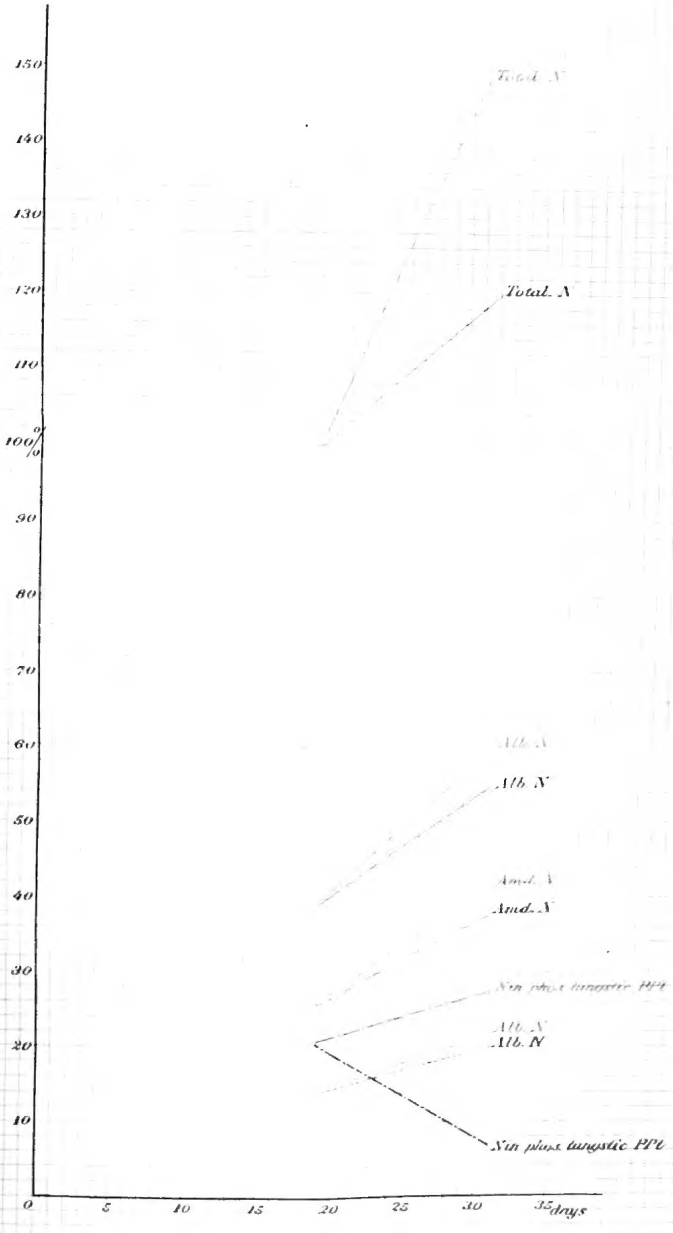

This plate shows the increase of organic bases (N. in phos. tungstic ppt.), especially arginin, in the shoots of Pinus Thunbergii by the addition of ammonium salts. The black lines refer to shoots treated with half saturated gypsum solution in the full day-light and the red lines to those treated with 0.5% ammonium chloride solution, half saturated with gypsum in the full day light.

東京帝國大學

農科大學

學術報告

第四卷

THE BULLETIN

OF THE

College of Agriculture,

TOKYO IMPERIAL UNIVERSITY,

JAPAN.

Vol. IV.

1900—1902.

CONTENTS OF VOLUME IV.

明治三十五年三月十七日印刷
明治三十五年三月二十日發行

農科大學

編輯兼發行者　佐久間衡治　東京市牛込區市ヶ谷加賀町一丁目十二番地

印刷者　株式會社秀英舍第一工場　東京市牛込區市ヶ谷加賀町一丁目十二番地

印刷所

A Contribution to the Knowledge of Arginin.

BY

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The occurrence of organic bases in the seeds as well as in the germinating shoots of many plants has already been shown many years ago by E. Schulze and others. Thus in the year 1886 E. Schulze and Steiger[1] found arginin in the cotyledons of germinating Lupinus, and at the same time, cholin was found by E. Schulze[2] in the seeds and in the etiolated shoots of Lupinus luteus, Soja hispida and Cucurbita pepo. Still afterwards he[3] found guanidin, cholin and betain in the germinating shoots of Vicia faba. But these bases were present in such small quantities that they appeared hardly worth a closer investigation. In the year 1894 S. G. Hedin[4] discovered arginin in the decomposition products of horn and it was soon asserted by him that arginin is a normal constituent of the decomposition products of proteids. A still more interesting result was soon afterwards obtained by A. Kossel[5] on his protamine, in the decomposition products of which he found, almost exclusively, the basic compounds, especially arginin. About the same time, E. Schulze[6] also found a considerable quantity of organic bases (chiefly arginin) in the shoots of coniferous plants as well as in the decomposition products of the proteids prepared from the seeds of several plants, (Picea excelsa, Abies pectinata, and Pinus sylvestris). Further, it was

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(1) E. Schulze und E. Steiger, Zeits. f. Physiol. Chem. Bd. XI, S. 43.

(2) E. Schulze. ibid. Bd. XI, S. 365.

(3) E. Schulze. ibid. Bd. XVII, S. 193.

(4) S. G. Hedin. ibid. Bd. XX, S. 186.

(5) A. Kossel. ibid. Bd. XXII, S. 176. A. Kossel and A. Mathews. ibid. Bd. XXII, S. 190.

(6) E. Schulze, Zeits. f. Physiol. Chem. Bd. XXII, S. 435. and Bd. XXIV, S. 276.

shown by Kutscher[1] that arginin results from the artificial trypsin digestion of proteids; and recently Ellinger[2] considered it to be the source of putrescins. Thus, the physiological importance of organic bases and especially of arginin has been more and more clearly brought to light by these authors, and consequently its study is now considered as one of the most important subjects in the whole domain of physiological chemistry.[3]

Although arginin has so often been made the object of investigation, yet many questions are left unsettled; and, among others, its behaviour, formation and transformation in the plant cells, its relation to the regeneration of proteids and the influence of light and other agents upon these processes have been studied very little. It was with the object of contributing something on these points that I made the present investigation.

I. On the decomposition product of the proteids prepared from the seeds of Japanese coniferous plants.

A) CRYPTOMERIA JAPONICA.

Proteids from the seeds of Cryptomeria japonica were prepared according to Ritthausen's method:—Powdered seeds[4] were first extracted with absolute alcohol and ether to free them

(1) Fr. Kutscher. ibid. Bd. XXV, S. 195.

(2) Ber. d. deutsch. chem. Ges. Bd. XXXI, S. 3183.

(3) Recently it was found that arginin, prepared from plants, differs somewhat, from that of animal origin and it was considered as isomerides, compare—Wl. Gulewitsch:—über das Arginin:—Zeits. f. Physiol. Chem. Bd. XXVII. S. 178.

(4) Seeds of Cryptomeria contain almost no other nitrogen compounds. Thus a fat-free sample yielded 1.80% nitrogen, of which 1.78% was albuminoid nitrogen.

It is also very important to see the chemical nature of the proteids contained in the seeds:—For this purpose 10 grams of the fat-free powder was mixed with 300.c. of a 10% sodium chloride solution, well stirred and after a few hours standing, filtered off, the clear filtrate gave by boiling, a white curdy precipitate, owing to the presence of some coagulable globulin. This precipitate was collected, while hot, on a filter washed with hot sodium chloride, dried and subjected to Kjeldahl's method for nitrogen determination. The filtrate was acidified with sulphuric acid and some phospho-tungstic acid was added, to precipitate the globulin not coagulable by boiling and this precipitate was also subjected to the method for nitrogen determination. The following results were obtained:

from fat and other impurities; then a dilute solution of caustic potash (0.2-0.4%) was added, and the whole was well stirred, and after 24 hours the upper portion of the solution, was slowly decanted. The remaining portion was once more extracted with potash solution and the upper portion decanted. This operation was repeated 3 times and the residue filterd with fine cloth, and the filtrate was mixed with the decanted solution. On neutralizing the potash extract thus obtained with dilute acetic acid, a brown flocculent precipitate was produced in a large quantity. This precipitate was first washed by decantation and afterwards collected on a filter, where it was again washed with water. The crude proteids thus obtained, were once more dissolved in the potash solution and reprecipitated with acetic acid, collected on a filter, washed with water, dilute alcohol, absolute alcohol and ether and dried over sulphuric acid. The crude proteids prepared in this way still contained some impurities of a reddish brown colour, which could be removed with difficulty. The dry proteids yielded 8.03% nitrogen, therefore if must have been still mixed with a considerable quantity of non-nitrogenous substances. The impure proteids were finely powdered and boiled with 20% hydrochloric acid (sp. gr 1.10) and some stannous chloride in a reflux cooler, at first very gently and afterwards directly over the flame. After 30 hours boiling, they were cooled and filtered. The filtrate was diluted with about 5 times its own bulk of water, the dissolved tin was precipitated with sulphuretted hydrogen, and the solution filtered once more. The filtrate was evaporated to a small volume to expell the greater part of hydrochloric acid, and then diluted

---

1. 10 g. air dry = 8.28 g. dry sample yielded :—
   a) Soluble in 10% NaCl, and coagulable by boiling .....0.00093 g N.
   b) „ „ „ „ not „ „ „ .....0.01414 g N.
2. 10 g. air dry = 8.28 g. dry sample yielded :—
   a) Soluble in 10% NaCl, and coagulable by boiling .....0.00112 g N.
   b) „ „ „ „ not „ „ „ .....0.0141 g N.

∴ We have on the average 0.18% nitrogen in the form of proteids soluble in 10% sodium chloride.

As the fat free sample contains 1.8% nitrogen so we see that nearly 10% of the total proteids is soluble in 10 % sodium chloride.

Therefore we must conclude that the greater part of the bases come from the proteids, which are not soluble in sodium chloride.

with water to a certain volume, and the determination of nitrogen was made. The results were as follows.

a). 19.75 grams dry crude proteids (=1.5825 g. N.) boiled with 20% HCl for 30 hours.

Total dissolved nitrogen.	1.5476.....	100.0
Nitrogen, in copper hydrate precipitate.	0.1040.....	6.7
Nitrogen, directly precipitated by phospho-tungstic acid.	0.5506.....	35.6
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate precipitate.	0.4316.....	28.0
Nitrogen in ammonia.	0.1936.....	12.5
Nitrogen in organic bases.	0.2380.....	**15.5**

b). 3.32 g. dry crude proteids (=0.2632 g. N.) boiled with 20% HCl for 96 hours.

Total dissolved nitrogen.	0.2604.....	100.0
Nitrogen in copper hydrate precipitate.	0.0158.....	6.1
Nitrogen directly precipitated by phospho-tungstic acid.	0.0884.....	34.0
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate.	0.0726.....	27.9
Nitrogen in ammonia.	0.0335.....	12.8
Nitrogen in organic bases.	0.0391.....	**15.1**

We see from the above results that nearly 15% of the nitrogen in the proteids are found in the form of organic bases (by the action of hydrochloric acid). No difference was observed between 30 and 96 hours boiling, both yielding the same result.

Note:—The total amount of nitrogen was determind by Kjeldahl's method.

Nitrogen in copper hydrate precipitate was determined to see whether some proteids or peptone still remain undecomposed, for this purpose the hydrochloric acid extract was exactly neutralized with caustic soda and then a few drops of freshly precipitated copper hydrate were added. The precipitate was collected on a filter, washed well, dried, and the nitrogen determined. In this way, I always found that a small quantity of nitrogen

I have tried also to see whether the splitting of organic bases is performed by a very dilute acid instead of a strong one. For this purpose 0.5% hydrochloric acid (1.25 c.c. conc. HCl to 100 c.c. H_2O) was mixed with powdered proteids and boiled for 96 hours. But as I found still some peptone remaining undecomposed, the boiling was continued for 50 hours more until no trace of peptone was present.

c). 10.27 g. crude proteids (0.822 g N) boiled with 0.5% hydrochloric acid for 146 hours (with addition of 1% $SnCl_2$).

Total dissolved nitrogen.	0.7888.....	100.0
Nitrogen in copper hydrate precipitate.	0.0148.....	5.6
Nitrogen directly precipitated by phospho-tungstic acid.(1)	0.2698.....	34.2
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate.	0.2272.....	28.8
Nitrogen in ammonia.	0.1033.....	13.1
Nitrogen in organic bases.	0.1239.....	**15.7**

Thus by boiling with 0.5% hydrochloric acid we obtain nearly the same result as with 20% hydrochloric acid.

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was present in the copper hydrate precipitate. But I afterwards satisfied myself that there are neither proteids nor peptone present; so it must be something else.

Phospho-tungstic acid precipitates both ammonia and organic bases; so we must subtract the nitrogen in ammonia from the nitrogen in phospho-tungstic precipitate and the difference is to be considered to belong to the organic bases.

Ammonia in the hydrochloric acid extract was distilled off by magnesia in the usual way.

For the determination of nitrogen in phospho-tungstic acid precipitate, the precipitate was collected on a filter, washed with 5% sulphuric acid, dried and subjected to Kjeldahl's method.

(1) It is absolutely necessary to prove the absence of peptone in this case, because it is precipitated by phospho-tungstic acid and would thus give a higher percentage of nitrogen. For this purpose the original hydrochloric acid extract was mixed with an excess of strong caustic soda and a few drops of very dilute solution of copper sulphate was added; but no violet colouration was observed. Further the phospho-tungstic precipitate was decomposed with caustic baryta, the excess of baryta removed by carbonic acid, filtered and evaporated and filtered once more. The concentrated filtrate gave no biuret reaction. Therefore peptone must have been entirely absent.

B) GINGKO BILOBA.(1)

Fresh seeds of Gingko biloba were freed from the hard shell and the juicy kernel was crushed in a mortar and extracted with a 0.2-0.4% solution of caustic soda. The soda extract was neutralized with dilute acetic acid and the crude proteids were prepared in the same way as was described before. The preparation was comparatively pure and contained 15.1% nitrogen.

a). 18.775 g. air dry=15.045 g. dry crude proteids (=2.2718 g. N.) boiled with 20% hydrochloric acid for 30 hours :—

Total dissolved nitrogen.	2.2396.....	100.0
Nitrogen in copper hydrate precipitate.	0.0372.....	1.7
Nitrogen in phospho-tungstic precipitate.	0.8184.....	36.5
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate.	0.7588.....	33.9
Nitrogen in ammonia.	0.2236.....	10.0
Nitrogen in organic bases.	0.5356.....	**23.9**

b). 10.143 g. air dry=8.135 g. dry crude proteids (=1.2285 g N) boiled with 0.5% hydrochloric acid for 146 hours.

Total dissolved nitrogen.	1.2276.....	100.0
Nitrogen in copper hydrate precipitate.	0.0372.....	3.0
Nitrogen in phospho-tungstic precipitate.	0.4383.....	35.7
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate.	0.4000.....	32.9
Nitrogen in ammonia.	0.1265.....	10.3
Nitrogen in organic bases.	0.2735.....	**22.6**

(1) Recent investigation has shown that Gingko biloba does not belong to the Coniferae and must be considered to form an independent family, Gingoaceae. This plant is peculiar to Japan.

C) PINUS THUNBERGII.

Preparation of crude proteids in the same way as before, was tolerably pure, and contained 15.6% nitrogen.

a). 12.36 g. crude proteids (=1.928 g N) boiled for 30 hours with 20% hydrochloric acid.

Total dissolved nitrogen.	1.9020.....	100.0
Nitrogen in copper hydrate precipitate.	0.0400.....	2.1
Nitrogen in phospho-tungstic precipitate.	0.7056.....	37.1
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate.	0.6581.....	34.6
Nitrogen in ammonia.	0.1541.....	8.1
Nitrogen in organic bases.	0.5040.....	**26.5**

b). 7.52 g. crude proteids (=1.173 g N) boiled with 0.5% hydrochloric acid for 146 hours.

Total dissolved nitrogen.	1.156	100.0
Nitrogen in copper hydrate precipitate.	0.021	1.8
Nitrogen in phospho-tungstic precipitate.	0.4451.....	38.5
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate.	0.4185.....	36.2
Nitrogen in ammonia.	0.0983.....	8.5
Nitrogen in organic bases.	0.3202.....	**27.7**

From the above results, we see that the organic bases are splitted off very easily by the action of dilute acid, the quantity of the bases produced by the action of dilute acid is always the same with that of strong acid.

II. Organic bases especially arginin in the shoots of coniferous plants.

1. Cryptomeria japonica.

Since E. Schulze found much organic bases especially arginin in the shoots of Picea excelsa, Abies pectinata and Pinus sylvestris, it is naturally to be expected that the shoots of other Coniferae also contain the same substance.

Seeds of Cryptomeria japonica were sown in the purified sea sand and kept in the dark in a warm house, where the temperature ranged between 15°C and 35°C. After one month they began to germinate and after three weeks more, the shoots were 6.–7cm. high. Hereupon they were removed from the sand, washed well, dried and analyzed :—

a). In 100 parts of dry matter.

Total nitrogen.	7.77.....	..100.0
Albuminoid nitrogen.	3.82.....	49.2
Asparagine nitrogen.	0.56.....	7.2
Nitrogen in phospho-tungstic precipitate.	**1.65**.....	**21.2**
Other nitrogen.	1.74.....	22.4

b). Another sample gave :—

Albuminoid nitrogen.	3.77
Nitrogen in phospho-tungstic precipitate.	**1.44**

We see here that nearly $\frac{1}{5}$ of the total nitrogen in the shoots belongs to the phospho-tungstic precipitate.

