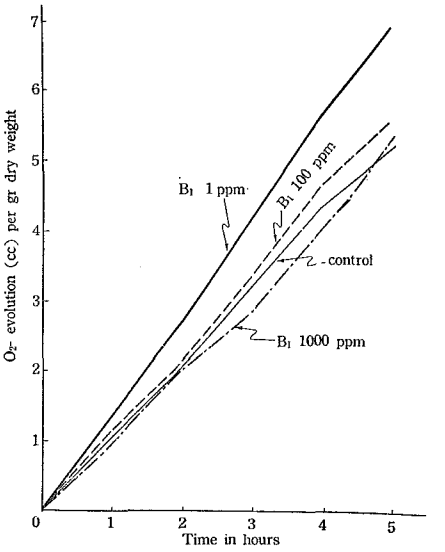

Fig. 34. Relation between thiamine application and the assimilation of cabbage leaves when determined by manometry. (Cumulative curves.)

Experiment at Ina in April, 1956. As the material of this experiment, the largest leaf in each seedling having attained an average weight of 5.6 gr and 5 fully grown leaves was used.

SECTION V*

RESPIRATION

1. Materials and Methods

The plants used as the material were sweet potato (var. Nōrin No. 1), potatoes (var. Dashaku), and kidney beans (var. Masterpiece). Every plant was sown or planted on May 2, 1955 and uniformly cultivated in pots with sand supplied with the KASUGAI's water culture solution for dry field crops (see p. 203); the experiments were carried out from July 20 to August 10, 1955.

The rate of respiration was determined by the WARBURG manometry.(176) The temperature of the cistern was 30±0.1°C. The concentrations of buffer and thiamine solution were the same as those used in the experiments on photosynthesis (CHAP. IV, SECT. IV). The final pH value of the solution was kept to be 4.16 by adjusting with HCl. Special materials for respiration were not added.

In the case of leaves 10 cm^2 leaf pieces were floated on the solution keeping the lower leaf surfaces in touch with the surface of solution, and in the case of roots 1 gr of fresh rootlets was floated on the solution.

The vessels of manometer were wrapped with black cloth for protecting against assimilation. And then, O_2-uptake during a 20 minute period was determined 5 times consecutively.

Respiratory quotient was determined according to the WARBURG's direct method.(176)

After repeating the measurement 5~13 times, the average values were compared.

2. Results and Considerations

(1) Thiamine application and the respiration of leaves and roots.

a. *Leaves.* The fourth fully expanded leaf (B_4) of the sweet potato was tested. The results shown in Fig. 35 indicate that the respiration in the 1 ppm plot was promoted remarkably, while in the 1000 ppm plot, it was decreased.

The results on potato leaves shown in Fig. 36 indicate that in every concentration the thiamine application promoted the respiration, especially the effect was remarkable at high concentration.

The results on kidney beans (Fig. 37) were similar to those of the potatoes just mentioned above.

b. *Roots.* In low concentration plots, the respiration of the roots of sweet potato and potato was promoted remarkably, but in the 1000 ppm plot it was almost equal to that of the control plot (Figs. 38 and 39). The respiration of kidney bean roots was promoted by thiamine application at any concentration, but the effect was most remarkable at 100 ppm (Fig. 40).

* The argument of this section was reported at the meeting of the Horticultural Association in October, 1955.

The dry matter of the materials used for the tests are shown in Fig. 41. It seems that the respiration of the materials with a high dry weight percentage (kidney bean) was comparatively advanced at a high thiamine concnetration, while that of the materials with a low dry weight percentage (sweet potato) was rather advanced at a low thiamine concentration.

In any case, a moderate thiamine application is effective on promoting the respiration of plants.

(2) Qo_2.

From the data obtained above, Qo_2 of leaves and roots were calculated by the next formula :

$Qo_2 = \mu l O_2$ uptake/mg dry weight/ hr

The results (Figs. 42~47) indicate that the moderate thiamine application is effective for promoting the metabolic velocity of the plants.

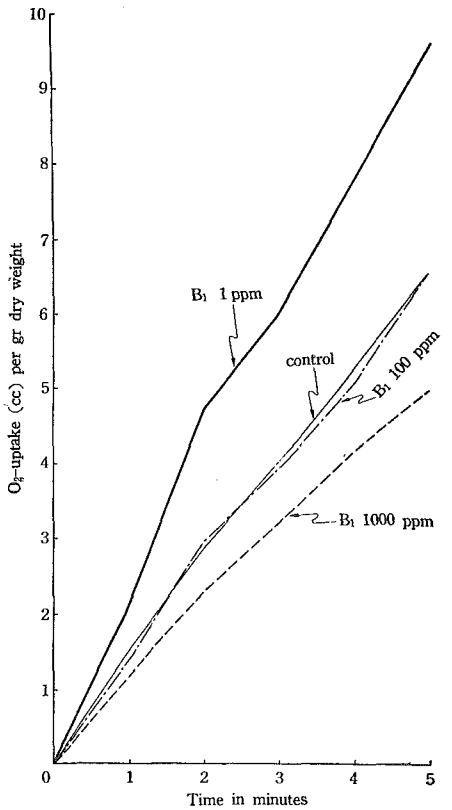

Fig. 35. Relation between thiamine application and the respiration of sweet potato leaves. (Cumulative curves.)

As the material, the B_4 leaves in each slip having attained 10 expanded leaves were used.

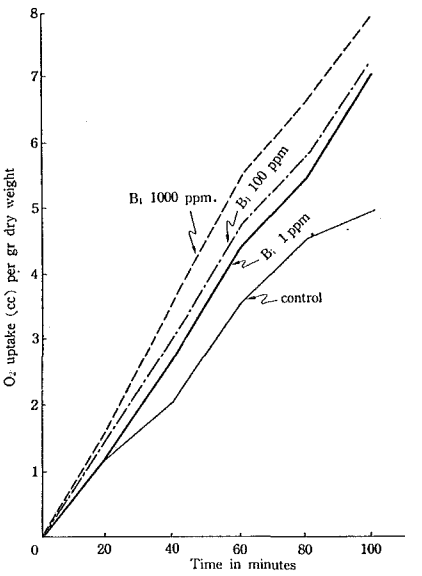

Fig. 36. Relation between thiamine application and the respiration of potato leaves. (Cumulative curves.)