2. Gingko biloba.

Seeds of Gingko biloba were sown in the purified sea sand and kept in the dark in a warm house, where the temperature ranged between 15°C and 35°C. When the etiolated shoots were 30cm. high on the average, and the roots 10-15cm. long, there being no open leaves yet, one portion of the shoots was removed from the sand, washed, dried and analyzed. The other portion was then exposed to day-light and a solution

containing 0.1% K_2HPO_4, KH_2PO_4, $MgSO_4$ and trace of $FeCl_3$, and $CaSO_4$ to half saturation, was added. After twenty one days, the shoots were 36cm. high, with many green open leaves, the diameter of which was on the average 5cm. This second portion was then removed from the sand, washed, dried and analyzed.

a). Etiolated shoots of (*so many days*) growth.

In 100 parts of dry matter.

Total nitrogen.	3.00.....	100.0
Albuminoid nitrogen.	1.44.....	48.0
Asparagine nitrogen.	0.48.....	16.0
Nitrogen in phospho-tungstic precipitate.	**0.14**.....	**4.7**
Other nitrogen.	0.94.....	31.3

Every 100 shoots contain :—

Total nitrogen.	0.5775
Albuminoid nitrogen.	0.2772
Asparagine nitrogen.	0.0924
Nitrogen in phospho-tungstic precipitate.	**0.0270**
Other nitrogen.	0.1809

b). Etiolated shoots of (*so many days*) growth exposed to day-light for the last 3 weeks.

In 100 parts of dry matter.

Total nitrogen.	2.97	100.0
Albuminoid nitrogen.	1.99.....	67.0
Asparagine nitrogen.	0.30.....	10.1
Nitrogen in phospho-tungstic precipitate.	**0.16**.....	**5.4**
Other nitrogen.	0.52.....	17.5

Every 100 shoots contain : —[1]

Total nitrogen.	1.1234
Albuminoid nitrogen.	0.7393

(1) The increase of the absolute quantity of nitrogen in the shoots of b) was due to the migration of the reserve materials from the seeds to the shoots.

Asparagine nitrogen.	0.1115
Nitrogen in phospho-tungstic precipitate.	0.0594
Other nitrogen.	0.2132

In this case, contrary to my expectation, I found very little organic bases both in the etiolated shoots and in the green plants.

3. Pinus Thunbergii.

Seeds were sown in the purified sea sand, and kept in the dark in a warm house where the temperature varied between 15°C and 30°C. On the 24th February, they began to germinate, and after 28 days they were 10-15cm. high. They were then removed from the sand, washed, dried and analyzed.

In 100 parts of dry matter.

Total nitrogen.	9.53.....	100.0
Albuminoid nitrogen.	2.94.....	30.9
Asparagine nitrogen.	2.35.....	24.7
Nitrogen in phospho-tungstic precipitate.	1.88.....	19.7
Other nitrogen.	2.36.....	25.7

III. Isolation of arginin.

1. Cryptomeria japonica.

a).

Isolation of arginin from the decomposition products of the proteids prepared from the seeds by the action of hydrochloric acid :—The hydrochloric extract was at first freed from dissolved tin by hydrogen sulphide. The filtrate was then evaporated off to a small volume, to expell the greater part of hydrochloric acid, and diluted with water. Phospho-tungstic acid solution was then added in excess, when a white precipitate was formed in considerable quantities. This was collected on a filter, washed with 5% sulphuric acid until no trace of hydrochloric

acid was present,[1] strongly pressed between the filter, to remove the excess of sulphuric acid, then put in a large porcelain basin, and mixed with water to the consistency of paste. Some caustic lime and baryta were then added, and the whole well rubbed in the basin (during the operation, the basin must be cooled with water), and filtered after a few hours standing. The dissolved lime and baryta in the filtrate were precipitated by carbonic acid. The filtrate from the insoluble carbonates was evaporated to drive off the dissolved carbonic acid, which dissolves some calcium and barium carbonate, and after 24 hours standing, filtered. The filtrate was neutralized with a known quantity of sulphuric acid and further evaporated to a small volume. After cooling, the sulphuric acid, formerly added, was precipitated with a calculated quantity of baryta water, and the insoluble barium sulphate filtered off (during the filtration, the vessels were well covered to prevent the absorption of carbonic acid by the bases). The clear filtrate which contains free bases, was treated according to S. G. Hedin's method, with a concentrated silver nitrate solution, by which some white flocculent precipitate was formed. This was removed by filtration, and the clear filtrate was evaporated on the water bath to a small volume, filtered once more, and left standing in a dessicator. After a short time, fine crystals of arginin silver nitrate was formed, which increased very much after two or three days. The crystals were then separated from the mother liquor, well washed with cold water, and recrystallized from a hot water solution. The crystals thus obtained were dried over sulphuric acid and the percentage of silver was determined by direct ignition :—

0.083 g. crystals yielded by ignition 0.026 g. $Ag = 31.3\%$[2]
Calculated as $C_6H_{14}N_4O_2$, $AgNO_3 + \frac{1}{2}H_2O$.....$Ag = 30.59\%$

A portion of the crystals was dissolved in hot water and, after the precipitation of silver by hydrogen sulphide, evaporated

(1) As it is very difficult to remove hydrochloric acid by simple washing, the precipitate was once more mixed with a large quantity of 5% sulphuric acid, well rubbed in a mortar, and then collected on a filter and well washed with 5% sulphuric acid. In this way all traces of hydrochloric acid was removed.

(2) As a small quantity of silver is reduced during the crystalization, it was very difficult to prepare absolutely pure crystals.

to a small volume. The solution gave the following reactions:—

With phospho-tungstic acid .....Voluminous white precipitate.
,, phospho-molybdic acid.....Yellow precipitate, soluble in excess.
,, potassio-mercury iodide.....No precipitate, on addition of much caustic soda, white precipitate was formed.
,, potassio-bismuth iodide.....Red precipitate.

The solution was further concentrated and after several days standing over sulphuric acid, fine white prismatic crystals, were obtained, which agree perfectly well with E. Schulze's description of arginin nitrate. A solution of these arginin nitrate crystals was mixed with some copper hydrate, and on being warmed a portion of the copper hydrate was dissolved with a dark blue colour. The solution was filtered while hot, and the filtrate was evaporated to a small volume and let stand over sulphuric acid. After three or four days, dark blue prismatic crystals were obtained, which after careful washing, were dried over sulphuric acid and analyzed.

0.1075 g. crystals became on drying at 100°C. 0.0975 g.
$=9.3\%$ water of crystallization.
0.1075 g. crystals yielded 0.016 g. CuO = 0.0126 g. Cu
$=11.7\%$ Cu.[1]

Calculated as $(C_6H_{14}N_4O_2)_2\ Cu(NO_3)_2\ 3H_2O$.....
9.13%.....water of crystallization.
..... 10.75% Cu.

A somewhat higher percentage of copper in my preparation

(1) For the determination of copper the crystals were first ignited and weighed. The CuO thus obtained was dissolved in sulphuric acid and precipitated with a dilute soda solution. The copper hydrate precipitate was collected on a filter, washed very well, dried, ignited and weighed. No difference was observed between the two weighings.

may be due to experimental error; so there is no doubt that the crystals obtained were those of *arginin*. The quantity of the arginin crystals obtained amounted to about 5% of the proteids used, (calculated as pure proteids), that is to say, nearly 10% of the nitrogen in the proteids was contained in the arginin. As I found that about 15% of the nitrogen in the proteids are contained in the phospho-tungstic precipitate (organic bases) formed by the action of hydrochloric acid, we may conclude that nearly $\frac{2}{3}$ of the organic bases formed, consist of *arginin*.

The mother liquor of basic arginin silver nitrate crystals was diluted with water, and silver was precipitated with hydrogen sulphide. The filtrate from the silver sulphide was evaporated and concentrated, and precipitated with phospho-tungstic acid. The precipitate was washed with 5% sulphuric acid, strongly pressed between the filters and then decomposed with caustic baryta. The dissolved baryta was removed by carbonic acid, evaporated, once more filtered, and a mercuric chloride solution was added, whereby a white precipitate was obtained, perhaps due to histidin (?) but too little to confirm. The filtrate from the mercuric chloride precipitate was diluted with water and the dissolved mercury was removed by hydrogen sulphide, filtered, and the filtrate was evaporated and concentrated. No crystals were obtained after long standing. Therefore lysin was probably not present.

b). Etiolated shoots of Cryptomeria japonica.

Etiolated shoots 6-8cm. high, grown in the purified sea sand, were dried and powdered. 40 grams of this dry powder were extracted with about one litre of water at 50–60°C. After two hours, the extract was cooled and filtered. To the filtrate, an excess of basic lead acetate was added until no more precipitate was formed, and filtered. The excess of lead in the filtrate was removed by sulphuric acid. The filtrate from the lead sulphate, was precipitated by phospho-tungstic acid, whereby a white precipitate was formed in considerable quantities. The precipitate was treated in the same way as described before, and crystals of basic arginin silver nitrate were obtained in a tolerably large quantity. But in this case it was too difficult to get the crystals pure, owing to the excessive reduction of silver. Therefore it was impossible to decide how much arginin was

present in the shoots. But any how I have no doubt that the chief product is arginin.

2. GINGKO BILOBA.

The isolation of arginin according to the usual method was in this case encountered with much difficulties by the strong reduction of silver and the separation of pure crystals was almost impossible. So I tried to convert it to the neutral silver salts according to Wl. Gulewitsch's method.[1] The latter compound was rather stable and it proved to be successful. For this purpose, the basic solution containing free bases, was at first neutralized exactly with dilute nitric acid, and then some concentrated silver nitrate solution was added. The evaporated and concentrated syrupy liquid became soon after cooling a white crystalline mass which after recrystalization became uniform long needles, most of them being united to bundles. It was easily soluble in water, but little soluble in absolute alcohol. The microscopical examination proved it to be exactly identical with Gulewitsch's description. After drying over sulphuric acid, the crystals were subjected to the following determinations.

1. 0.0808 g. crystals yielded on ignition
0.0220 g=27.2% Ag.
2. 0.090 g. crystals of another preparation
yielded on ignition 0.0245 g=27.5% Ag.
3. On drying at 100°C, no loss in weight.....no water of crystallization.

Calculated as $C_6H_{14}N_4O_2$, HNO_3+AgNO_3 =26.5% Ag.

4. Melting point was found in one case to be 181-183°C. and in the other 182-184°C. Above 130-140°C, the crystals became gradually brownish black, but did not melt until 181°C exactly. This result exactly coincides with Gulewitsch's observation.

5. A part of the neutral salt was dissolved in water and so much baryta water was added as to remove exactly half of the nitric acid to convert it into the basic salts. Evaporated to a small volume and let stand over sulphuric acid, whereby

(1) Wl. Gulewitsch. "Ueber das Arginin" :—Zeits. f. Physiol. Chem. Bd. XXVII. Heft. 3. 1899, s. 178.

the basic salt was formed which after recrystallization became fine needles, uniting to bundles.

0.113 g dry crystals yielded on ignition 0.0345 g = 30.53% Ag.
Calculated as $C_6H_{14}N_4O_2$, $AgNO_3 + \frac{1}{2}H_2O$ = 30.55% Ag.

We may now safely conclude that the base was arginin.

I did not calculate how much arginin was present in the decomposition products of the proteids, yet I am sure that the greater part of the bases was arginin. It is not yet decided whether some other bases can be isolated or not. I hope to take up this question later on.

3. Pinus Thunbergii.

In the decomposition products of the proteids and in the etiolated shoots, arginin was isolated just in the same way as in Cryptomeria japonica and was found to amount also to more than half of the entire bases.

IV. Organic bases in the decomposition products of the proteids prepared from the shoots of Pinus Thunbergii.

It will be very interesting to see whether the chemical nature of the proteids in the shoots of coniferous plants is different from that of the proteids in seeds or not. For this purpose, the shoots of Pinus Thunbergii 10-12cm. high grown in the full day-light in the purified sea sand were dried and powdered and its composition was determined first :—

Average dry weight of 1000 shoots. 6.54 gram.

In 100 parts of dry matter.

Total nitrogen.	7.74
Albuminoid nitrogen.	3.40
Asparagine nitrogen.	1.42
Nitrogen in phospho-tungstic precipitate.(1)	0.34
Other nitrogen.	2.58

About 50 grams of the finely powdered sample were mixed with about 1000c.c. of 0.2-0.3% caustic soda solution, well stirred and let stand for 24 hours, then filtered with clean cloth, and

(1) By long exposure to day-light, organic bases had been reduced to the minimum quantity.

the residue once more extracted with soda solution. The soda extract was neutralized with dilute acetic acid, whereby a flocculent precipitate was formed in abundance. This was washed at first by decantation and afterwards collected on a filter, washed with water, alcohol and ether, and dried over sulphuric acid. The crude proteids thus obtained were still mixed with chlorophyll and other impurities. The dried and powdered proteids were then boiled with 20% hydrochloric acid (sp. gr. 1.10) for 20 hours (with addition of some $SnCl_2$) in the reflux cooler, and the solution filtered. The filtrate was treated in the usual way, and the following result was obtained.

Total dissolved nitrogen.	0.2700.....	100.0
Nitrogen directly precipitated by phospho-tungstic acid. (α)	0.0891.....	33.0
Nitrogen in ammonia. (β)	0.0203.....	7.5
(α)—(β)...Nitrogen in organic bases.	0.0688.....	**25.5**

We see that this result nearly coincides with the one obtained for the seeds; therefore it is very probable that the chemical nature of the proteids in the shoots is the same as in the seeds. From want of time, I could not isolate the bases, but I am sure that the chief base is arginin. I suppose also that the proteids in the shoots of other coniferous plants can produce the bases in the same way. I hope to make some further communication on this subject.

SUMMARY OF RESULTS.

1. The proteids prepared from the seeds of Cryptomeria japonica, Pinus Thunbergii and Gingko biloba, produce much organic bases by the action of acids, the chief of which is arginin.

2. Organic bases are produced by the action of even very dilute acids.

3. The etiolated shoots of Cryptomeria and Pinus, contain much organic bases, especially arginin; but the shoots of Gingko contain only a very small quantity of the bases.

4. The chemical nature of the proteids prepared from the shoots of coniferous plants are most probably the same as that of the seed proteids, inasmuch as they give the same decomposition products as those of the seeds.

ANALYTICAL DATA.

I. Cryptomeria japonica.

(a). Boiled with 20% hydrochloric acid for 30 hours.

50 grams air dry = 36.50 g. dry crude proteids (= 1.582 g N) were boiled with hydrochloric acid. The extract was diluted to 400 c.c. from which the following determinations were made :—

	Solutions used.	Baryta water replaced.	Nitrogen found.	Absolute quantity in total solution.	Ratio.
	c.c.				
Total nitrogen	10	20.6	0.0387	1.548	100.0
	„	20.8			
Nitrogen in copper hydrate precipitate	„	1.3	0.0026	0.104	6.7
	„	1.5			
Nitrogen directly precipitated by phospho-tungstic acid	„	7.4	0.0138	0.551	35.6
	„	7.4			
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate	„	5.6	0.0108	0.432	28.0
	„	6.0			
Nitrogen in ammonia	„	2.5	0.0048	0.194	12.5
	„	2.7			
Nitrogen in organic bases	„	—	—	0.238	15.5
	„	—			

1 c.c. baryta water = 0.00186 g. N.

(b). Boiled with 20% hydrochloric acid for 96 hours.

4.2 g. air dry = 3.32 g. dry crude proteids (=0.263 g. N) were boiled with hydrochloric acid of 20%. The extract was diluted to 400 c.c. from which the following determinations were made :—

	Solutions used.	Baryta water replaced.	Nitrogen found.	Absolute quantity in total solution.	Ratio.
	c.c.				
Total nitrogen	40	13.8	0.0260	0.2604	100.0
	„	14.2			
Nitrogen in copper hydrate precipitate	„	0.8	0.0016	0.0160	6.1
	„	0.9			
Nitrogen directly precipitated by phospho-tungstic acid...	„	4.6	0.0088	0.0883	34.0
	„	4.9			
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate	„	3.7	0.0073	0.0730	27.9
	„	4.1			
Nitrogen in ammonia	„	1.7	0.0033	0.0334	12.8
	„	1.9			
Nitrogen in organic bases ...	„	—	—	0.0391	15.1
	„	—			

1 c.c. baryta water = 0.00186 g. N.

(c). Boiled with 0.5% hydrochloric acid for 146 hours.

10.27 g. dry crude proteids (=0.822 g N) were boiled with 0.5% hydrochloric acid. The extract was diluted to 400 c.c

	Solutions used.	Baryta water replaced.	Nitrogen found.	Absolute quantity in total solution.	Ratio.
	c.c.				
Total nitrogen	10	10.6	0.0197	0.7888	100.0
	„	10.6			
Nitrogen in copper hydrate precipitate	„	0.5	0.0011	0.0448	5.6
	„	0.7			
Nitrogen directly precipitated by phospho-tungstic acid...	„	3.6	0.0067	0.2698	34.2
	„	3.7			
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate	„	3.0	0.0057	0.2272	28.8
	„	3.1			
Nitrogen in ammonia	„	1.4	0.0026	0.1033	13.1
	„	1.3			
Nitrogen in organic bases ...	„	—	—	0.1239	15.7
	„	—			

1 c.c. baryta water = 0.00186 g. N.

II. Gingko biloba.

(a). Boiled for 30 hours with 20% hydrochloric acid.

18.775 g. air dry = 15.045 g. dry crude proteids (= 2.2718 g. N) were boiled with 20% hydrochloric acid. The extract diluted to 400 c.c.

	Solutions used.	Baryta water replaced.	Nitrogen found.	Absolute quantity in total solution.	Ratio.
	c.c.				
Total nitrogen	10	30.4	0.0560	2.2396	100.0
	„	29.8			
Nitrogen in copper hydrate precipitate	„	0.5	0.0009	0 0372	1.7
	„	0.5			
Nitrogen directly precipitated by phospho-tungtic acid ...	„	11.2	0.0205	0.8184	36.5
	„	10.8			
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate	„	10.1	0.0190	0.7588	33.9
	„	10.3			
Nitrogen in ammonia	„	2.9	0.0056	0.2236	10.0
	„	3.2			
Nitrogen in organic bases ...	„	—	—	0.5356	23.9
	„	—			

1 c.c. baryta water = 0.00186 g. N.

(b). Boiled with 0.5% hydrochloric acid for 146 hours.

10.143 g. air dry = 8.128 g. dry crude proteids (= 1.229 g. N) were boiled with 0.5% hydrochloric acid. The extract diluted to 400 c.c.

	Solutions used.	Baryta water replaced.	Nitrogen found.	Absolute quantity in total solution.	Ratio.
	c.c.				
Total nitrogen	10	16.5	0.0307	1.2276	100.0
	„	16.5			
Nitrogen in copper hydrate precipitate	„	0.5	0.0009	0.0372	3.0
	„	0.5			
Nitrogen directly precipitated by phospho-tungstic acid...	„	5.8	0.0110	0 4383	35.7
	„	6.0			
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate	„	5.3	0.0100	0.4000	32.9
	„	5.4			
Nitrogen in ammonia	„	1.8	0.0032	0.1265	10.3
	„	1.7			
Nitrogen in organic bases ...	„	—	—	0.2735	22.6
	„	—			

III. Pinus Thunbergii.

(a). Boiled for 30 hours with 20% hydrochloric acid.

12.36 g. dry crude proteids (= 1.928 g. N) were boiled for 30 hours with 20% hydrochloric acid. The extract diluted to 400 c.c.

	Solutions used.	Baryta water replaced.	Nitrogen found.	Absolute quantity in total solution.	Ratio.
	c.c.				
Total nitrogen	10	25.4	0.0476	1.902	100.0
	„	25.7			
Nitrogen in copper hydrate precipitate	„	0.5	0.0010	0.040	2.1
	„	0.6			
Nitrogen directly precipitated by phospho-tungstic acid...	„	9.3	0.0176	0.7056	37.1
	„	9.7			
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate	„	8.8	0.0165	0.6581	34.6
	„	8.9			
Nitrogen in ammonia	„	2.0	0.0039	0.1541	8.1
	„	2.1			
Nitrogen in organic bases ...	„	—	—	0.5040	26.5
	„	—			

1 c.c. baryta water = 0.00186 g. N.

(b). Boiled with 0.5% hydrochloric acid for 146 hours.

7.52 g. dry crude proteids (= 1.173 g. N) were boiled with 0.5% hydrochloric acid. The extract diluted to 400 c.c.

	Solutions used.	Baryta water replaced.	Nitrogen found.	Absolute quantity in total solution.	Ratio.
	c.c.				
Total nitrogen	10	15.5	0.0289	1.156	100.0
	„	15.6			
Nitrogen in copper hydrate precipitate	„	0.25	0.0005	0.0210	1.8
	„	0.30			
Nitrogen directly precipitated by phospho-tungstic acid...	„	5.95	0.0111	0.4451	38.5
	„	6.00			
Nitrogen in phospho-tungstic precipitate in the filtrate of copper hydrate	„	5.5	0.0105	0.4185	36.2
	„	5.7			
Nitrogen in ammonia	„	1.3	0.0025	0.0983	8.5
	„	1.4			
Nitrogen in organic bases ...	„	—	—	0.3202	27.7
	„	—			

1 c.c. baryta water = 0.00186 g. N.

II. Etiolated Shoots.

I. (a). Cryptomeria japonica.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
	c.c.			
Total nitrogen	1. 0.462	19.2	0.0359	7.77
	2. „	19.4		
Albuminoid nitrogen.	1. 0.462	9.4	0.0177	3.82
	2. „	9.6		
Asparagine nitrogen...	1. „	0.7	0.0012	0.28 = ½ Asp. N.
	2. „	0.6		
Nitrogen in phospho-tungstic precipitate.	1. „	4.2	0.0076	1.65
	2. „	4.0		

(b)

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
	c.c.			
Albuminoid nitrogen.	1. 0.517	10.4	0.0195	3.77
	2. „	10.6		
Nitrogen in phospho-tungstic precipitate.	1. „	3.8	0.00744	1.44
	2. „	4.2		

II. Gingko biloba.

(a). Etiolated shoots (dried on the 15th May).

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
	c.c.			
Total nitrogen	1. 0.387	10.7	0.0117	3.00
	2. „	10.9		
Albuminoid nitrogen.	1. 0.774	10.2	0.0111	1.44
	2. „	10.4		
Asparagine nitrogen...	1. „	1.7	0.0018	0.24 $=\frac{1}{2}$ Asp. N.
	2. „	1.7		
Nitrogen in phospho-tungstic precipitate.	1. „	1.1	0.0011	0.14
	2. „	0.8		

(b). Etiolated shoots were exposed to the day-light (from 15th May to 5th June).

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
	c.c.			
Total nitrogen	1. 0.473	13.1	0.0140	2.97
	2. „	12.9		
Albuminoid nitrogen.	1. 0.946	17.3	0.0188	1.99
	2. „	17.5		
Asparagine nitrogen...	1. „	1.3	0.0014	0.15 $=\frac{1}{2}$ Asp. N.
	2. „	1.3		
Nitrogen in phospho-tungstic precipitate.	1. „	1.2	0.0015	0.16
	2. „	1.5		

1 c.c. baryta water = 0.00108 g. N.

III. Pinus Thunbergii.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
	c.c.			
Total nitrogen	1. 0.474	24.2	0.0452	9.53
	2. „	24.4		
Albuminoid nitrogen.	1. 0.948	25.5	0.0279	2.94
	2. „	26.0		
Asparagine nitrogen...	1. „	10.1	0.0112	1.17 = ½ Asp. N.
	2. „	10.6		
Nitrogen in phospho-tungstic precipitate.	1. „	16.8	0.0178	1.88
	2. „	16.1		

1 c.c. baryta water = 0.00108 g. N.

IV. Decomposition products of the proteids prepared from the shoots of Pinus Thunbergii.

Boiled with 20% hydrochloric acid for 20 hours (with addition of 1% $SnCl_2$). The extract diluted to 250 c.c.

	Solutions used.	Baryta water replaced.	Nitrogen found.	Absolute quantity in total solution.	Ratio.
	c.c.				
Total nitrogen	25	25.2	0.0270	0.2700	100.0
	„	24.8			
Nitrogen directly precipitated by phospho-tungstic acid...	„	8.2	0.0089	0.0891	33.0
	„	8.3			
Nitrogen in ammonia	„	1.8	0.0020	0.0205	7.5
	„	2.0			
Nitrogen in organic bases ...	„	—	—	0.0686	25.5
	„	—			

1 c.c. baryta water = 0.00108 g. N.

On the Formation of Arginin in Coniferous Plants.

BY

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(PLATES. I.—VI.)

In my last article I have proved that the seeds of some coniferous plants contain certain proteids which produce by the action of acids large quantities of organic bases, especially arginin; and also that the shoots of these plants contain arginin in considerable quantities. The distribution of this substance seems to be very wide, and as far as our experience goes, it is found in almost all coniferous plants. So it is highly probable that this substance plays an important rôle in the metabolism of nitrogen compounds in coniferous plants, especially during germination. E. Schulze supposes that this substance arises not only from the hydrolytic decomposition of reserve proteids in the seeds during germination, but also from the transformation of other decomposition products.* So we may assume that this substance has an intimate relation to the decomposition and regeneration of proteids in the plant cells. As it seems to be very interesting to make a closer observation on this substance and to explain its fate more fully, I have directed my endeavours to the solution of the following questions:—

* Compare E. Schulze:—"Ueber die beim Umsatz der Proteinstoffe in den Keimpflanzen einiger Coniferae-Arten entstehenden Stickstoffverbindungen" (Zeitsch. f. Physiol. Chem. XXII. s. 445.) He says;—"Man muss vielmehr annehmen, dass die starke Anhäufung des Arginins erst eine Folge der Umwandlungen ist, denen die beim Proteinzerfall zuerst entstandenen Produkte im Stoffwechsel der Keimpflanzen unterlagen. Dass für das Stattfinden solcher Umwandlungen, welche selbstverständlich auch mit der Regeneration von Eiweissstoffen im Zusammenhang stehen können, auch die an anderen Keimpflanzen gemachten Beobachtungen sprechen, ist aus der vorhergehenden Abhandlung zu ersehen."

1. Is arginin synthetically formed from ammonium salts or nitrates offered to the coniferous plants, in a similar way as asparagine is formed in the other plants?

2. Is it also synthetically formed from ammonium salts or nitrates in plants not belonging to the Coniferae?

3. What is the effect of light and mineral nutriments upon the formation and transformation of this substance?

4. Is it the primary decomposition product of proteids only or is it also formed from other decomposition products?

5. Can it be used directly for the regeneration of proteids in the plant cells?

I. Arginin as Synthetical Product from Ammonium Salts.

The following experiments were made to settle the question whether arginin can be formed synthetically from the ammonium salts offered to the plants. The first three experiments were made with coniferous plants and the next four with plants not belonging to the Coniferae, to see whether there is any difference between the two sets.

A) CONIFEROUS PLANTS.

1. PINUS THUNBERGII.

Seeds of Pinus Thunbergii were sown in three wooden boxes containing purified sea sand, moistened with distilled water. On the 25th February they began to germinate and from that time on a half saturated gypsum solution was occasionally added to each box, to keep it constantly moist. On the 15th March, the shoots of one box were carefully removed from the sand, washed well, dried and analyzed. The other two were treated with the following solutions:—

1) Half saturated gypsum solution.

The shoots were kept in a warm house in full day-light, the temperature varying between 30°c and 15°c.

2) 2% ammonium chloride solution, half saturated with gypsum.*

After twelve days (on the 27th), they were removed from the sand, washed very well, until entirely free of ammonia, dried and analyzed.

As the temperature was too high, and the ammonium salt too concentrated, some of the shoots treated with the ammonium salt began to show signs of suffering. These affected shoots were rejected and only the healthy ones were analyzed.

	a) Shoots dried on the 15th March.	b) Shoot treated with ½ sat. gypsum for 12 days (15th-27th).	c) Shoots treated with 2% ammonium chloride, half saturated with gypsum for 12 days (15th-27th).
Number of shoots.	710	1656	1340
Length	6–8cm.	10–15	10–15
Total dry weight.	4.250g.	15.051	12.353
Dry weight of 100 shoots.	0.599g.	0.909	0.922

In 100 parts of dry matter.

	a)	b)	c)
Total nitrogen.	8.73	6.90	8.45
Albuminoid nitrogen.	3.42	3.18	3.35
Asparagine nitrogen.	1.24	1.15	1.20
Nitrogen in phospho-tungstic precipitate.	**1.81**	**0.38**	**1.55**
Other nitrogen	2.26	2.19	2.35

In 100 parts of total nitrogen.

	a)	b)	c)
Total nitrogen.	100.0	100.0	100.0
Albuminoid nitrogen.	39.2	46.1	39.6

* A 2% ammonium chloride solution is evidently too strong for the young shoots, but I prefered it, because a more dilute solution is too favorable to the growth of the shoots, and the absorbed ammonium salts may be converted into proteids too rapidly, the consequence being that no intermediate product can be detected. I had failed twice in this way; so it is necessary to give a concentrated solution, and to make the shoots suffer a little, so that the regeneration of proteids may be retarded. Otherwise it is too difficult to find out the intermediate product, stored up as such in the plant cells.

Asparagine nitrogen.	14.2	16.7	14.2
Nitrogen in phospho-tungstic precipitate.	**20.7**	**5.5**	**18.3**
Other nitrogen.	25.9	31.7	27.9

Every 100 shoots contain :—

	a)	b)	c)
Total nitrogen.	0.0523	0.0627	0.0779
Albuminoid nitrogen.	0.0210	0.0289	0.0309
Asparagine nitrogen.	0.0074	0.0105	0.0111
Nitrogen in phospho-tungstic precipitate.	**0.0109**	**0.0035**	**0.0143**
Other nitrogen.	0.0135	0.0199	0.0217

The shoots dried on the 15th March a) were not fully developed, and the reserve materials in the seeds were not yet completely transported to the growing shoots. Therefore the absolute quantity of every 100 shoots is considerably lower in a), but after 12 days, that is in b) and c), almost all reserve material was transported to the shoots. The considerable increase of the absolute quantity of nitrogen in c) compared with b) may be due to the absorption of the ammonium salt offered to the shoots. Anyhow, we see from the above tables that even when exposed to full day-light much organic bases* (that is, nitrogen precipitated by phospho-tungstic acid) are accumulated in the first stage of germination a), while they disappear very rapidly on further exposure to day-light, the disappearance being most probably due to their being directly used for the regeneration of proteids, as we see in the shoots b). But we observe that the shoots treated with ammonium chloride solution still contain a considerable quantity of organic bases c). The greater part of these organic bases must be synthetically formed from the ammonium salt absorbed by the shoots. We shall see the relation more clearly if we calculate the total nitrogen of a) as 100. Thus we have :—

* About 20% of the nitrogen must have been transported from the seeds to the shoots during the experiments (15th to 27th), and we see that in c) about 29% of the nitrogen had been absorbed from the ammonium salts offered.

	a)	b)	c)
Total nitrogen.	100.0	120.0	149.0
Albuminoid nitrogen.	39.2	55.3	59.6
Asparagine nitrogen.	14.2	20.0	21.1
Nitrogen in phospho-tungstic precipitate.	20.7	6.6	27.3
Other nitrogen.	25.9	38.1	41.4

We can now safely conclude that the greater part of the nitrogen absorbed as ammonium salts, is accumulated as organic bases in the shoots, and as we found no noticeable increase of asparagine even when treated with ammonium chloride solution, it is highly probable that in coniferous plants, organic bases (arginin !) are synthetically formed, instead of asparagine.

Plate I. shows the increase of organic bases (N. in phos. tungstic ppt.), especially arginin, in the shoots of Pinus Thunbergii by the addition of ammonium salts. The black lines refer to shoots treated with half saturated gypsum solution in the full day-light. The red lines refer to shoots treated with 0.5% ammonium chloride solution, half saturated with gypsum in the full day-light.

Note:—The total nitrogen was determined by Kjeldahl's method, and albuminoid nitrogen by that of Stutzer's. The filtrate of copper hydrate precipitate was acidified with sulphuric acid and some excess of phospho-tungstic acid solution was added, when a strong white precipitate was formed. The precipitate was collected on a filter, washed with 5% sulphuric acid, dried and subjected to Kjeldahl's method for nitrogen determination. The nitrogen in phospho-tungstic precipitate chiefly consists of that of organic bases.

The filtrate of phospho-tungstic precipitate served for the determination of asparagine nitrogen. For this purpose caustic baryta was added to the filtrate until the solution became alkaline and caused the phospho-tungstic acid in solution to precipitate. The solution was then filtered, and the filtrate was acidified with sulphuric acid to precipitate the excess of baryta as barium sulphate. To the filtrate from barium sulphate, was added some pure concentrated hydrochloric acid (10 c.c. conc. hydrochloric acid to each 100 c.c. of the solution), and the mixture boiled in a reflux cooler for 2 hours and distilled in the usual way, care being taken to neutralize the solution with caustic soda until it became faintly acid and then to make it slightly alkaline by the addition of a little magnesia.

It is also quite important to prove the absence of ammonia in the shoots, either mechanically adhering to them or preserved unchanged in the plant cells, because phospho-tungstic acid precipitates it together with the organic bases, and thus becomes a source of error. For the detection of ammonia, the powdered sample was extracted with cold water, and to the extract, a few drops of Nessler's reagent was added. But the

It is also quite important to investigate what organic base is formed by the assimilation of ammonium salts. As the germinating shoots of Coniferae produce large quantities of arginin, it is highly probable that it is the synthetical product in this case.

For isolation of the base, a powdered sample of the shoots (weighing 8.8 grams) treated with ammonium chloride solution, was extracted with about 300 c.c. of warm water, to the extract was added an excess of basic lead acetate, until no more precipitate was formed, filtered, and to the clear filtrate was added some sulphuric acid to remove the excess of lead; this filtrate of lead sulphate may contain bases. It was precipitated with phospho-tungstic acid, the precipitate was collected on a filter, washed with 5% sulphuric acid, pressed between the filter and the precipitate then decomposed with baryta, and the base was crystallized as basic arginin silver nitrate, according to S. G. Hedin's method. It was thus found that there is formed a tolerably large quantity of arginin silver nitrate. Its quantity was found to be more than 0.4 grams, that is, more than half of the total organic bases consists of arginin.*

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presence of a little sugar and other reducing compounds in the plants interferes with the reaction; and as no other certain test could be made, I adopted the following procedure:—The phospho-tungstic precipitate was decomposed at the ordinary temperature with caustic baryta, filtered, and the baryta in the filtrate was removed by carbonic acid, and filtered, the filtrate was tested for ammonia by Nessler's reagents, but no colouration!; so we may be sure that no ammonia was present in the phospho-tungstic precipitate. Also, the filtrate was tested for peptone by adding an excess of strong caustic soda and one drop of copper sulphate; but no violet colouration! Therefore we may conclude safely that the phospho-tungstic precipitate consisted of organic bases only.

Besides the above tests, I made also quantitative determination of ammonia (though the absence of it was formerly proved!) in the following way;—a sample of 1–2 grams was warmed in 50 c.c. distilled water at 50–60° C. for half an hour, cooled, and after cooling, basic lead acetate was added in excess, filtered, to the filtrate was added some sulphuric acid to remove the dissolved lead, filtered, the filtrate was nearly neutralized with dilute caustic soda and some magnesia was added until it was slightly-alkaline, and distilled off. I found in this way that a little ammonia always comes off, but its quantity being less than 0.1% of the dry matter. I found afterwards that this ammonia comes from the slight decomposition of bases and amides and was not present previously.