As the material, the fully grown leaves situated at the center part of each plant 32 days after germination were used.

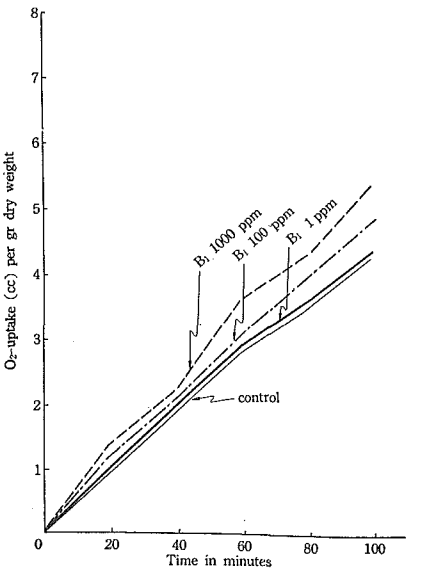

Fig. 37. Relation between thiamine application and the respiration of kidney bean leaves. (Cumulative curves.)

As the material, the fully grown leaves situated at the center part of each plant 26 days after germination were used.

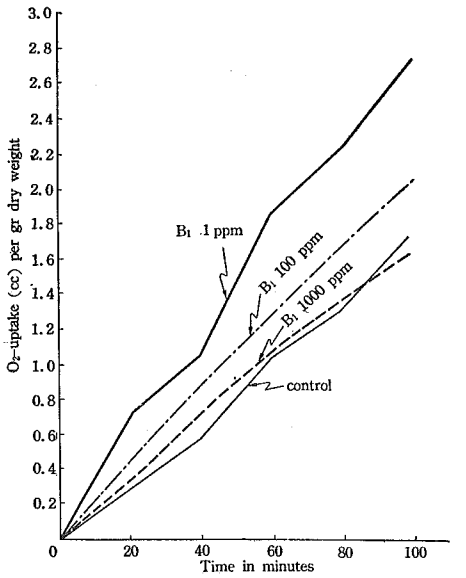

Fig. 38. Relation between thiamine application and the respiration of sweet potato roots. (Cumulative curves.)

As the material, small roots of each plant 20 days after planting were used.

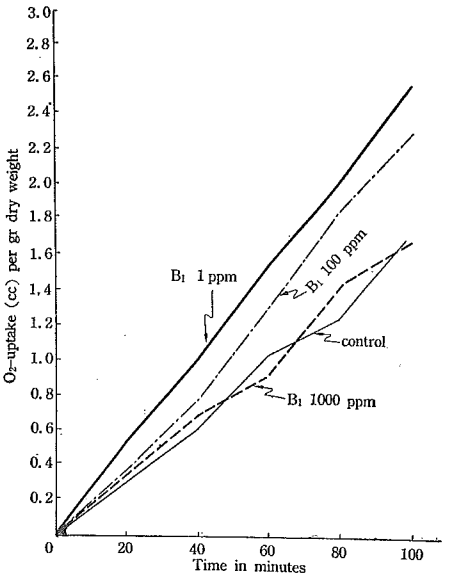

Fig. 39. Relation between thiamine application and the respiration of potato roots. (Cumulative curves.)

As the material, small roots of each plant 18 days after germination were used.

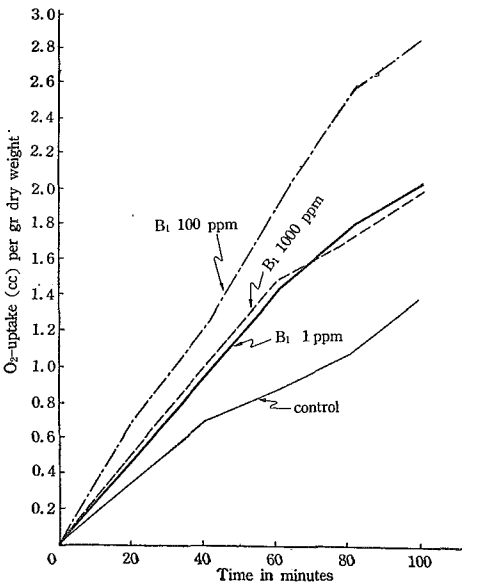

Fig. 40. Relation between thiamine application and the respiration of kidney bean roots. (Cumulative curves.)

As the material, small roots of each plant 35 days after germination were used.

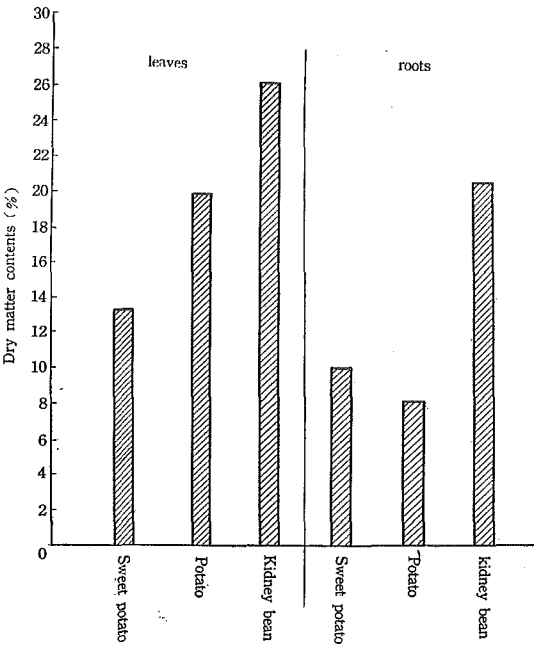

Fig. 41. Dry matter contents of the materials used.

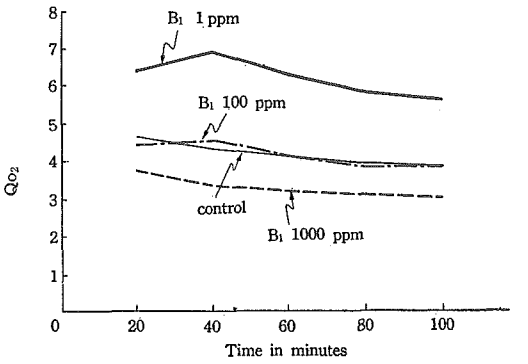

Fig. 42. Relation between thiamine application and Qo_2 of sweet potato leaves. The remarks are the same as in Fig. 35.

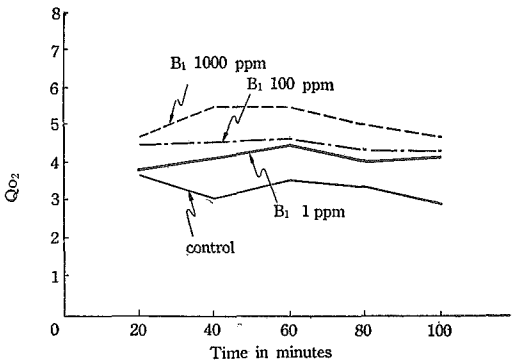

Fig. 43. Relation between thiamine application and Qo_2 of potato leaves. The remark are the same as in Fig. 36.

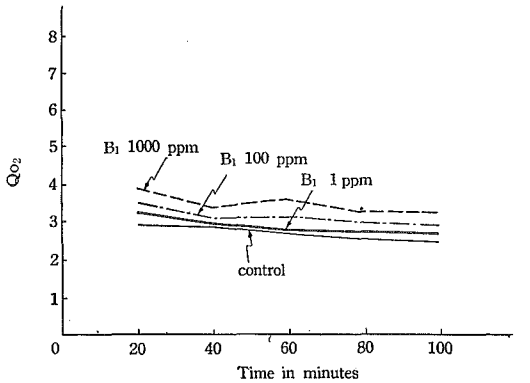

Fig. 44. Relation between thiamine application and Qo_2 of kidney bean leaves. The remarks are the same as in Fig. 37.

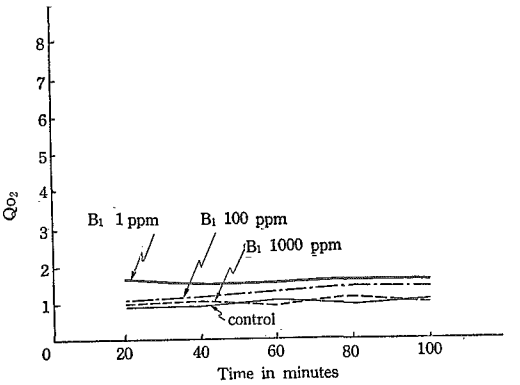

Fig. 45. Relation between thiamine application and Qo_2 of potato roots. The remarks are the same as in Fig. 38.

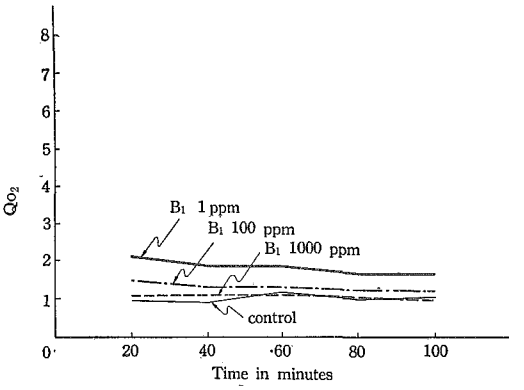

Fig. 46. Relation between thiamine application and Qo_2 of potato roots. The remarks are the same as in Fig. 39.

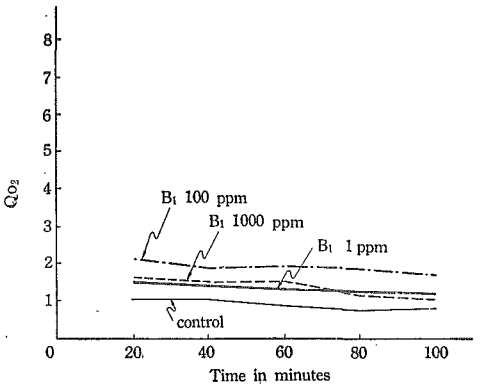

Fig. 47. Relation between thiamine application and Qo_2 of kidney bean roots. The remarks are the same as in Fig. 40.

(3) Thiamine application and respiratory quotient.

The respiratory quotient of the plants used in the experiments described in (1) was determined by the WARBURG direct method.(176) The thiamine concentrations applied in this experiment were the optimum ones to the respiration of each plant.

From the results shown in Figs. 48~50, it may be evident, that the respiratory quotient of the leaves supplied with optimum thiamine, approaches to 1.0. viz. to that of sugars.

It seems that the moderate thiamine spray smoothes the utilization of sugar, the main material for respiration, and thus promotes the growth of plants or increases the yield and improves the quality of crops.

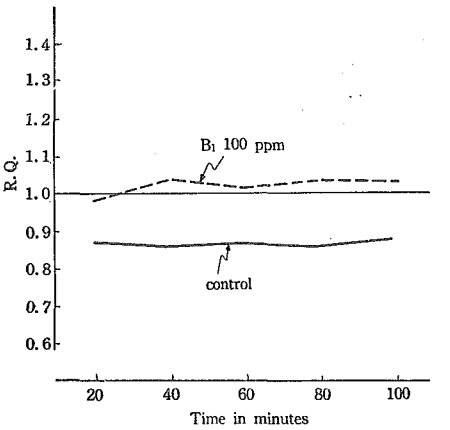

Fig. 48. Relation between thiamine application and the respiratory quotient of sweet potato leaves.

As the material, the B_4 leaves in each slip having grown to 12 expanded leaves were used.

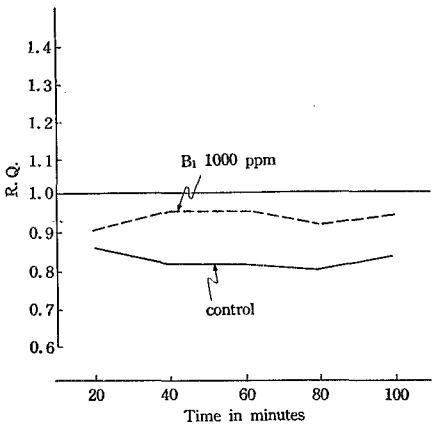

Fig. 49. Relation between thiamine application and the respiratory quotient of potato leaves.

As the material, the fully grown leaves situated at the center part of each plant 35 days after germination were used.