* As the basic arginin silver nitrate easily undergoes reduction and changes to the neutral silver nitrate salt, which is twelve times more soluble in water than the basic salt, it is certain that a tolerable quantity of arginin still remains in the mother liquor. We may therefore assume that almost $\frac{2}{3}$ or more consists of arginin.

I have also tried to isolate arginin from the shoots treated with gypsum solution only (b), but I found only a very small quantity of arginin, while in the shoots dried on the 15th March (a), there was a tolerably large quantity. In both cases, however, it was found to be less than in the shoots treated with ammonium salts (c).

2. Second experiment with the shoots of Pinus Thunbergii.

Young shoots, grown in the purified sea sand, were carefully removed from the sand ; one portion was directly dried, and the other portion was divided into 2 parts and put in the following solutions :—

1) Half saturated gypsum solution.

2) 0.5% ammonium choride solution, half saturated with gypsum.

This second portion was kept in the laboratory in diffuse day-light, at the temperature ranging between 10° C and 16° C. After 12 days (from 27th March to 8th April), the shoots were removed from the solutions, washed very well, until no trace of ammonia was left, dried and analyzed. During the experiment, the solutions were renewed four times to prevent bacterial turbidity to appear. But, as toward the end of the experiment, the shoots put in the ammonium chloride solution began to suffer, while those kept in the gypsum solution were still healthy, I could not keep them any longer.

The analysis gave the following results :—

	a) Shoots dried on the 27th March.	b) Shoots kept in gypsum solution for 12 days (27th March-8th April).	c) Shoots kept in ammonium chloride solution for 12 days (27th March-8th April).
Number of shoots.	1656	400	446
Length.	12–15cm.	12–15	12–15
Total dry weight.	15.051g.	3.955	4.296
Dry weight of every 100 shoots.	0.909g.	0.989	0.963

In 100 parts of dry matter.

	a)	b)	c)
Total nitrogen.	6.90	6.64	7.88
Albuminoid nitrogen.	3.29	3.13	3.17
Asparagine nitrogen.	1.15	1.16	1.42
Nitrogen in phospho-tungstic precipitate.	**0.38**	**0.45**	**1.37**
Other nitrogen.	2.08	1.90	1.92

In 100 parts of total nitrogen.

	a)	b)	c)
Total nitrogen.	100.0	100.0	100.0
Albuminoid nitrogen.	47.7	47.1	40.2
Asparagine nitrogen.	16.7	17.5	18.0
Nitrogen in phospho-tungstic precipitate.	**5.5**	**6.8**	**17.4**
Other nitrogen.	30.1	28.6	24.4

Every 100 shoots contain :—

	a)	b)	c)
Total nitrogen.	0.0647	0.0656	0.0759
Albuminoid nitrogen.	0.0309	0.0310	0.0306
Asparagine nitrogen.	0.0108	0.0115	0.0137
Nitrogen in phospho-tungstic precipitate.	**0.0036**	**0.0045**	**0.0132**
Other nitrogen.	0.0195	0.0187	0.0185

Calculating the total nitrogen of a) as 100, we have :—

	a)	b)	c)
Total nitrogen.	100.0	101.4	117.3
Albuminoid nitrogen.	47.7	47.9	47.2
Asparagine nitrogen.	16.7	17.7	21.1
Nitrogen in phospho-tungstic precipitate	**5.5**	**6.9**	**20.4**
Other nitrogen.	30.1	28.9	28.6

We shall see the difference more clearly in the following table :—

	a)	b)	c)
Total nitrogen.	100.0	101.4	117.3
Albuminoid nitrogen.	100.0	100.1	99.0
Asparagine nitrogen.	100.0	106.3	127.0
Nitrogen in phospho-tungstic precipitate.*	**100.0**	**125.0**	**370.2**
Other nitrogen.	100.0	96.0	94.8

This result is more decisive than that of the former experiment. Almost all the ammonia absorbed by the shoots c), amounting to 17% of the total nitrogen, was converted into the organic base, and there was only a little increase of asparagine.

Plate II. shows the increase of organic bases, especially arginin, in the shoots of Pinus Thunbergii by the addition of ammonium salts. The black lines refer to shoots cultured in the half saturated gypsum solution, in the diffused day-light. The red lines refer to shoots cultured in 0.5% ammonium chloride solution, half saturated with gypsum, in the diffused day-light.

3. Cryptomeria japonica.

Etiolated shoots of Cryptomeria japonica, grown in the purified sea sand in a warm house, and 6-8cm. high (10th April), were exposed to direct sun-light in two lots. To one lot, a half saturated gypsum solution was added and to the other a 0.5% ammonium chloride solution half saturated with gypsum. After

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* In this case also, the absence of ammonia and peptone in the phospho-tungstic precipitate was proved in the same way as in the former experiment.

As the sample was too little, the isolation of arginin was impossible; but the following examination was made;—From 1.5 grams of each of the three samples phospho-tungstic precipitate was obtained in the usual way, and the basic arginin silver salt was microscopically examined. I found only a very little of arginin crystals in a) and b), but three or four times more in c). So I conclude that the arginin was synthetically formed from ammonium salts in c).

10 days (on the 20th April) they were carefully removed from the sand, washed well to free them from all trace of ammonia, dried and analyzed.*

	a) Shoots, treated with gypsum for 10 days (10-20th April).	b) Shoots, treated with ammonium chloride solution for 10 days (10-20th April).
Number of shoots.	727	667
Length.	8–10 cm.	8–10 cm.
Total dry weight.	1.590 g.	1.538
Dry weight of 100 shoots.	0.219 g.	0.231

In 100 parts of dry matter.

	a)	b)
Total nitrogen.	6.69	7.62
Albuminoid nitrogen.	3.17	3.05
Asparagine nitrogen.	0.42	0.62
Nitrogen in phospho-tungstic precipitate.	**1.12**	**1.77**
Other nitrogen.	1.98	2.18

In 100 parts of total nitrogen.

	a)	b)
Total nitrogen.	100.0	100.0
Albuminoid nitrogen.	47.4	40.0
Asparagine nitrogen.	6.3	8.1
Nitrogen in phospho-tungstic precipitate.	**16.7**	**23.3**
Other nitrogen.	29.6	28.6

Every 100 shoots contained :—

	a)	b)	Ratio. a)	b)
Total nitrogen.	0.0147	0.0176	100:	120.0

* During the experiment, the temperature varied between 15° C. and 38° C. The shoots treated with the ammonium chloride solution began to suffer, (perhaps owing to the concentration of the solution due to evaporation) and so they could not be kept any longer.

Albuminoid nitrogen.	0.0069	0.0070	100: 101.5
Asparagine nitrogen.	0.0009	0.0014	100: 155.7
Nitrogen in phospho-tungstic precipitate.*	**0.0025**	**0.0041**	**100: 166.7**
Other nitrogen.	0.0043	0.0050	100: 116.1

In this case, some increase of asparagine nitrogen was also observed, but the absolute increase was only $\frac{1}{3}$ as compared with the increase of organic bases.

From the above three experiments we may safely conclude that the ammonium salts offered to the coniferous plants are chiefly converted into arginin and only a small quantity of asparagine is formed synthetically, which latter is the chief product of the assimilation of ammonium salts in other plants.† Now let us try to see whether arginin can be synthetically formed in other plants, not belonging to the Coniferae.

B) PLANTS, NOT BELONGING TO THE CONIFERAE.

1. Brassica rapa.

On the 7th November, young plants, 10-12 cm. high, grown in the field was carefully removed, washed well, a portion of it was directly dried and the other portion was divided into 2 parts and grown in the following solutions:—

1) Half saturated gypsum solution.

2) 0.5% ammonium sulphate solution, half saturated with gypsum.

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* Neither ammonia nor peptone was likewise found in this case.

† Compare my article "On the Formation of Asparagine etc." Bull. II. No. 7. of the Coll. of Agri., Tokyo.

I also made the same experiment twice with Pinus Thunbergii, adding 0.2% sodium nitrate instead of the 0.5% ammonium chloride solution, but failed to prove the increase of arginin, because by exposure to direct sun-light the nitrate offered to the shoots was too quickly transformed into proteids, and no intermediate product remained in the plant cells.

They were kept in the laboratory in diffuse day-light, the temperature varying between 10°C. and 15°C. After 7 days, they were removed from the solutions, washed well, dried and analyzed. The solutions were renewed twice and no bacterial growth was observed. Toward the end of the experiment, some of the leaves began to suffer, and the top became yellow. These weaklings were removed. The analysis gave the following results :—

In 100 parts of dry matter :—

	a) Plants, dried on 7th Nov.	b)* Plants kept in gypsum solution (7-14th).	c)* Plants kept in ammonium sulphate solution (7-14th).
Total nitrogen.	4.90	6.12	8.35
Albuminoid nitrogen.	4.21	2.88	3.68
Asparagine nitrogen.	0.18	1.48	2.52
Nitrogen in phospho-tungstic precipitate.	0.28	0.30	0.36
Other nitrogen.	0.23	1.46	1.79

Here we found no increase of organic bases ; on the contrary, much increase of asparagine was observed.

2. Hordeum distichon.

On the 7th November, young barley plants 10 cm. high, were carefully removed from the field and washed well. A portion was directly dried, while the other portion was divided into two lots and grown in the following solutions :—

1) Half saturated gypsum solution.

2) 0.5% ammonium sulphate solution, half saturated with gypsum.

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* During the experiment, much decomposition of proteids took place, in consequence of which much increase of asparagine was observed in the control plants b). The increase was, however, much greater in c), which must be due partly to synthetical formation from the ammonium salt.

The higher percentage of total nitrogen in b) was due to the decrease of dry matter during the experiment and in c) and partly to the absorption of ammonium salt by the roots.

Here we need not calculate the absolute quantity of 100 shoots etc., the result being too decisive.

They were kept in the laboratory in diffuse day-light, the temperature ranging between 10° C. and 15° C. After 7 days they were taken out from the solutions, washed, dried and analyzed.

In 100 parts of dry matter :—

	a) Plants, dried on the 7th.	b)* Plants, kept in gypsum solution for 7 days (7th-14th).	c)* Plants, kept in ammonium sulphate solution for 7 days (7th-14th).
Total nitrogen.	5.40	5.56	6.60
Albuminoid nitrogen.	4.52	3.04	4.27
Asparagine nitrogen.	0.30	1.52	1.42
Nitrogen in phospho-tungstic precipitate.	**0.42**	**0.52**	**0.64**
Other nitrogen.	0.16	0.48	0.27

We found again no increase of organic bases in this case.

3. Second experiment with the shoots of Barley.

(Hordeum distichon.)

On the 2nd November, grains of barley were sown in four wooden boxes containing purified saw dust. Two were kept in the dark and the other two in full day-light in a warm house. Germination had fairly set in and on the 14th the shoots were 6-8 cm. high. One of the boxes of either set was then treated with a half saturated gypsum solution and the other with a 0.5% ammonium sulphate solution half saturated with gypsum. After 7 days (on the 21st) the shoots were carefully removed from the saw dust, washed well, dried and analyzed. No ammonia was stored up in the shoots. The analysis gave the following results :—

In 100 parts of dry matter.
Plants kept in :—

* During the experiment much decomposition of proteids took place, in consequence of which much asparagine was formed. But we found no difference in the amount of asparagine in b) and c); the asparagine once formed being perhaps transformed into proteids.

	Dark.		Light.	
	a) Treated with gypsum.	b) Treated with ammonium sulphate.	a) Treated with gypsum.	b) Treated with ammonium sulphate.
Total nitrogen.	3.82	4.58	3.72	4.40
Albuminoid nitrogen.	2.48	2.56	2.21	2.40
Asparagine nitrogen.	**0.70**	**0.90**	**0.94**	**1.52**
Nitrogen in phospho-tungstic precipitate.	**0.26**	**0.36**	**0.24**	**0.27**
Other Nitrogen.	0.38	0.76	0.33	0.21

We see that the shoots treated with the ammonium sulphate solution, produced both in the dark and in day light, much asparagine, but no organic bases.

4. CUCURBITA MELOPEPO (pumpkin).

On the 2nd November, pumpkin seeds were sown in purified sea sand; one portion was kept in the dark and the other portion in day light in a warm house, where the temperature ranged between 15° C. and 35° C. On the 16th, the shoots kept in the dark were 5-13 cm. high and those kept in day light 4-8.5 cm. high; then one-half of the shoots in both plots was removed from the sand, washed and analysed, and the remaining half was treated with a 0.5% ammonium sulphate solution, half saturated with gypsum. After 7 days (22nd) the shoots in the dark were 18 cm. high, while those in day light were 12 cm. high. Both were then removed from the sand, washed well, dried and analyzed.

In 100 parts of dry matter.

Shoots kept in :—

	Dark.		Light.	
	Control (dried on the 16th).	Treated with ammonium sulphate (16-22).	Control.	Treated with ammonium sulphate (16-22).
Total nitrogen.	6.74	8.65	6.96	8.62
Albuminoid nitrogen.	5.85	5.59	5.72	4.87
Asparagine nitrogen.	**0.28**	**1.36**	**0.38**	**2.09**

Nitrogen in phospho-tungstic precipitate.	0.17	0.49	0.30	0.60
Other nitrogen.	0.44	1.21	0.56	1.06

During the experiment, the shoots treated with the ammonium sulphate solution grew rapidly, in consequence of which much decomposition of proteids and accumulation of asparagine must have taken place. Therefore the considerable increase of asparagine both in the dark and in day-light may be due partly to the decomposition of proteids and partly to the direct assimilation of the ammonium salt. Only a little increase of organic bases in the shoots treated with the ammonium salt was observed.

The above four experiments suffice to prove that the plants, not belonging to the Coniferae, can not produce arginin from ammonium salts, and asparagine alone seems to play an important rôle in the metabolism of nitrogen compounds. This result agrees exactly with that of my former investigation (Compare my article, "On the Formation of Asparagine in Plants, etc." Bull. Agr. Coll., Tokyo. Vol. II. No. 7.)

II. On the Influence of Light and Mineral Nutriments upon the Formation and Transformation of Arginin.

Since the investigation of E. Schulze and of myself have shown that in the shoots of Coniferae, a considerable amount of organic bases, especially arginin, is formed, and since further my last investigation has demonstrated that the bases are not only formed by the decomposition of reserve proteids, but also synthetically from ammonium salts, it seems highly probable that arginin in Coniferae plays an important rôle in the formation of proteids. So I tried to observe more closely the behaviour of it in the shoots during the germination process, and further the influence of light and mineral nutriments on it. As the object of the experiment, young shoots of Pinus Thunbergii were used. The seeds were soaked with water for 3 days and then sown in purified sea sand moistened with distilled water, and kept in a warm house, where the temperature ranged between 15° C. and 30° C. The sand was

occasionally irrigated with a half saturated gypsum solution to keep it constantly moist. On the 24th February the germination had fairly set in, and the shoots were subjected to the following treatments:

1. The shoots were kept in *perfect darkness* until the 23rd March (4 weeks after germination) and the gradual change in nitrogen compounds was observed.

	a) 2 weeks shoots (dried on the 9th March).	b) 4 weeks shoots (dried on the 23rd March).
Number of Shoots.	1220	1224
Length.	6-8 cm.	10-15 cm.
Total dry weight.	7.437 g.	7.730
Dry weight of 100 shoots.	0.610 g.	0.631

In 100 parts of dry matter.

	a)	b)
Total nitrogen.	9.13	9.53
Albuminoid nitrogen.	3.11	2.94
Asparagine nitrogen.	2.30	2.35
Nitrogen in phospho-tungstic precipitate.	**1.60**	**1.88**
Other nitrogen.	2.12	2.36

In 100 parts of total nitrogen.

	a)	b)
Total nitrogen.	100.0	100.0
Albuminoid nitrogen.	34.1	30.9
Asparagine nitrogen.	25.2	24.7
Nitrogen in phospho-tungstic precipitate.	**17.5**	**18.7**
Other nitrogen.	23.2	25.7

Every 100 shoots contained:—

	a)	b)
Total nitrogen.	0.0557	0.0601
Albuminoid nitrogen.	0.0190	0.0186
Asparagine nitrogen.	0.0140	0.0148
Nitrogen in phospho-tungstic precipitate.	**0.0098**	**0.0119**
Other nitrogen.	0.0129	0.0149

Calculating the total nitrogen of a) as 100 we have :—

	a)	b)
Total nitrogen.	100.0	108.0
Albuminoid nitrogen.	34.1	33.3
Asparagine nitrogen.	25.2	26.6
Nitrogen in phospho-tungstic precipitate.	**17.5**	**21.3**
Other nitrogen.	23.2	26.3

In the 2 weeks shoots, the reserve material in the seed has not yet been completely transported to the shoots and a portion still remained undissolved. Therefore we find a little increase of total nitrogen in the 4 weeks shoots. We see from the above results that a considerable amount of organic bases is already formed in the first 2 weeks and after-wards increases only very gradually.

Plate III. shows the change of various nitrogenous compounds (especially nitrogen in phospho-tungstic precipitate) during the developement of the shoots of Pinus Thunbergii in perfect darkness.