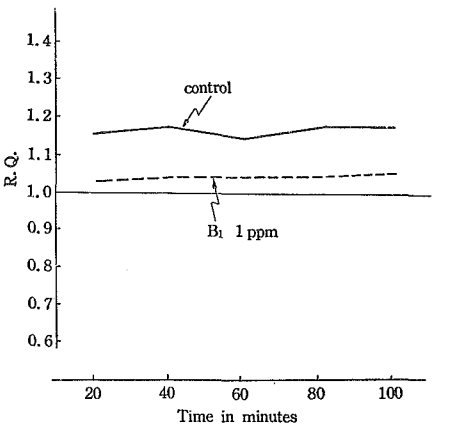

Fig. 50. Relation between thiamine application and the respiratory quotient of kidney bean leaves.

As the material, the fully grown leaves situated at the center part of each plant 28 days after germination were used.

CONCLUSIONS

The present study has dealt with physiological actions of thiamine and the methods of its application in cultivating sweet potatoes, potatoes, and kidney beans.

The application of moderate concentration of thiamine on seeds promotes the germination, especially that of old seeds. The cause of this phenomenon may be explained as follows:

In the process of seed germination under natural conditions, the stored thiamine is transformed from free form to ester form and consumed, the decrease being nearly parallel to that of the dry weight in the dark as the germination process advances. The thiamine content of old seeds is also decreased, and closely related to the rate of germination.

A part of thiamine absorbed by seeds is also transformed to the ester form and consumed. The respiration of seeds is promoted by the application of thiamine. The general activation in physiologic processes, esp. the activation in energy metabolism, may have direct bearing upon the germination.

A moderate amount of thiamine also activates the transpiration and photosynthesis of the leaves, and its promoting effects on the root and tuber development, or on increasing the yields and improving the quality of crops, may be explained by the same reason as in the seed germination. Thiamine is to be the primal requisite for the energy metabolism.

The thiamine sprayed on leaves is absorbed in a short time and accumulated in the tissues and organs of high metabolic activity. It is gradually transformed into ester form and this will be utilized in plants. The values of respiratory quotient approaches to 1.0.

A moderate thiamine spray raises the C–N ratio in plants, and promotes the initiation and development of the tuber, and the flower bud differentiation. It shortens the growing period of plants.

Thiamine is highly effective under unfavorable conditions, but also to some extent under 'normal' conditions. The plant or its organ seems to be susceptible to thiamine deficiency. A high metabolic activity requires more thiamine, and more thiamine will again increase the physiologic processes. Thus the actively metabolizing plants usually run after thiamine, a limiting factor in these conditions.

SUMMARY

The present study has been carried out with the intention of elucidating the physiological action of thiamine in higher plants and the methods of its utilization for cultivating garden crops.

The main materials were: sweet potatoes (*Ipomoea batatas* LAM.), potatoes (*Solanum tuberosum* LINN.), and kidney beans (*Phaseolus vulgaris* LINN.).

(1) Thiamine application at moderate concentration promotes the germination of seeds or increases the germination rate of old seeds.

(2) In the germinating seeds the thiamine stored is transformed from free form to ester form and is consumed in the dark nearly in parallel to the decrease of dry weight. By the artificial application of thiamine, the rate of respiration is remarkably increased.

(3) The thiamine content of old seeds is decreased nearly in proportion to the time elapsed; the rate of germination is closely related to the thiamine content.

(4) Foliar thiamine spray at moderate concentration is effective for the initiation and development of root, flower bud differentiation, and the increase of the yield of crops; larger tubers and weighty pods were produced and the flavor of crops was improved.

(5) The optimum concentration of thiamine solution for seed soaking, as well as for foliar spray, lies at 1 ppm or thereabouts. Spraying is more effective in the first half than in the latter half of the growing period of plants.

(6) Under unfavorable conditions of light, temperature, and fertilizer, the thiamine application is especially effective for the growth of plants.

(7) When applied in optimum quantity, phosphorus is the most effective of the three fertilizer elements, i.e. nitrogen, phosphorus and potassium, for the thiamine activity in plants.

(8) The foliar thiamine spray combined with a low concentration sugar solution is more effective than the individual sprays.

(9) Foliar thiamine spray mixed with acidic agricultural chemicals is desirable; alkaline chemicals decrease the thiamine stability remarkably.

(10) The foliar thiamine spray at moderate concentration increases the sugar, starch, and thiamine contents of crops, and raises the C–N ratio of plants.

(11) The thiamine sprayed on leaves is absorbed by plants in a short time, and is mostly accumulated in the parts of high metabolic activity.

(12) A greater part of the thiamine absorbed by leaves is its free form, but a part is transformed to the ester form as time elapses.

(13) Foliar thiamine spray at moderate concentration promotes the transpiration, the water absorption, the photosynthesis and the respiration of plants, and makes the values of respiratory quotient to approach to 1.0.

(14) Some thiamine deficiency in the actively metabolic portion of plants, especially in the early growing period, seems to be responsible for the smoothing effects of thiamine through sugar metabolism.

ACKNOWLEDGEMENTS

In pursuing these studies, the author has been much indebted to prof. Dr. Shunichirō IMAMURA of the agricultural Faculty, Kyoto University for his kind guidance and useful criticism.

The author also owes a great debt to ex-Prof. Dr. Kumaichi MATSUMOTO, Prof. Dr. Akira KOBAYASHI, Prof. Dr. Yōtarō TSUKAMOTO, Asst. Prof. Dr. Minoru HAMADA, Lect. Dr. Michio KONISHI in the Kyoto University, prof. Dr. Susumu KŌMA in the Shimane Agricultural College, Mr. Taikichi TAKANO in the Nagoya University, Prof. Dr. Sumio SHIMIZU, Asst. Prof. Toshiaki TAKAHASHI, Asst. Prof. Katsumi KUMASHIRO, Lect, Kyō MASUZAWA, Mr. Shoji KOBAYASHI, Mr. Kadomi HOSODA, Mr. Kōichi HOSODA, and all the seminarists and farm workers in the Agricultural Faculty of Shinshu University.

A part of this work has been aided by the Scientific Reseach Fund of the Education Ministry of Japan, to which the author offers his many thanks.

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EXPLANATION OF PLATE I

Fig. I. Effect of foliar thiamine spray on the growth of young sweet potato plants. Water culture experiment at Ina (Shinshu Univ.) in 1953. C: control. B:B_1 spray. Experimental deta see Table 7 (p. 206).

Fig. II. Effect of foliar thiamine spray on the growth of young sweet potato plants. Sand culture experiment at Kyoto in 1954. Photo 20 days after planting. C: control. B: B_1 spray. See Table 8 (p. 206).

Fig. III. Effect of foliar thiamine spray on the growth in thickness of sweet potato tubers. Field experiment at Ina in 1953. Photo : tubers per 3 plants at the harvest time. C : control. B : B_1 spray. Other explanations see Table 10 (p. 208).

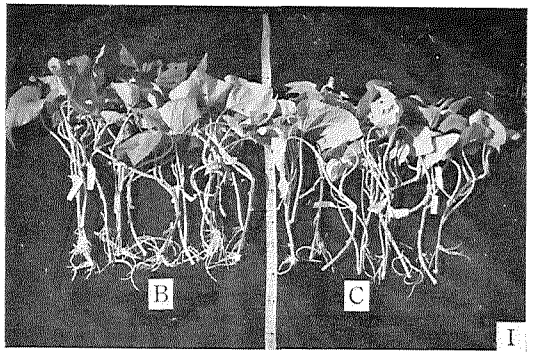
Fig. IV. Effect of foliar thiamine spray on the formation of sweet potato tubers. Sand culture experiment at Kyoto in 1954. C : control. B : B_1 spray. a : photo 35 days after planting (tubers per 5 plants). b : photo 49 days after planting. Other explanations see Table 12 (p. 209).

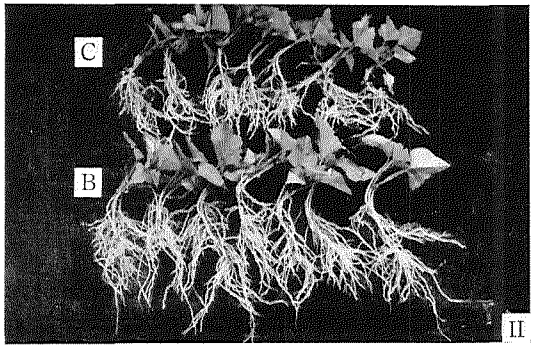
Fig. V. Relation between the thiamine concentration for foliar spray and the initiation and development of young sweet potato roots. Sand culture experiment at Ina in 1952. Photo 30 days after planting. 0, 0.01, 1, 100, 1000: concentration (ppm) of thiamine sprayed. Other explanations see Textfig. 8 (p. 211).

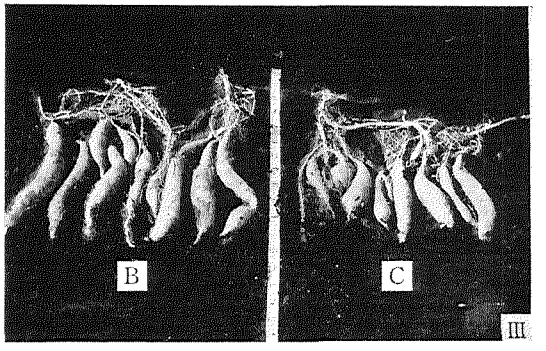
Fig. VI. Relation between the concentration of thiamine for foliar spray and the formation and growth in thickness of sweet potato tubers. Field experiment at Ina in 1952. Photo 70 days after planting.
A : control. B : 100 ppm B_1 spray. C : 1 ppm B_1 spray. D : 0.01 ppm B_1 spray. Other explanations see Textfig. 10 (p. 212).

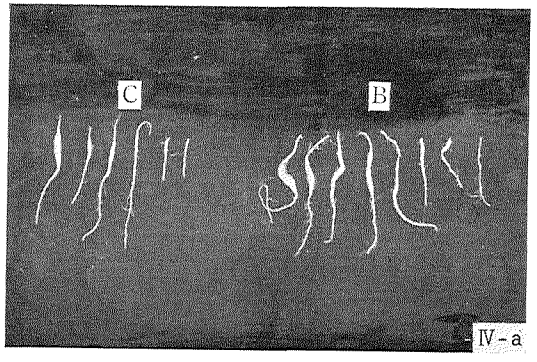
Fig. VII. Effect of foliar thiamine application on the growth of kidney bean roots. Water culture experiment at Ina in 1951. a : control. b : B_1 applied plot. Variety: Burpee's Stringless Green Pod. The leaves in the plot applied with thiamine were coated with 1cc of 1 ppm thiamine solution per plant 5 times during a 15 day period. The seeds were sown on September 2, photo September 28. See page 217.