2. The shoots were kept in *full day-light* and harvested in 4 different periods to see the change in nitrogen compounds.

	a)	b)	c)	d)
Shoots dried after	14 (9th March)	19 (14th)	28 (23rd)	32 days (27th)
Number of shoots.	1348	710	780	1656
Length.	6–8	8–10	10–15	12–16
Total dry weight.	8.672	4.750	7.185	15.533
Dry weight of 100 shoots.	0.643	0.669	0.921	0.938

In 100 parts of dry matter.

	a)	b)	c)	d)
Total nitrogen.	8.81	8.73	6.95	6.90
Albuminoid nitrogen.	3.51	3.81	3.27	3.29
Asparagine nitrogen.	1.32	1.24	1.26	1.15
Nitrogen in phospho-tungstic precipitate.	**1.54**	**1.62**	**0.44**	**0.38**
Other nitrogen.	2.44	2.06	1.98	2.08

In 100 parts of total nitrogen

	a)	b)	c)	d)
Total nitrogen.	100.0	100.0	100.0	100.0
Albuminoid nitrogen.	39.8	43.6	47.1	47.7
Asparagine nitrogen.	15.0	14.2	18.1	16.7
Nitrogen in phospho-tungstic precipitate.	**17.5**	**18.6**	**6.3**	**5.5**
Other nitrogen.	27.7	23.6	28.5	30.1

Every 100 shoots contained :—

	a)	b)	c)	d)
Total nitrogen.	0.0567	0.0584	0.0640	0.0647
Albuminoid nitrogen.	0.0226	0.0255	0.0301	0.0309
Asparagine nitrogen.	0.0085	0.0083	0.0116	0.0108
Nitrogen in phospho-tungstic precipitate.	**0.0099**	**0.0107**	**0.0040**	**0.0036**
Other nitrogen.	0.0157	0.0137	0.0182	0.0195

Calculating the total nitrogen of a) as 100 we have :—

	a)	b)	c)	d)
Total nitrogen.	100.0	103.0	112.9	114.2
Albuminoid nitrogen.	39.8	45.0	53.2	54.3
Asparagine nitrogen.	15.0	14.6	20.5	19.0
Nitrogen in phospho-tungstic precipitate.	**17.5**	**19.2**	**7.1**	**6.3**
Other nitrogen.	27.7	24.2	32.2	34.2

We see from the above results that the shoots produce, even in *full day-light*, in the first stage of germination much organic bases, the quantity of which is almost equal to that which is produced in the *dark*. They increased for 19 days after germination, but afterwards decreased rapidly with the increase of proteids. The transformation of the bases is *far greater and quicker* than that of asparagin.*

* Prianischnikow found in his experiment ("Zur Kenntniss der Keimungsvorgänge bei Vicia sativa," Landw. Vers. Stationen Bd. XLV, 1896, S. 247), that the organic bases in the germinating shoots of Vicia sativa undergo no noticeable change both in the dark and in day-light. Thus, he says, "Die in Phosphorwolframsäure-Niederschlag eingegangene Stickstoffmenge zeigt keine besondere Schwankungen, indem sie etwa $\frac{1}{12}$

Plate IV. shows the change of various nitrogenous compounds during the development of the shoots of Pinus Thunbergii, under the full day-light.

3. The shoots were at first kept in the *dark* and afterwards exposed to *full day-light*, and the change in nitrogen compounds observed.

a) Etiolated shoots dried on the 9th March.....
14 days after germination.

b) Etiolated shoots dried on the 23rd March.....
28 days after germination.

c) Etiolated shoots were exposed to *full day-light* from 9th to 23rd March, that is, kept in dark the first 2 weeks and for the next 2 weeks exposed to day-light and treated with half saturated gypsum solution.

d) Treated in the same way as c) and with a solution containing 0.1% K_2HPO_4 0.1%KH_2PO_4 0.1% $MgSO_4$, half saturated with gypsum.

Analysis gave the following results :—

	a)	b)	c)	d)
Number of shoots.	1220	1224	1500	1342
Length.	8–10	15–17	10–12	10–12
Total dry weight.	7.437	7.730	11.668	10.284
Dry weight of 100 shoots.	0.610	0.631	0.778	0.766

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des Gesamtstickstoffs beträgt." The chief organic bases in Vicia sativa are cholin, betain and guanidin etc. We may also suppose that the organic bases, except arginin, play no important rôle in coniferous plants, and the principal cause of the increase or decrease of nitrogen in phospho-tungstic precipitate is chiefly due to the change of arginin.

Prianischnikow also found that during the first stage of germination (first 10 days) the greater part of proteids decomposes, and amido-compounds, especially asparagine, accumulate in large quantities, but that afterwards, both the decomposition of proteids and the accumulation of asparagine etc. are only very gradual. The change in nitrogen compounds proceeds in the first period almost equally both in the dark and in day-light. If we compare my result with that of Prianischnikow, we shall find out a closer resemblance between asparagine and arginin in the mode of formation and transformation into proteids and in its behavior toward light.

In 100 parts of dry matter.

	a)	b)	c)	d)
Total nitrogen.	9.13	9.53	8.11	8.19
Albuminoid nitrogen.	3.11	2.94	3.18	3.25
Asparagine nitrogen.	2.30	2.35	1.64	1.60
Nitrogen in phospho-tungstic precipitate.	**1.60**	**1.88**	**1.11**	**0.96**
Other nitrogen.	2.12	2.36	2.18	2.38

In 100 parts of total nitrogen.

	a)	b)	c)	d)
Total nitrogen.	100.0	100.0	100.0	100.0
Albuminoid nitrogen.	34.1	30.9	39.2	39.7
Asparagine nitrogen.	25.2	24.7	20.2	19.5
Nitrogen in phospho-tungstic precipitate.	**17.5**	**18.7**	**13.7**	**11.7**
Other nitrogen.	23.2	25.7	26.9	29.1

Every 100 shoots contained :—

	a)	b)	c)	d)
Total nitrogen.	0.0557	0.0601	0.0621	0.0637
Albuminoid nitrogen.	0.0190	0.0186	0.0244	0.0249
Asparagine nitrogen.	0.0140	0.0148	0.0127	0.0123
Nitrogen in phospho-tungstic precipitate.	**0.0098**	**0.0119**	**0.0085**	**0.0075**
Other nitrogen.	0.0129	0.0149	0.0167	0.0191

Calculating the total nitrogen of a) as 100 we have :—

	a)	b)	c)	d)
Total nitrogen.	100.0	108.0	111.6	114.5
Albuminoid nitrogen.	34.1	33.3	43.8	44.7
Asparagine nitrogen.	25.2	26.6	22.5	22.0
Nitrogen in phospho-tungstic precipitate.	**17.5**	**21.3**	**15.3**	**13.4**
Other nitrogen.	23.2	26.8	30.0	34.4

Here we observe that when the etiolated shoots were exposed to full day-light organic bases decreased with the increase of proteids ; but in this case, the decrease of the bases

was not so remarkable as in the 2 former experiments. This may be explained by considering that the shoots, having at first been kept in the dark, lost the greater part of carbohydrates by respiration and also that the chlorophyll bodies being decomposed by keeping in the dark, the assimilation of the carbonic acid gas did not proceed so energetically as to prepare enough carbohydrates necessary for the regeneration of arginin into proteids. Also the energy of living protoplasm might have been greatly diminished by keeping it in the dark. Therefore if I had kept them still longer then, the shoots would have recovered from the abnormal condition and much decrease of the bases would have been observed! We found also that the shoots treated with mineral nutriments transformed much organic bases into proteids.

Plate V. shows the influence of light upon the change of nitrogenous compounds in the shoots of Pinus Thunbergii. The shoots were kept for 14 days in perfect darkness and a portion was afterwards, exposed to the day-light for 14 days, while the other portion was still kept in the dark. The black lines refer to shoots kept in the dark until the end of the experiment. The red lines refer to shoots, previously kept in the dark, was exposed to the full day-light for 14 days.

4. The shoots which was first exposed to *full day-light*, were afterwards kept in *the dark*, and the change in nitrogen compounds observed :—

a) The shoots kept in full day-light until 15th March (18 days after germination).

b) The shoots were afterwards kept in the dark, from 15th to 27th, with a half saturated gypsum solution.

	a)	b)
Number of shoots.	710	560
Length.	8–10 cm.	10–12
Total dry weight.	4.750	3.819
Dry weight of 100 shoots.	0.669	0.681

In 100 parts of dry matter.

	a)	b)
Total nitrogen.	8.73	9.16

Albuminoid nitrogen.	3.81	3.13
Asparagine nitrogen.	1.24	1.58
Nitrogen in phospho-tungstic precipitate.	1.62	1.59
Other nitrogen.	2.06	2.81

In 100 parts of total nitrogen.

	a)	b)
Total nitrogen.	100.0	100.0
Albuminoid nitrogen.	43.6	34.2
Asparagine nitrogen.	14.2	17.2
Nitrogen in phospho-tungstic precipitate.	18.6	17.4
Other nitrogen.	23.6	31.2

Every 100 shoots contained :—

	a)	b)
Total nitrogen.	0.0584	0.0624
Albuminoid nitrogen.	0.0255	0.0213
Asparagine nitrogen.	0.0083	0.0108
Nitrogen in phospho-tungstic precipitate.	0.0109	0.0108
Other nitrogen.	0.0137	0.0191

Calculating the total nitrogen of a) as 100, we have :—

	a)	b)
Total nitrogen.	100.0	106.8
Albuminoid nitrogen.	43.6	36.5
Asparagine nitrogen.	14.2	18.4
Nitrogen in phospho-tungstic precipitate.	18.6	18.6
Other nitrogen.	23.6	32.7

Here, by keeping in the dark, some decrease of proteids and increase of asparagine and other amido-compounds was observed but no increase of organic bases.

Plate VI. shows the influence of light upon the change of nitrogenous compounds. Shoots were at first exposed to the full day-light for 19 days, then a portion was kept in the dark for 9 day, while the other portion was still exposed to the day-light. The black lines refer to shoots exposed to the full day-light. The red lines refer to shoots afterwards kept in the dark for 9 days.

The chief results obtained from the above four experiments are :—

1). Arginin accumulates in a large quantity, in the dark as well as in full day-light, in the first stage of germination but disappears quickly when the shoots are further exposed to day-light, most probably being directly used for the regeneration of proteids. It, however, increases gradually when the shoots are kept still further in the dark.

2). The formation and transformation of arginin in coniferous plants are far greater and quicker than those of asparagine.

3). The addition of mineral nutriments accelerates the transformation of arginin.

4). Asparagine is also present in a tolerably large quantity in the shoots of coniferous plants. It must have also an important function.

Although we have not been able to prove experimentally the transformation of other amido-compounds into arginin, yet we can deduce its probability from the following facts :—

a) In the shoots, we find sometimes so much arginin that we can not regard it as merely the primary decomposition product. Thus I found in the shoots of Cryptomeria japonica about 21% of the total nitrogen in the form of organic bases ; while, when the proteids of the seed are treated with dilute or strong hydrochloric acid, the nitrogen of the organic bases amounts to only 15.5% of the whole. Therefore if arginin came only from the hydrolytic decomposition of proteids, then we should never find more than 15.5% of the total nitrogen in the shoots. Further, I found that the proteids prepared from the shoots of Pinus Thunbergii, have also the same chemical nature, and produce, by the action of hydrochloric acid, almost the same quantity of organic bases as those of the seeds. This fact may hold good also in the shoots of other Coniferae. Now

in the shoots of Cryptomeria I found still 49% of the total nitrogen in the form of albuminoid nitrogen, from which we can obtain at least $49 \times 15.5\% = 7.6\%$ organic bases. If we suppose that the entire proteids in the shoots were split, then we must have at least $21. + 7.6 = 28.6\%$ of organic bases. Such a large quantity of the bases can never come from mere hydrolytic decomposition!*

b) As I have already shown that the coniferous plants can converts ammonium salts into arginin, so it is highly probable that the ammonia formed by the decomposition of other amido-compounds in the plant cells may be easily converted into arginin, just in the same way as asparagine is formed in the other plants. The question whether arginin is formed at the cost of asparagine or whether the former can be converted into asparagine before it is used for the regeneration of proteids, is still left open.

SUMMARY AND CONCLUSIONS.

1. Arginin in coniferous plants not only comes from the decomposition of proteids but also can be synthetically formed from the ammonium salts (and also from nitrates!) offered to the plants.

2. Plants not belonging to the Coniferae do not produce arginin by the assimilation of ammonium salts, asparagine being the only product.

3. The synthetical formation of arginin proceeds in full as well as in diffuse day-light. But whether it is formed in the dark is not yet proved.

4. Arginin accumulates in a large quantity in the shoots of Coniferae in the dark as well as in full day-light in the first

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* E. Schulze found in the etiolated shoots of Picea excelsa 54.6% albuminoid nitrogen and 29.3% nitrogen in phospho-tungstic precipitate. But by the action of hydrochloric acid, he found that about 30% of the total nitrogen comes as the nitrogen in phospho-tungstic precipitate; so we can get about 30% nitrogen in phospho-tungstic precipitate only when all proteids are decomposed, if we assume that the bases are formed only by the hydrolytic decomposition. But we have here still 54.6% of nitrogen in the form of proteids which can split off at least $54.6 \times 30\% = 16.4\%$ nitrogen in phospho-tungstic precipitate. (Compare E. Schulze:—Zeits. f. Physiol. Chem. XXII. 1896. S. 441.)

stage of germination, but soon diminishes on further exposure to light and gradually increases on further sojourn in the dark. Its transformation into proteids under the influence of light can be accelerated by the addition of mineral nutriments.

5. Although the greater part of arginin in the shoots of Coniferae comes from the hydrolytic decomposition of reserve proteids, yet a portion of it must come also from the transformation of other amido-compounds, that is, it is not only a primary product, but also a secondary or transitory product.

6. Arginin is probably directly used for the regeneration of proteids; but its relation to other amido-compounds still needs further elucidation.

ANALYTICAL DATA.

I. Arginin as the Assimilation product from Ammonium salts.

A). CONIFEROUS PLANTS.

1. PINUS THUNBERGII.

TOTAL NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.433	20.6	0.0387	8.73
	2. „	20.8		
b)	1. 0.480	18.0	0.0331	6.90
	2. „	17.5		
c)	1. 0.469	21.2	0.0396	8.45
	2. „	21.3		

ALBUMINOID NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.886	16.2	0.0303	3.42
	2. „	16.4		
b)	1. 0.960	16.5	0.0305	3.18
	2. „	16.3		
c)	1. 0.938	16.9	0.0314	3.35
	2. „	16.9		

ASPARAGINE NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen $=\frac{1}{2}$ Asp. N.
		c.c.		
a)	1. 0.886	3.1	0.0056	0.62
	2. „	2.8		
b)*	1. 0.960	5.2	0.0055	0.57
	2. „	5.0		
c)	1. 0.938	3.0	0.0056	0.60
	2. „	3.0		

NITROGEN IN PHOSPHO-TUNGSTIC PRECIPITATE.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.886	8.5	0.0160	1.81
	2. „	8.7		
b)*	1. 0.960	3.5	0.0037	0.38
	2. „	3.3		
c)	1. 0.938	7.5	0.0145	1.55
	2. „	7.4		

1 c.c. baryta water =0.00189 gram nitrogen.

* 1 c.c. baryta water =0.00108 gram nitrogen.

2. Pinus Thunbergii.

TOTAL NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of Nitrogen.
		c.c.		
a)	1. 0.480	18.0	0.0331	6.90
	2. „	17.5		
b)	1. 0.466	16.5	0.0309	6.64
	2. „	16.8		
c)	1. 0.459	19.5	0.0359	7.83
	2. „	19.1		

ALBUMINOID NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.960	29.0	0.0316	3.29
	2. „	29.4		
b)	1. 0.932	26.8	0.0292	3.13
	2. „	27.2		
c)	1. 0.918	26.8	0.0292	3.17
	2. „	27.2		

ASPARAGINE NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen $= \frac{1}{2}$ Asp. N.
		c.c.		
a)	1. 0.960	5.2	0.0055	0.57
	2. „	5.0		
b)	1. 0.932	4.9	0.0054	0.58
	2. „	5.0		
c)	1. 0.918	6.0	0.0065	0.71
	2. „	6.1		

NITROGEN IN PHOSPHO-TUNGSTIC PRECIPITATE.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.960	3.5	0.0037	0.38
	2. „	3.3		
b)	1. 0.932	3.8	0.0042	0.45
	2. „	4.0		
c)	1. 0.918	11.4	0.0125	1.37
	2. „	11.0		

1 c.c. baryta water = 0.00108 gram nitrogen.

3. CRYPTOMERIA JAPONICA.

TOTAL NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.176	10.8	0.0118	6.69
	2. „	11.0		
b)	1. 0.182	12.9	0.0138	7.62
	2. „	12.7		

ALBUMINOID NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.433	12.7	0.0137	3.17
	2. „	12.7		
b)	1. 0.421	11.5	0.0129	3.05
	2. „	12.3		

ASPARAGINE NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen $= \frac{1}{2}$ Asp. N.
		c.c.		
a)	1. 0.433	0.8	0.0009	0.21
	2. „	0.8		
b)	1. 0.421	1.1	0.0013	0.31
	2. „	1.3		

NITROGEN IN PHOSPHO-TUNGSTIC PRECIPITATE.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.433	4.3	0.0049	1.12
	2. „	4.7		
b)	1. 0.421	6.8	0.0075	1.77
	2. „	7.0		

1 c.c. baryta water = 0.00108 gram nitrogen.

B) PLANTS NOT BELONGING TO THE CONIFERAE.

1. Brassica rapa.

TOTAL NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.434	8.6	0.0212	4.90
	2. „	8.6		
b)	1. 0.432	10.5	0.0264	6.12
	2. „	10.9		
c)	1. 0.438	14.9	0.0366	8.35
	2. „	14.7		

ALBUMINOID NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.868	14.6	0.0366	4.21
	2. „	14.9		
b)	1. 0.866	10.0	0.0250	2.88
	2. „	10.2		
c)	1. 0.876	13.0	0.0322	3.68
	2. „	13.1		

ASPARAGINE NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen $=\frac{1}{2}$ Asp. N.
		c.c.		
a)	1. 1.736	0.5	0.0015	0.09
	2. „	0.7		
b)	1. 0.866	2.5	0.0064	0.74
	2. „	2.7		
c)	1. 1.752	8.8	0.0219	1.26
	2. „	8.9		

NITROGEN IN PHOSPHO-TUNGSTIC PRECIPITATE.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.868	1.0	0.0025	0.28
	2. „	1.0		
b)	1. 1.732	2.0	0.0052	0.30
	2. „	2.2		
c)	1. 0.876	1.2	0.0031	0.36
	2. „	1.3		

1 c.c. baryta water = 0.00247 gram nitrogen.

2. HORDEUM DISTICHON.

TOTAL NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.440	9.5	0.0237	5.40
	2. „	9.7		
b)	1. 0.440	9.8	0.0245	5.56
	2. „	10.0		
c)	1. 0.441	10.7	0.0264	6.60
	2. „	10.7		

ALBUMINOID NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.880	16.0	0.0398	4.52
	2. „	16.2		
b)	1. 0.879	10.8	0.0267	3.04
	2. „	10.8		
c)	1. 0.882	15.3	0.0377	4.27
	2. „	15.4		

ASPARAGINE NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen $= \frac{1}{2}$ Asp. N.
		c.c.		
a)	1. 1.760	1.0	0.0027	0.15
	2. „	1.2		
b)	1. 1.758	5.2	0.0133	0.76
	2. „	5.6		
c)	1. 1.764	5.0	0.0125	0.71
	2. „	5.1		

NITROGEN IN PHOSPHO-TUNGSTIC PRECIPITATE.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 1.760	3.1	0.0074	0.42
	2. „	2.9		
b)	1. 1.758	3.6	0.0091	0.52
	2. „	3.8		
c)	1. 1.764	4.5	0.0113	0.64
	2. „	4.6		

1 c.c. baryta water = 0.00247 gram nitrogen.

3. HORDEUM DISTICHON.

TOTAL NITROGEN.

		Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
			c.c.		
Dark	a)	1. 0.459	7.0	0.0175	3.82
		2. „	7.2		
	b)	1. 0.458	8.8	0.0210	4.58
		2. „	8.2		
Light	a)	1. 0.458	6.9	0.0170	3.72
		2. „	6.9		
	b)	1. 0.454	8.0	0.0200	4.40
		2. „	8.2		

ALBUMINOID NITROGEN.

		Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
			c.c.		
Dark	a)	1. 0.917	9.0	0.0227	2.48
		2. „	9.4		
	b)	1. 0.916	9.5	0.0235	2.56
		2. „	9.5		
Light	a)	1. 0.915	8.5	0.0202	2.21
		2. „	8.7		
	b)	1. 0.907	8.7	0.0217	2.40
		2. „	8.9		

ASPARAGINE NITROGEN.

		Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen $= \frac{1}{2}$ Asp. N.
			c.c.		
Dark	a)	1. 1.834	2.5	0.0064	0.35
		2. „	2.8		
	b)	1. 1.832	3.1	0.0082	0.45
		2. „	3.4		
Light	a)	1. 1.830	3.4	0.0087	0.47
		2. „	3.6		
	b)	1. 1.814	5.1	0.0129	0.71
		2. „	5.3		

NITROGEN IN PHOSPHO-TUNGSTIC PRECIPITATE.

		Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
			c.c.		
Dark	a)	1. 1.834	1.8	0.0047	0.26
		2. „	2.0		
	b)	1. 1.832	2.8	0.0067	0.36
		2. „	2.5		
Light	a)	1. 1.830	1.9	0.0044	0.24
		2. „	1.6		
	b)	1. 1.814	2.0	0.0050	0.27
		2. „	2.0		

1 c.c. baryta water = 0.00247 gram nitrogen.

4. CUCURBITA MELOPEPO.

TOTAL NITROGEN.

		Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
			c.c.		
Dark	a)	1. 0.462	12.5	0.0311	6.74
		2. „	12.7		
	b)	1. 0.451	15.6	0.0390	8.65
		2. „	16.0		
Light	a)	1. 0.458	12.9	0.0319	6.96
		2. „	13.0		
	b)	1. 0.444	15.7	0.0383	8.62
		2. „	15.3		

ALBUMINOID NITROGEN.

		Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
			c.c.		
Dark	a)	1. 0.924	21.7	0.0541	5.85
		2. „	22.1		
	b)	1. 0.902	20.3	0.0504	5.59
		2. „	20.5		
Light	a)	1. 0.916	21.2	0.0524	5.72
		2. „	21.2		
	b)	1. 0.887	17.4	0.0432	4.87
		2. „	17.6		

ASPARAGINE NITROGEN.

		Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen $= \frac{1}{2}$ Asp. N.
			c.c.		
Dark	a)	1. 1.848	0.9	0.0026	0.14
		2. „	1.2		
	b)	1. 1.804	4.5	0.0112	0.68
		2. „	4.7		
Light	a)	1. 1.832	1.6	0.0035	0.19
		2. „	1.2		
	b)	1. 1.774	7.8	0.0186	1.04
		2. „	7.2		

NITROGEN IN PHOSPHO-TUNGSTIC PRECIPITATE.

		Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
			c.c.		
Dark	a)	1. 1.848	1.3	0.0032	0.17
		2. „	1.3		
	b)	1. 1.804	3.2	0.0084	0.47
		2. „	3.5		
Light	a)	1. 1.832	2.2	0.0054	0.30
		2. „	2.3		
	b)	1. 1.774	4.2	0.0106	0.60
		2. „	4.5		

1 c.c. baryta water = 0.00247 gram nitrogen.

II.

1. PINUS THUNBERGII

TOTAL NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)*	1. 0.450	22.0	0.0411	9.13
	2. „	22.3		
b)*	1. 0.474	24.2	0.0452	9.53
	2. „	24.4		

* 1 c.c. baryta water = 0.00186 gram nitrogen.

ALBUMINOID NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.900	25.4	0.0280	3.11
	2. „	26.4		
b)	1. 0.948	25.4	0.0279	2.94
	2. „	26.1		

ASPARAGINE NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen $=\frac{1}{2}$ Asp. N.
		c.c.		
a)	1. 0.900	9.5	0.0104	1.15
	2. „	9.8		
b)	1. 0.948	10.1	0.0112	1.17
	2. „	10.6		

NITROGEN IN PHOSPHO-TUNGSTIC PRECIPITATE.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage o nitrogen.
		c.c.		
a)	1. 0.900	13.7	0 0144	1.60
	2. „	12.9		
b)	1. 0.948	16.8	0.0178	1.88
	2. „	16.1		

1 c.c. baryta water = 0.00108 gram nitrogen.

2.

TOTAL NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.458	21.5	0.0404	8.81
	2. „	21.9		
b)	1. 0.443	20.5	0.0387	8.73
	2. „	20.8		
c)	1. 0.487	18.0	0.0339	6.95
	2. „	18.4		
d)	1. 0.480	18.0	0.0331	6.90
	2. „	17.5		

ALBUMINOID NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.916	17.0	0.0322	3.51
	2. „	17.7		
b)	1. 0.886	18.0	0.0338	3.81
	2. „	18.3		
c)*	1. 0.974	29.7	0.0319	3.27
	2. „	29.2		
d)*	1. 0.960	29.0	0.0316	3.29
	2. „	29.4		

ASPARAGINE NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen = ½ Asp. N.
		c.c.		
a)	1. 0.916	3.2	0.0061	0.66
	2. „	3.3		
b)	1. 0.886	3.1	0.0056	0.62
	2. „	2.8		
c)*	1. 0.974	5.6	0.0062	0.63
	2. „	5.9		
d)*	1. 0.960	5.2	0.0055	0.57
	2. „	5.0		

NITROGEN IN PHOSPHO-TUNGSTIC PRECIPITATE.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.916	7.8	0.0141	1.54
	2. „	7.4		
b)	1. 0.886	7.8	0.0144	1.62
	2. „	7.6		
c)*	1. 0.974	4.1	0.0043	0.44
	2. „	3.9		
d)*	1. 0.960	3.5	0.0037	0.38
	2. „	3.3		

1 c.c. baryta water = 0.00186 gram nitrogen.

* 1 c.c. baryta water = 0.00108 gram nitrogen.

3.

TOTAL NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)*	1. 0.450	22.0	0.0411	9.13
	2. „	22.3		
b)*	1. 0.474	24.2	0.0452	9.53
	2. „	24.4		
c)*	1. 0.486	21.3	0.0394	8.11
	2. „	21.0		
d)*	1. 0.479	21.2	0.0393	8.19
	2. „	21.0		

ALBUMINOID NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.900	25.4	0.0280	3.11
	2. „	26.4		
b)	1. 0.948	25.5	0.0279	2.94
	2. „	26.0		
c)*	1. 0.972	16.6	0.0309	3.18
	2. „	16.7		
d)	1. 0.958	28.6	0.0311	3.25
	2. „	30.0		

ASPARAGINE NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen $= \frac{1}{2}$ Asp. N.
		c.c.		
a)	1. 0.900	9.5	0.0104	1.15
	2. „	9.8		
b)*	1. 0.948	10.1	0.0112	1.17
	2. „	10.6		
c)*	1. 0.972	4.2	0.0080	0.82
	2. „	4.4		
d)	1. 0.958	7.0	0.0077	0.80
	2. „	7.2		

NITROGEN IN PHOSPHO-TUNGSTIC PRECIPITATE.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.900	13.7	0.0144	1.60
	2. „	12.9		
b)	1. 0.948	16.8	0.0178	1.88
	2. „	16.1		
c)*	1. 0.972	6.0	0.0108	1.11
	2. „	5.5		
d)	1. 0.958	8.7	0.0092	0.96
	2. „	8.2		

1 c.c. baryta water = 0.00108 gram nitrogen.

* 1 c.c. baryta water = 0.00186 gram nitrogen.

4.

TOTAL NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.443	20.5	0.0387	8.73
	2. „	20.8		
b)	1. 0.481	23.6	0.0441	9.16
	2. „	23.8		

ALBUMINOID NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen.
		c.c.		
a)	1. 0.886	18.0	0.0338	3.81
	2. „	18.3		
b)	1. 0.962	16.0	0.0301	3.13
	2. „	16.5		

ASPARAGINE NITROGEN.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen $=\frac{1}{2}$ Asp. N.
		c.c.		
a)	1. 0.886	3.1	0.0056	0.62
	2. „	2.8		
b)	1. 0.962	4.2	0.0076	0.79
	2. „	4.0		

NITROGEN IN PHOSPHO-TUNGSTIC PRECIPITATE.

	Dry matter used.	Baryta water replaced.	Nitrogen found.	Percentage of nitrogen = ½ Asp. N.
		c.c.		
a)	1. 0.886	7.8	0.0144	1.62
	2. „	7.6		
b)	1. 0.962	8.0	0.0153	1.59
	2. „	8.3		

1 c.c. baryta water = 0.00186 gram nitrogen.

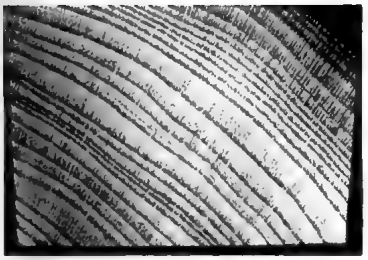


Fig. 1.

Fig. 2.

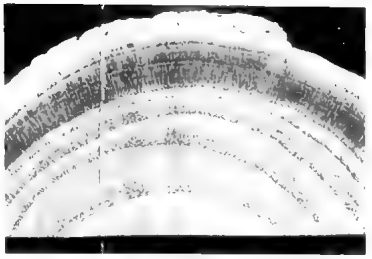


Fig. 3.

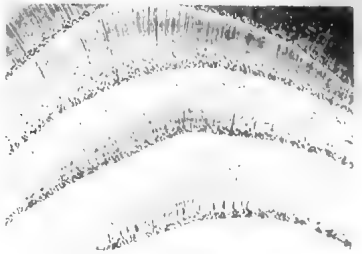


Fig. 4.

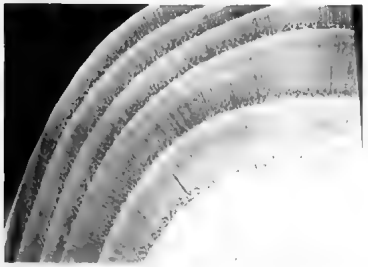


Fig. 5.

Fig. 6.

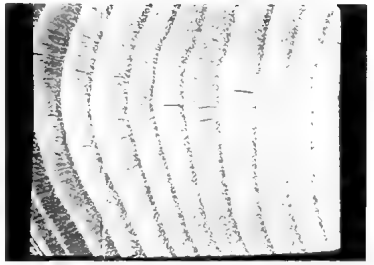


Fig. 7.

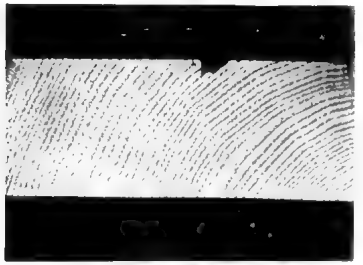


Fig. 8.

Fig. 9.

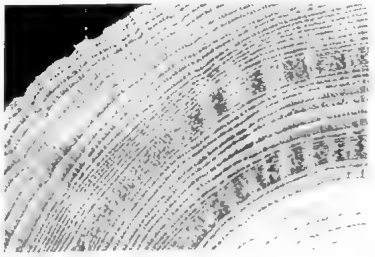


Fig. 10.

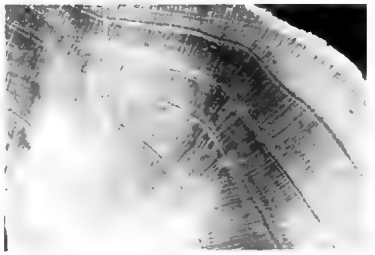


Fig. 11.

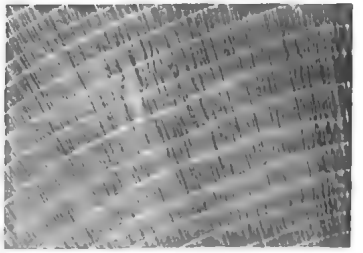


Fig. 12.

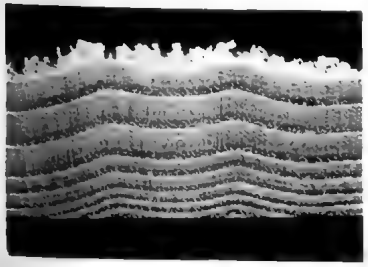


Fig. 13.

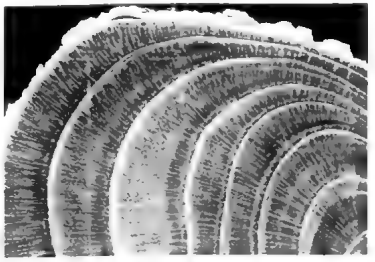


Fig. 14.

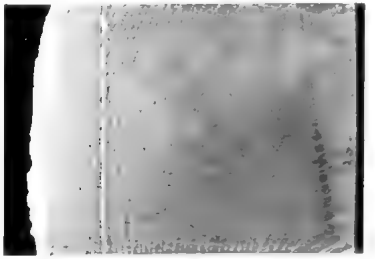


Fig. 15.