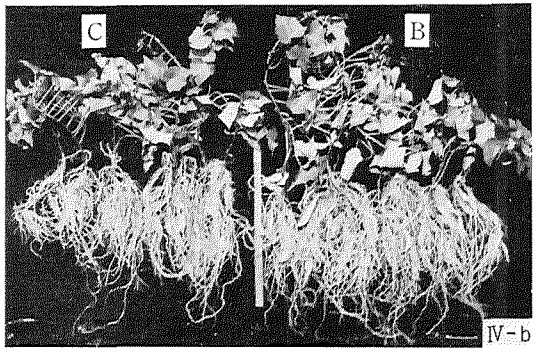
PLATE I

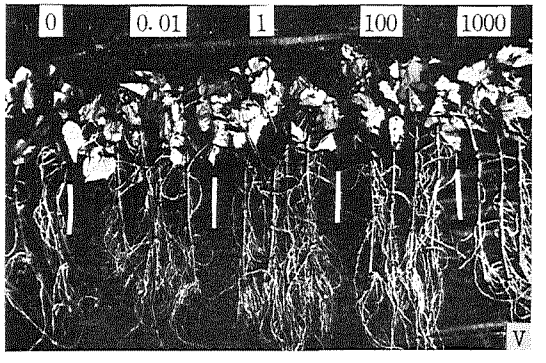


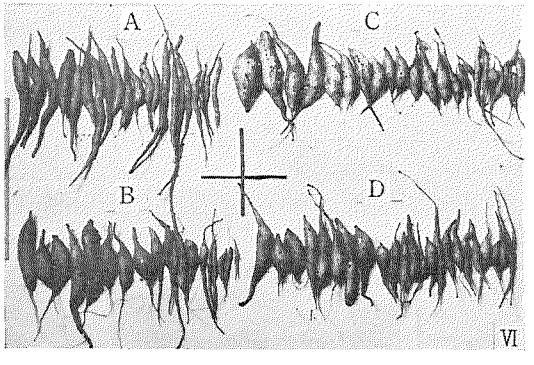


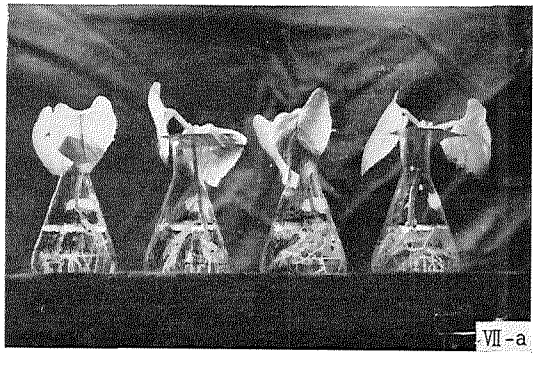


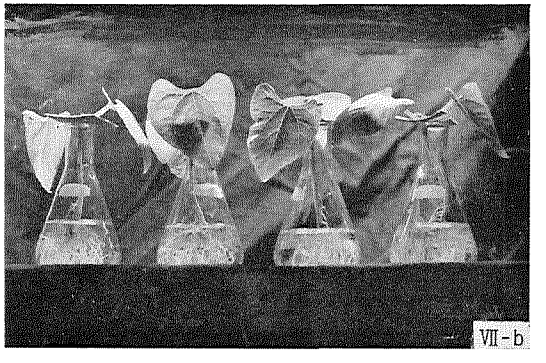












EXPLANATION OF PLATE II

Fig. VIII. Effect of foliar thiamine spray on podding of kidney beans (photo per 10 plants). Sand culture experiment at Kyoto in 1954. Variety : Masterpiece. C : control. B : B_1 spray. Other explanations see Table 21 p. 219.

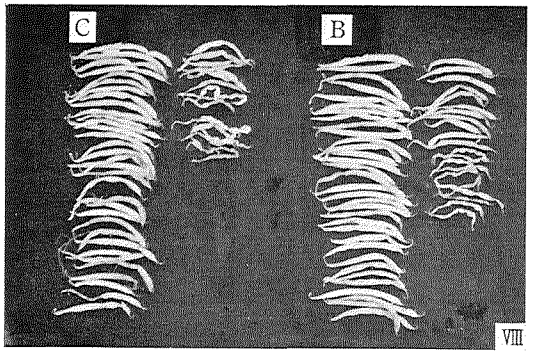
Fig. IX. Relation between the concentration of thiamine solution sprayed and the growth of kidney beans. Photo at the harvest time. Soil culture cxperiment in pots at Ina in 1952. From left to right : plants in plot sprayed with 0, 0.01 and 100 ppm thiamine solution. The variety used was Masterpiece. Other explanations see Textfig. 23 (p. 238).

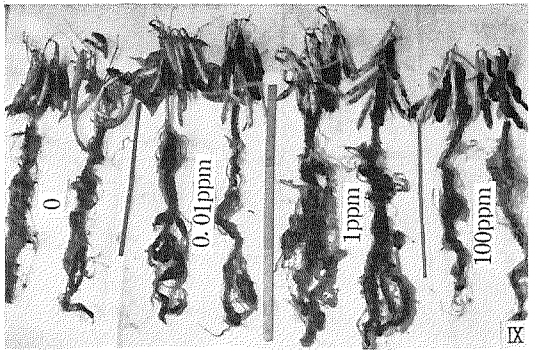
Fig. X. Light, surplus nitrogenous fertilizer and thiamine for the growth of sweet potato roots. Sand culture experiment at Kyoto in 1954. L : in the light. D : in the dark. O : no thiamine, no fertilizer. B : 1 ppm thiamine (foliar spray). N :ammonium sulphate (22.5 kg N per 0.1 ha) in sand. Photo : total roots from each of 8 plants 10 days after planting. See page 228.

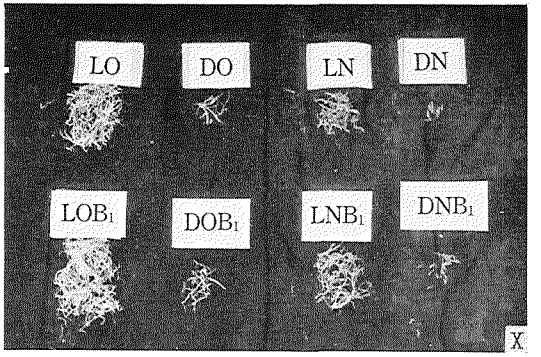
Fig. XI. Temperature, light and thiamine for the growth of young sweet potato plants. Photo 15 days after planting. Each plots of a : the total roots of 8 plants. b : the young plants treated in the dark. Other explanations see Table 28 (p. 229).

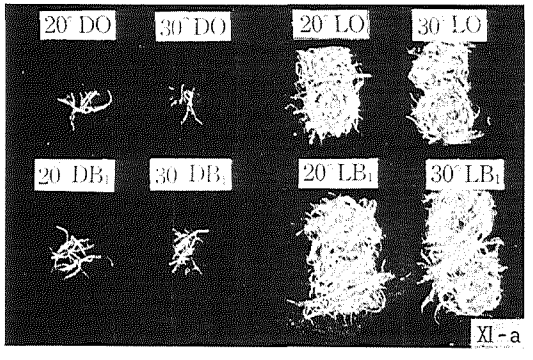
Fig. XII. Sugar and thiamine for the growth of young sweet potato plants in the light. Photo 20 days after planting. Sand culture experiment at Kyoto in 1954. C : control. S : 20 ppm sucrose (foliar spray). B : 1 ppm thiamine (foliar spray). SB : foliar spray of thiamine with sucrose. Each plot of b shows the total roots from 8 plants. See page 230.

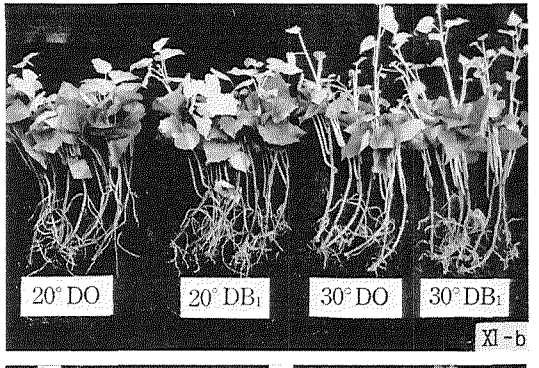
Fig. XIII. Sugar and thiamine for the growth of young sweet potato plants in the dark. Photo 20 days after planting. Each plot of b sows the total roots from 8 plants. Other explanations see Fig. XII (p. 230).

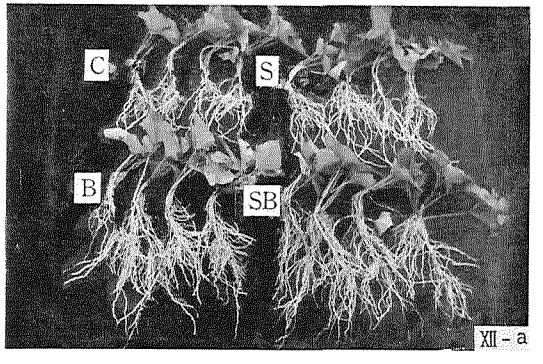

C
B
VIII

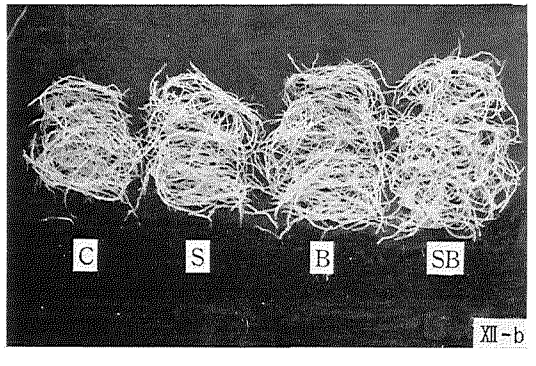

0
0.01ppm
1ppm
100ppm
IX


LO
DO
LN
DN
LOB1
DOB1
LNB1
DNB1
X


20° DO
30° DO
20° LO
30° LO
20° DB1
30° DB1
20° LB1
30° LB1
XI-a


20° DO
20° DB1
30° DO
30° DB1
XI-b


C
S
B
SB
XII-a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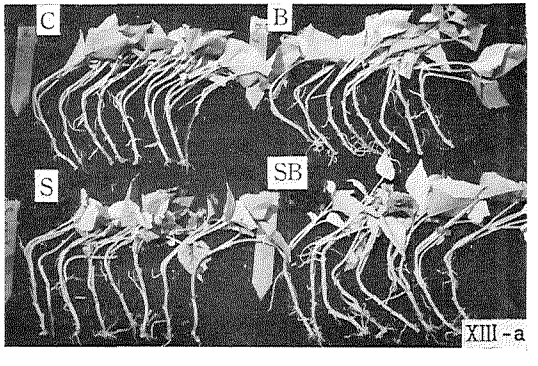
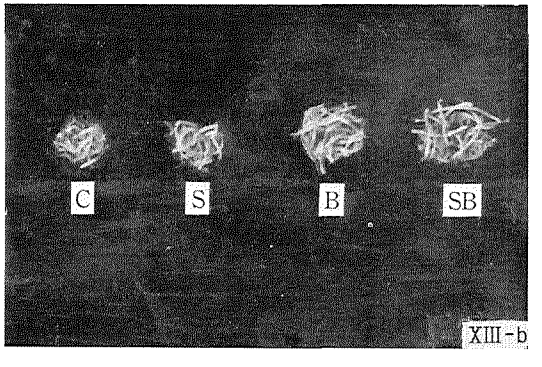
S
B
SB
XII-b


C
B
S
SB
XIII-a


C
S
B
SB
XIII-b

EXPLANATION OF PLATE III

Fig. XIV. Sugar, surplus nitrogenous fertilizer and thiamine for the growth of sweet potato roots. Photo 10 days after planting. Each plot of this photograph shows the total roots from 8 plants. Other explanations see Table 29 (p. 231).

Fig. XV. Kind of sugar and thiamine for the root development and tuber formation of sweet potatoes. Photo 49 days after planting. Each plot of these photographs shows the total tubers (a) or roots (b) from 8 plants. Other explanations see Table 32 (p. 233).

Fig. XVI. Relation between sugar and thiamine activity for the growth of young sweet potato plants. Photo 49 days after planting. B : 1 ppm thiamine foliar spray. S : 1 ppm sucrose foliar spray. See page 234.

Fig. XVII. Relation between surplus fertilizer elements and thiamine for the development of sweet potato roots. Photo 10 days after planting. The photograph of each plot shows the total roots from 8 plants. Other explanations see Table 34 (p. 235).

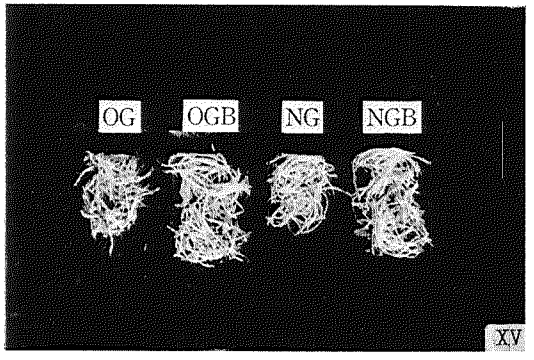
Fig. XVIII. Effect of thiamine and Bordeaux mixture on the yield and quality of potatoes. A : spray of Bordeaux mixture alone. B : spray of thiamine mixed with Bordeaux mixture. C. seperate sprays of thiamine and Bordeaux mixture. C : seperate sprays of thiamine and Bordeaux mixture. Each plot shows the total tubers from 6 plants. Every plot had no disease. See page 237.