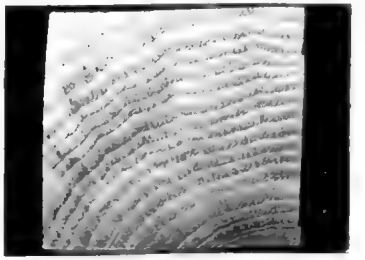
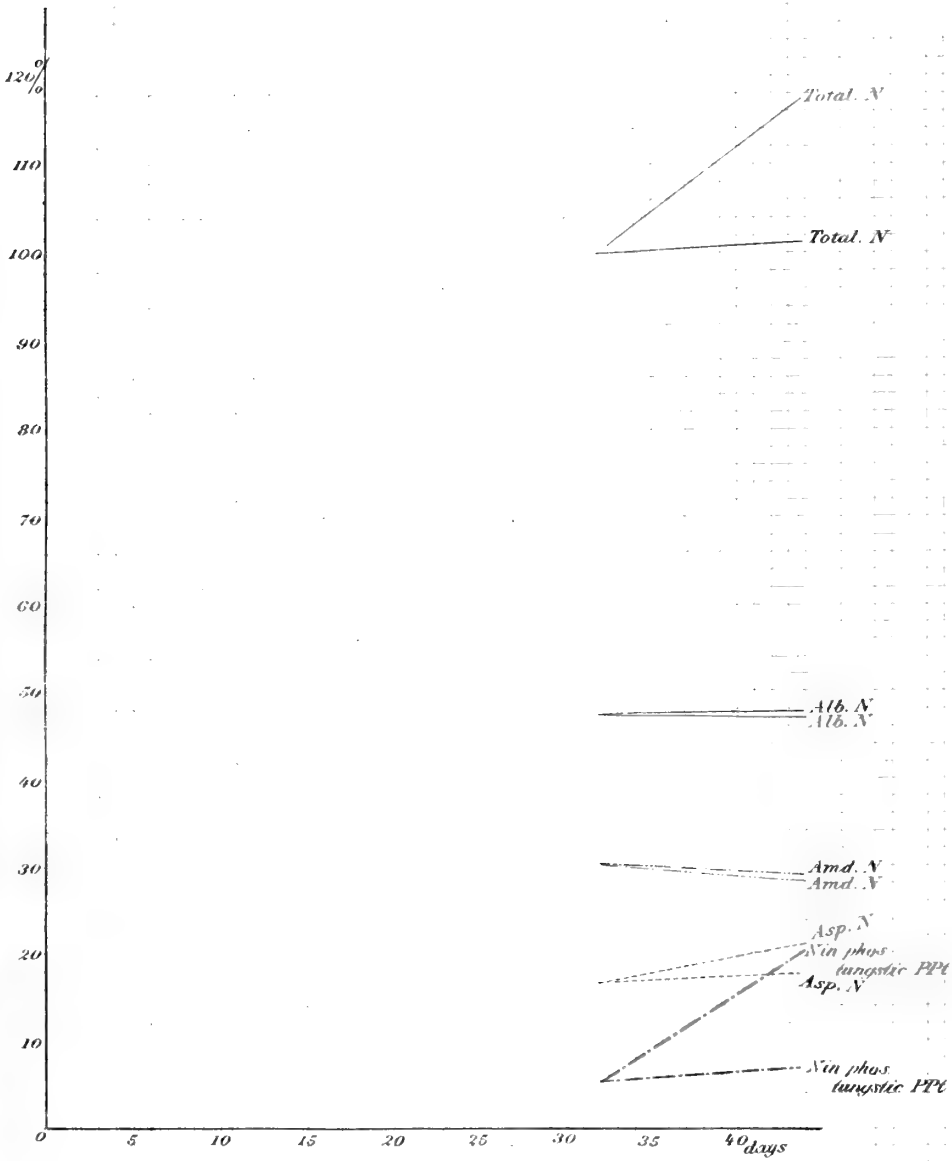
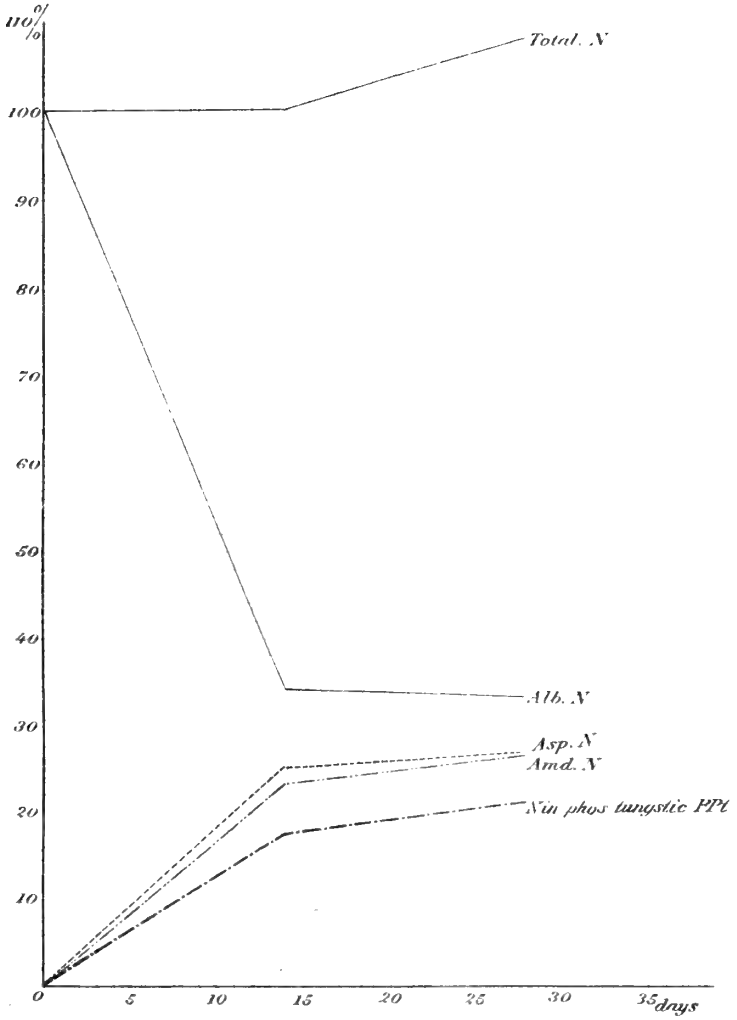


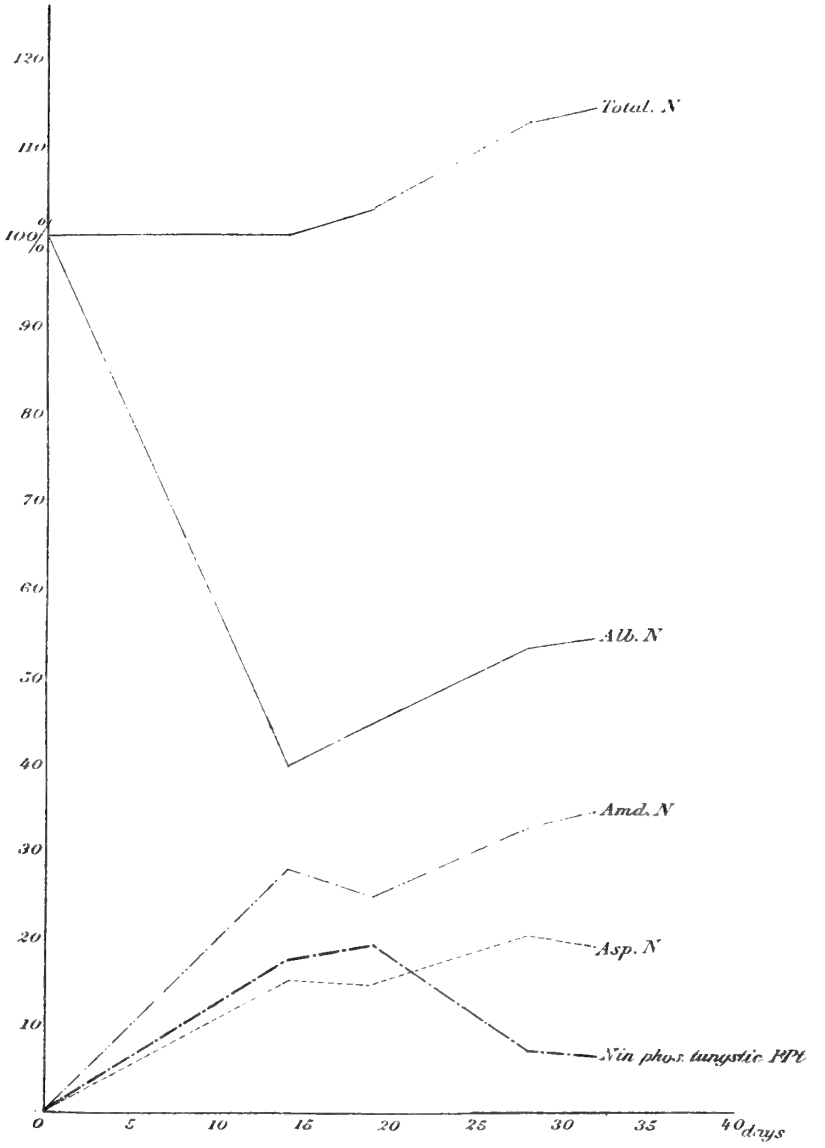
Fig. 16.



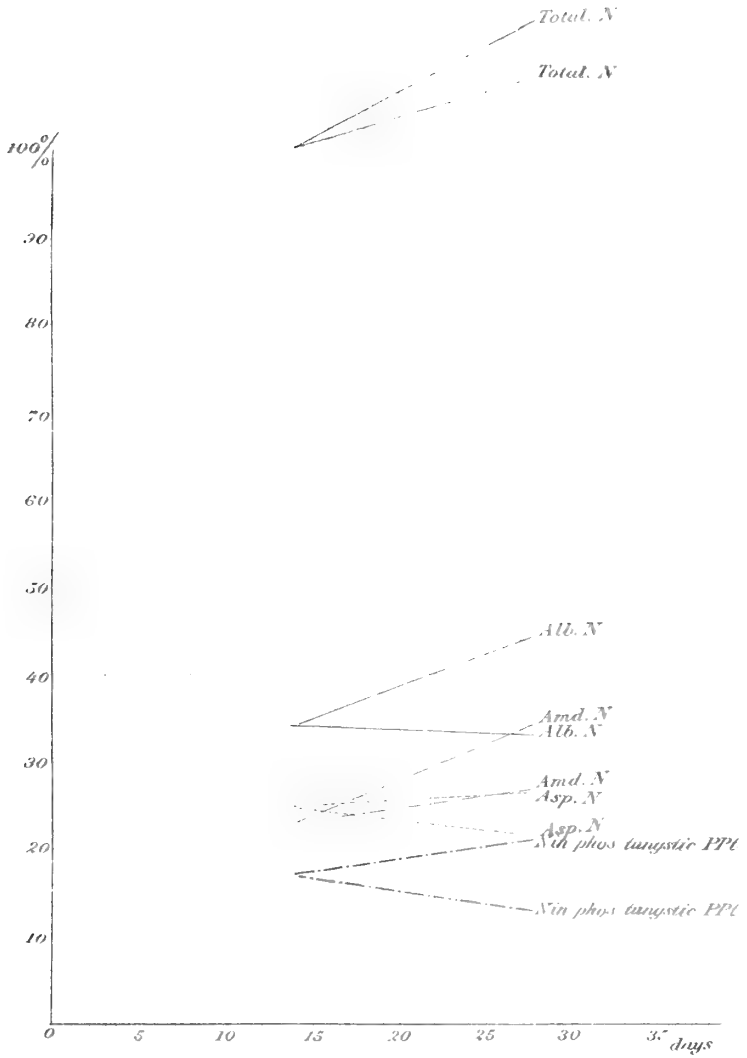
This plate shows the increase of organic bases, especially arginin, in the shoots of Pinus Thunbergii by the addition of ammonium salts. The black lines refer to shoots cultured in the half saturated gypsum solution, in the diffused day-light, and the red lines refer to those cultured in 0.5% ammonium chloride solution, half saturated with gypsum, in the diffused day-light.



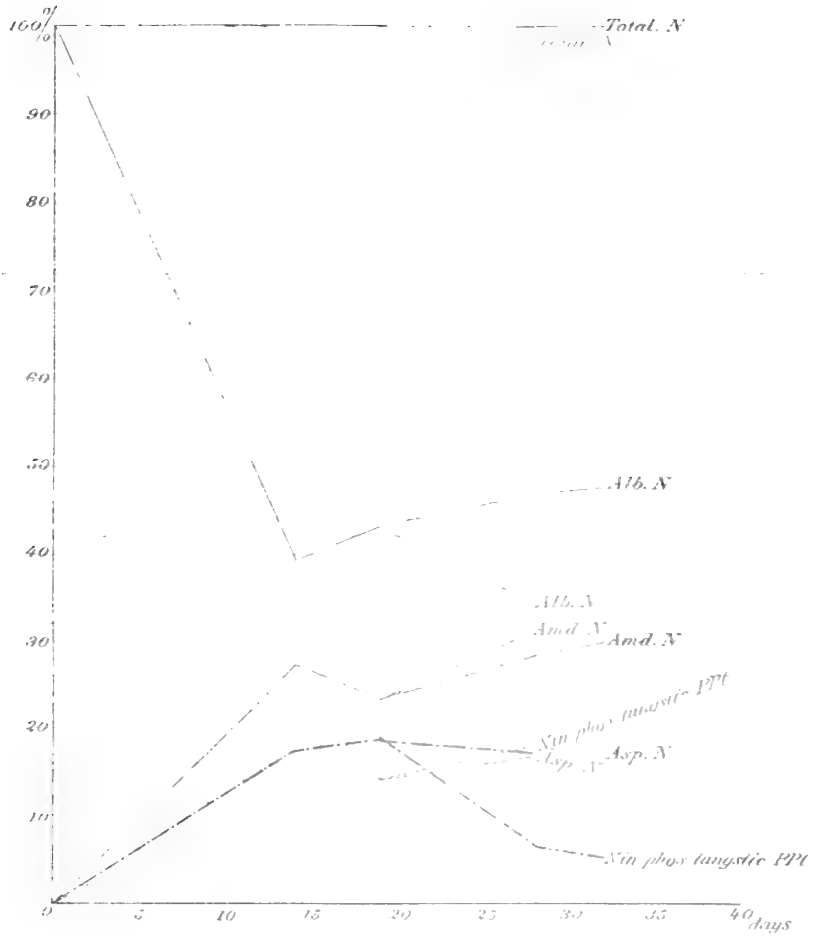
This plate shows the change of various nitrogenous compounds (especially nitrogen phospho-tungstic precipitate) during the development of the shoots of Pinus Thunbergii in perfect darkness.



This plate shows the change of various nitrogenous compounds during the development of the shoots of Pinus Thunbergii, under the full day-light.



This plate shows the influence of light upon the change of nitrogenous compounds in the shoots of Pinus Thunbergii. The shoots were kept for 14 days in perfect darkness and a portion was afterwards, exposed to the day-light for 14 days, while the other portion was still kept in the dark. The black lines refer to shoots kept in the dark until the end of the experiment, and the red lines to those, previously kept in the dark, and afterwards exposed to the full day-light for 14 days.



This plate shows the influence of light upon the change of nitrogenous compounds. Shoots were at first exposed to the full day-light for 19 days, then a portion was kept in the dark for 9 days, while the other portion was still exposed to the day-light. The black lines refer to shoots exposed to the full day-light, and the red lines to those afterwards kept in the dark for 9 days.

Can Strontium and Barium Replace Calcium in Phaenogams?

BY

U. Suzuki, *Nōgakushi.*

It is generally believed that the principal function of lime salts in plants consists in neutralizing oxalic acid or precipitating oxalic acid from soluble oxalates produced by the metabolism of the plant cells. The poisonous oxalic acid is thus removed from the solutions as insoluble oxalate of lime. But it may be objected that many plants do not produce oxalic acid, although lime salts are absolutely necessaay for them. Further the quantity of lime needed by the plants does not always correspond with the quantity of oxalic acid produced by them. These facts naturally lead us to look for the physiological function of lime salts in another direction.

Recently O. Loew* has propounded the view that the nucleus and chlorophyll bodies contain lime in organized protein compounds. Hence if deprived of lime or if lime is substituted by other elements, then those structures will suffer since the capacity for imbibition would change, which would lead to serious disturbances of structure.

If the principal function of lime salts merely consists in

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* O. Loew: Ueber die Physiologischen Functionen der Calciumsalze :—Botanisches Centrblit Bd. LXXIV. 1898.—His conclusion is as follows :—

"Meine Ansicht, dass Zellkerne bei einer gewissen Höhe der Entwickelung, und Chlorophyllkörper, sofern sie nicht auf primitiver Entwickelungsstufe stehen, des Kalks bedürfen und Kalk-Proteinverbindungen sich an deren Organisation betheiligen ist nicht wiederlegt. Sie ist im Gegentheil wahrscheinlicher, als die von Anderen vertheidigte Ansicht, nach welche den Kalksalzen bloss die Besorgung von Stoffwechselvorgängen zukäme."

"Strontiumsalze sind so lange den Pflanzen unschädlich, als diesen hinreichend Calciumsalze zur Verfügung stehen. Jenseits aber eines gewissen Verhältnises sind schädliche Wirkungen unverkennbar. Eine physiologische Vertretung von Verbindungen des Calcium durch solche des Strontiums findet nicht statt."

precipitating the oxalic acid produced in the cells, then such elements as strontium and barium, which form insoluble salts with oxalic acid, must prove equally available as calcium. On the contrary, if lime is an integral part of the nucleus and chlorophyll-bodies, then, as Loew says, it can never be replaced by other elements. This is a point which needs a thorough elucidation.

In 1893, some experiments on this lime was made by Haselhoff,* who concluded that strontium can replace calcium in phaenogams. But he cultivated his plants in a fertile soil, which contained naturally much lime, and also in his water culture, he added strontium-salts together with lime. Hence we can not be surprised that he observed no injurious effects. Loew holds the view that when strontium is used together with lime, then the plants will by elective absorption take up much more lime than strontium, and can exchange small doses of strontium taken into organic structures always against the lime present in solution. His observations on the poisonous action of oxalates upon plant-cells under the microscope led him to the view that the calcium is present in the nucleus and chlorophyll bodies in organized protein compounds, as above mentioned. But if strontium-salts alone are offered to the plant, then the effect must be quite different. In accordance with this view, he made two experiments with young branches of Tradescantia repens, and found that the poisonous action of strontium is, to a large extent, masked by the presence of lime, but that as soon as the lime content is diminished to a certain degree, then the poisonous effect of strontium-salts becomes at once apparent As this question appeared very interesting I have made also experiments with five species of phaenogams. The results of these experiments will be described in the following pages :—

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* Haselhoff ;—(Landw. Jahrbücher. Bd. XXII. 1893. S. 851.) His conclusion is as follows :—

1) Das Strontium wirkt nicht schädlich auf die Pflanzenentwickelung.
2) Das Strontium wird von der Pflanze aufgenommen und scheint bei der Ernährung die Stelle des Kalks zu vertreten.
3) Diese Substitution des Kalks durch Strontium bei der Pflanzenernährung scheint aber erst dann einzutreten, wann der Vorrath an Kalk und anderen Nährstoffen nicht mehr zum Aufbau der pflanzlichen Organismen ausreichen.

A) SAND CULTURE.

1. BUCKWHEAT. (Polygonum fagopyrum.)

Common sea sand was boiled with strong hydrochloric acid (sp. gr 1.15) for about two hours and then well washed at first with hot water and afterwards with distilled water until no trace of hydrochloric acid was present. The purified sand thus prepared was then transfered into four large funnels, of the diameter of about 25 cm., each of which was placed upon a tall glass cylinder, so that the excess of the solution added to the sand may pass through the funnel and drop down into the cylinder, without leaving any excess in it and yet keeping all equally moist.

Seeds of buckwheat were previously soaked with water and on March 26, they were equally distributed into the four funnels and kept in a warm house. By April 5, the germinating plants reached 3–8 cm. high. They were then treated with the following solutions prepared exactly after Haselhoff.