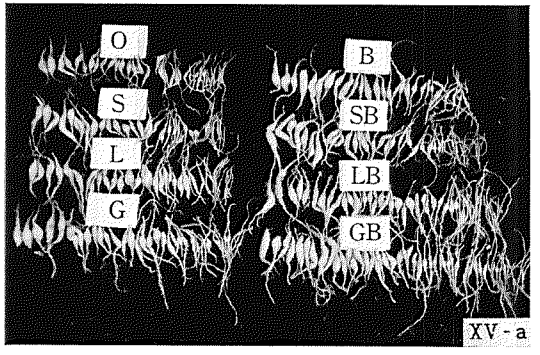
Fig. XIX. Effect of foliar thiamine spray on the growth in thickness of sweet potato tubers. Field experiment at Ina in 1952. Photo 60 days after planting. C : control. B : B_1 spray. Thiamine solution was sprayed on the leaves 6 times in all. Other procedures see Textfig. 9 (p. 211).

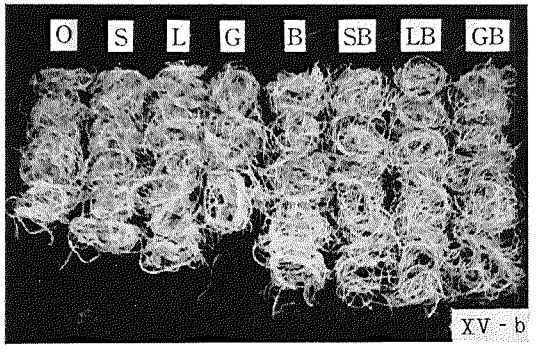
Fig. XX. Effect of foliar thiamine spray on the growth of kidney bean roots. Sand culture experiment at Ina in 1951. A : control. B:B_1 spray. Variety : Burpee's Stringless Green Pods. Two cc of 1 ppm thiamine solution per plant was sprayed on the leaves 6 times during a 30 day period. The seeds were sown on September 7 and investigation was carried out on October 15. See page 217.

Fig. XXI. Relation between fertilizer elements applied moderately and thiamine for the growth of sweet potato roots. Photo 15 days after planting. Each plot of this photograph shows the roots from 8 plants. Other explanations see Table 33 (p. 234).

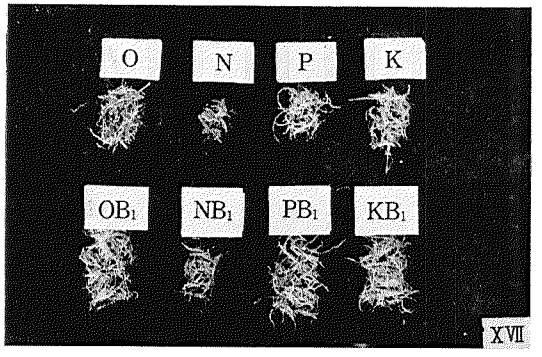
Fig. XXII. Relation between fertilizer elements applied moderately and thiamine for the formation of sweet potato tubers. Photo 49 days after planting. The remarks in this photograph are the same as in Table 31 (p. 232).

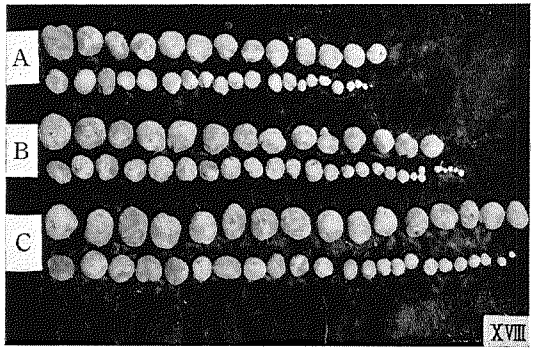

OG
OGB
NG
NGB
XV

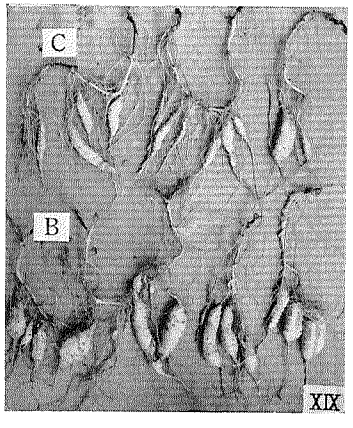

O
S
L
G
B
SB
LB
GB
XV - a

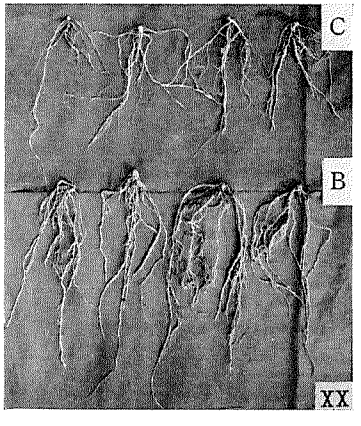

O
S
L
G
B
SB
LB
GB
XV - b

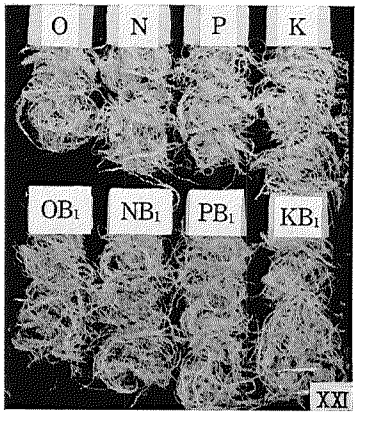

B
SB
XVI

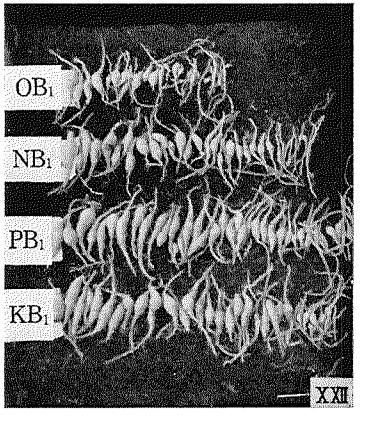

O
N
P
K
OB1
NB1
PB1
KB1
XVII


A
B
C
XVIII


C
B
XIX


C
B
XX


O
N
P
K
OB1
NB1
PB1
KB1
XXI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NB1
PB1
KB1
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