1)	2.02 g. KNO_3	with 0.94 g.	$K_2O + 1.08$ g. N_2O_5	in 1 litre water.
	3.28 g. $Ca(NO_3)_2$	„ 1.15 g.	$CaO + 2.16$ g. N_2O_5	
2)	2.02 g. KNO_3	„ 0.94 g.	$K_2O + 1.08$ g. N_2O_5	„ „ „
	4.23 g. $Sr(NO_3)_2$	„ 2.07 g.	$SrO + 2.16$ g. N_2O_5	
3)	2.02 g. KNO_3	„ 0.94 g.	$K_2O + 1.08$ g. N_2O_5	„ „ „
	5.22 g. $Ba(NO_3)_2$	„ 3.06 g.	$BaO + 2.16$ g. N_2O_5	
4)	2.02 g. KNO_3	„ 0.94 g.	$K_2O + 1.08$ g. N_2O_5	„ „ „
	1.64 g. $Ca(NO_3)_2$	„ 0.575 g.	$CaO + 1.08$ g. N_2O_5	
	2.12 g. $Sr(NO_3)_2$	„ 1.04 g.	$SrO + 1.08$ g. N_2O_5	
5)	5.26 g. $MgSO_4 + 7H_2O$	„ 0.86 g.	$MgO + 1.70$ g. SO_3	„ „ „
	3.36 g. K_2HPO_4	„ 1.82 g.	$K_2O + 1.08$ g. P_2O_5	
	Traces of $FeCl_3$			

From April 5, each funnel was treated with the solutions 1), 2), 3) and 4), respectively, and once in every 4–5 days, the solution 5) was added to each funnel, so that the plants in all the funnels may enjoy an equal amount of mineral salts except

Ca, Sr, and Ba (I shall hereafter call, for convenience, the plants treated with solution 1), Ca plants, with solution 2), Sr plants etc.).

The solutions were given almost every day and the details noticed. At first, there was no difference among the plants, but afterwards the Sr and Ba plants gradually showed signs of suffering, while the Ca and Ca + Sr plants grew on equally well. Toward the end of the experiment, the suffering of the Sr and Ba plants became very serious, many plants beginning to die off, many leaves falling down, and the stems unable to stand upright, so that I could not keep them any longer. On the 24th, the plants were carefully removed from the sand, washed well, and the following measurements made :

PLANTS TREATED WITH SOLUTION.

	1)	2)	3)	4)
Number of plants	Ca plants 20	Sr plants 15	Ba plants 28	Ca + Sr plants 17
Length of stem	12.9cm	11.4	10.8	11.4
Length of petiole	2.4–3.3cm	0.6–0.9	1.5–1.8	2.1–3.0
Length of roots	6.0–10.5cm	6.0	4.5–7.5	9.0
Total weight of fresh stems	7.045g	3.895	5.245	5.625
Total weight of fresh leaves	3.950g	1.195	1.395	3.285
Weight of 1 fresh stem	**0.352g**	**0.260**	**0.187**	**0.331**
Weight of 1 fresh leaf	**0.198g**	**0.080**	**0.050**	**0.193**
Diameter of cotyledon	3.6cm	2.4	2.4	3.0–3.6

In the Ca and Ca + Sr plants two or three new leaves developed in each plant, the diameter of which was in the former 2.1–3.6 cm. and in the latter 1.5–3.0 cm., while in the Sr and Ba plants no development of new leaves was observed. We see from the above results that the Ca plants were best, the Ca + Sr plants being nearly on the same footing with them, but somewhat inferior; Sr and Ba plants were nearly equal in length among themselves, but the stems were very small and slender, so that they could not stand upright. The growth of leaves was

especially bad in the Sr and Ba plants, no new leaves developing and the cotyledons remaining very small and afterwards beginning to fall down. The roots were not developed in both plants.

2) Barley. (Hordeum distichon.)

(See Pl. VII)

The treatment was exactly the same with buckwheat. The seeds were sown in purified sea sand contained in four large funnels and kept in a warm house. On the 5th April, the plants were 3-8 cm. high, and the solutions were then given. During the experiment, no obstacles were encountered. Until the 16th, no difference was observed among the plants, but afterwards the Sr and Ba plants began to suffer slowly. This was especially the case with the Ba plants. The tips of the leaves became yellow and afterwards chlorophyll was entirely destroyed, the leaves becoming white, as if the plants had been kept in the dark for several weeks, and no growth was observed. The Sr plants preserved their green colour a little longer, but afterwards they gradually became dirty green and the tips of the leaves turned yellow. The development of the roots was remarkably bad in both plants. The Ca plants grew most energetically, the roots reached their maximum length, and the leaves were a dark green in colour. The Ca+Sr plants also grew very well and at first showed no difference from the Ca plants, but afterwards the growth became rather slow and toward the end of the experiment, the difference between the two was quite distinct, especially as regards the development of the roots. The Ba and Sr plants suffered more and more as days passed on, and as I could not keep them any longer, the experiment was stopped on the 11th, and the plants were carefully removed from the sand, washed well, and the following measurements were made:—

PLANTS TREATED WITH SOLUTION.

	1)	2)	3)	4)
	(Ca plants)	(Sr plants)	(Ba plants)	(Ca + Sr plants)
Number of plants	62	60	57	46
Length of leaves.....	39–27cm aver. 32	18–15 aver. 13.5	21–12 aver. 15	33–18 aver. 22
Length of roots	15–30cm	18–9. aver. 13	18–9. aver. 13	24–15
Breadth of leaves	0.9cm	0.6	0.45	0.75
Total fresh weight.....	93.g	40.	29.	62.
Fresh weight of 1 plant	1.5g	0.67	0.51	1.35
Total dry weight	6.673	3.026	1.955	4.522
Dry weight of 1 plant	0.108	0.050	0.034	0.098
Ratio of dry weight	100.0	46.3	31.5	90.7

We see from the above results that strontium and barium salts are strongly poisonous, stopping all growth and gradually causing death. Barium salt destroys chlorophyll bodies and no assimilation can therefore take place.* The poisonous action of

* One of the reasons why barium salts are poisonous to plants may be the precipitation of sulphates in the plant cells which makes it impossible for them to enter into the organization of the protoplasm and chlorophyll bodies, etc.

The presence of barium in the plants treated with barium-salts was easily shown by dissolving the plant ash in hydrochloric acid, and adding some sulphuric acid to the hydrochloric extract, when a strong white precipitate of barium sulphate was formed.

In Ca plants I found no white precipitate formed on a similar treatment.

Barium-salt is sometimes found as a normal constituent in some plants. Compare R. Hornberger :—Über das Vorkommen des Bariums in der Pflanze und im Boden (Landw. Vers. Stationen. München. Bd. LI. Heft VI. S. 473.)

Compare also R. Kobert :—Kann ein in einem Pflanzenpulver gefundenes abnorm höher Barytgehalt erklärt werden durch direkte Aufnahme von Baryumsalz durch die lebende Pflanze aus dem Boden? :—(Chem. Ztg. 10. Juni 1899. Nr. 46). He says as follows :—

"Fast alle Pflanzen sind im Stande, gelegentlich aus dem Boden Baryumverbindungen aufzunehmen. In meinem Lehrbuche der Intoxicationen, heisst es. "Strontian, Baryt und Mangan werden ohne Nachtheil für den Pflanzenorganisms von Pflanzen aufgenommen, und die so barythaltiggewordenen Pflanzen können auf Menschen und Thiere giftig wirken." "Bei mikroscopischen Untersuchung des von Dr. Jonscher erwähnten Paprikapulvers muss sich ja zeigen, ob die Barytmenge nur zugemischt sind, oder, in der Pflanzen enthalten sind."

strontium was very much neutralized (?) by the presence of calcium-salt. This is the reason why Haselhoff was led to erroneous conclusion.

B) WATER CULTURE.

1) PHLOX PANICULATA. (Polemoni.)

Young plants of Phlox paniculata were cut off and put in the following solutions:

1) 3.28 g. $Ca(NO_3)_2$ in 1 litre of water .....1.12 g. $CaO + 2.16$ g. N_2O_5

2) 4.23 g. $Sr(NO_3)_2$ „ „ „ .....2.07 g. $SrO + 2.16$ g. N_2O_5

3) 5.22 g. $Ba(NO_3)_2$ „ „ „ .....3.06 g. $BaO + 2.16$ g. N_2O_5

4) { 1.64 g. $Ca(NO_3)_2$ „ „ „ .....0.56 g. $CaO + 1.08$ g. N_2O_5 }
{ 2.12 g. $Sr(NO_3)_2$ „ „ „ .....1.04 g. $SrO + 1.08$ g. N_2O_5 }

5) { 1.64 g. $Ca(NO_3)_2$ „ „ „ .....0.56 g. $CaO + 1.08$ g. N_2O_5 }
{ 2.61 g. $Ba(NO_3)_2$ „ „ „ .....1.53 g. $BaO + 1.08$ g. N_2O_5 }

The experiment was commenced on the 19th April, and by the 22nd Ba plants had already begun to show signs of suffering, the upper soft part of the stems turning at first brownish black and drying up, and the leaves could not flourish any longer, becoming yellow or brownish black, and on the 24th nearly all the leaves withered away. Sr plants fared somewhat better than Ba plants, but on the 24th they began to show the same signs as Ba plants, and no leaves remained alive until the 27th. Ca plants were quite healthy and showed no signs of suffering until the end of the experiment (1st May), on the contrary, many new leaves appeared during the experiment. Ca+Sr and Ca+Ba plants were far better than Sr or Ba plants. By the 24th they had shown only slight signs of suffering, many leaves remaining alive until the 27th; no new leaves, however, appeared, and on the 29th nearly all leaves except a few on the top, were dead. Nevertheless it is quite evident that the poisonous action of strontium and barium salts was much neutralized by the presence of calcium salt.

2) RUBUS IDAEUS L. VAR. STRIGASUS MAXIM.

The experiment was carried on exactly in the same way as with Phlox paniculata. But in this case, control experiment was also made with distilled water. The result was almost the same as in the former experiment, the only difference was that the Sr plants suffered most. They died already on the 24th and no leaf remained alive until the 27th, all having turned brownish black. The Ba plants also died, and nothing remained until the 24th. The Ca and control plants were quite healthy until the end of the experiment and no difference was observed between them. The Ca + Sr and Ca + Ba plants also remained alive until the end of the experiment (1st May), but by this time the edges of the leaves turned brownish black.

3) COREOPSIS TINCTORIA NUTL.

The same experiment was repeated with the young plants of this species having roots, and the same result as above was obtained. The Ca and control plants were the best; the Ca + Sr and Ca + Ba plants were also very good; but toward the end of the experiment (1st May) they began to show signs of suffering. The Sr and Ba plants, on the contrary, began to suffer already on the 24th, being unable to stand upright, and the leaves, with the exception of those on the upper part of the stems, nearly all dying off by the end of the experiment.

The above five experiments are enough to prove that strontium and barium salts are strongly poisonous to the higher plants and can never replace calcium salts. Their poisonous action can, however, be neutralized to a large extent in the presence of lime salts. In the experiment with Rubus and Coreopsis we observe that control plants, kept in distilled water, were equally healthy; so it is quite evident that the bad condition of the Sr and Ba plants in these species was due not to deficiency of the necessary lime salts, but to the poisonous action of strontium and barium salts. If the principal function of lime salts consists merely in neutralizing the oxalic acid formed during metabolism in the plant cells, and if it never enters into closer combination with the protoplasm, then we must have naturally quite different results from those above obtained!

My results exactly agree with those of Loew,* and as he has already discussed fully the physiological function of lime salts, I will not repeat it here.

SUMMARY.

1. Strontium and barium can never replace calcium in phaenogams; they are strongly poisonous and the poisonous action may, to a certain degree, be lessened by the addition of lime salts.

2. Haselhoff's view that strontium can replace calcium is incorrect.

3. Loew's view that calcium is contained in the nucleus and chlorophyll-bodies in organized compounds would agree best with my observations.

OBSERVATIONS DURING THE EXPERIMENTS.

1) BUCKWHEAT.

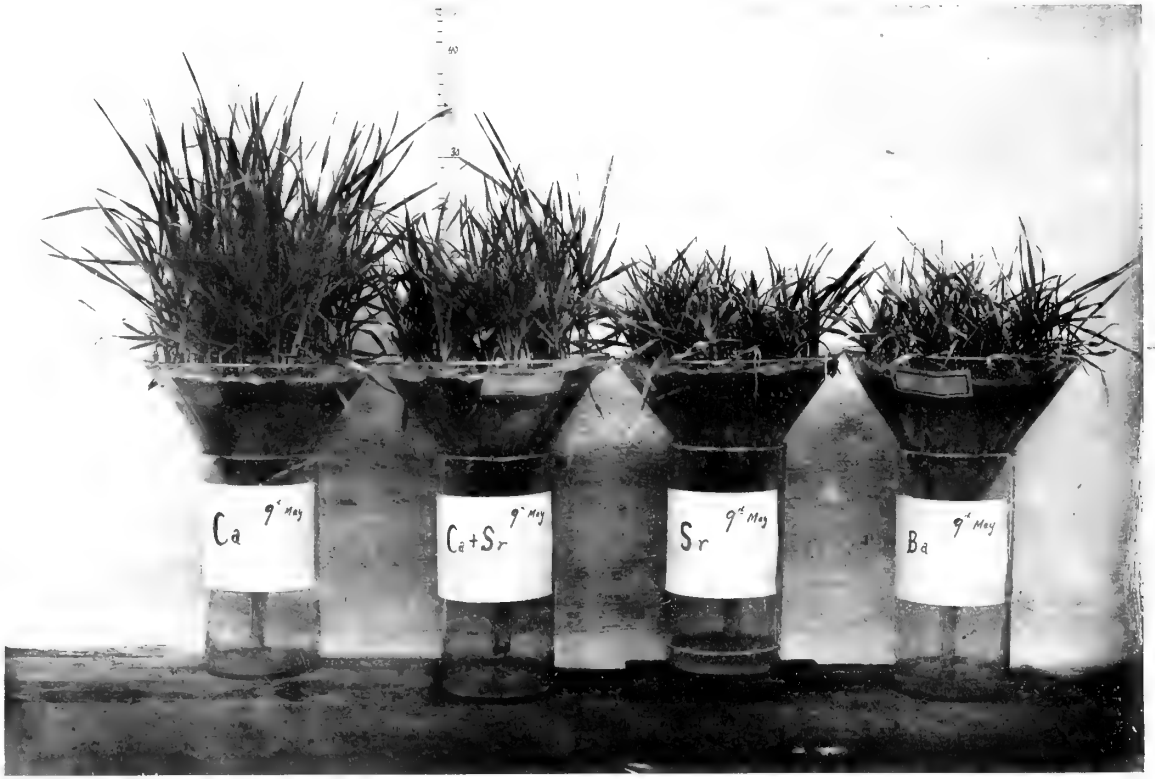
Date.	Weather.	Temp. (max.)	Ca plants.	Ca + Sr plants.	Sr plants	Ba plants.	Remarks.
April 5th	Rain	16°C	40 c.c. of the solution added.				3–8 cm high.
10	Cloudy	21	"	"	"	"	No difference yet observed 6–14 cm.
11	Fine	31	"	"	"	"	No difference yet observed 6–15 cm.
13	"	25	"	"	"	"	Ca plants became a little better.
15	"	32	"	"	"	"	
16	"	20	40 c.c. of the solution 5) added.				Ca plants were very well, small new leaves appeared, but none on other plants.
18	Cloudy	20	40 c.c. of the solution added.				
20	Fine	30	Distilled water added.				New leaves also appeared in Ca + Sr plants, Sr and Ba plants suffered much, many leaves falling down from Sr and Ba plants. Experiment stopped.
23	"	20	"	"	"		
24	"	25	"	"	"		

* Cf: Die chemische Energie der lebenden Zellen, Chapt. 3 und 4.

Date	Weather.	Temp. (Max.)	Ca plants.	Ca+Sr plants.
5th April	Rain	10°C.	40 c.c. of the solution added.	40c.c. of the solution added.
10	Cloudy	21	" " " "	" " " "
11	Fine	31	" " " "	" " " "
13	"	25	" " " "	" " " "
15	"	32	" " " "	" " " "
16	"	20	40c.c. of the sol. 7) added.	40c.c. of the sol. 5) added.
18	Cloudy	20	80c.c. of each solution added.	80c.c. of each solution added.
20	Fine	30	" " " "	" " " "
23	"	20	" " " "	" " " "
24	"	25	" " " "	" " " "
25	"	24	Distilled water added.	Distilled water added.
26	"	29	40c.c. of each solution and some distilled water added.	40c.c. of each solution and some distilled water added.
27	"	25	" " " "	" " " "
29	"	20	80c.c. of solution 5) and some distilled water added.	80c.c. of solution 5) and some distilled water added.
30	"	30	40c.c. of each solution and some distilled water added.	40c.c. of each solution and some distilled water added.
1st May	"	33	80c.c. of each solution and some distilled water added.	80c.c. of each solution and some distilled water added.
2	Cloudy	23	100c.c. of each solution and some distilled water added.	100c.c. of each solution and some distilled water added.
3	Rain	22	Dark green colour, growth best.	Dark green colour, little inferior.
4	"	20	100c.c. of each solution and some distilled water added.	100c.c. of each solution and some distilled water added.
6	"	22	100c.c. of solution 5) and some distilled water added.	100c.c. of solution 5) and some distilled water added.
7	Fine	25	100c.c. of each solution and some distilled water added.	100c.c. of each solution and some distilled water added.
8	"	25	Growth best, dark green.	Growth little slower.
9	"	28		
10	"	34	Pure water added.	Pure water, growth slower.
11	"	32	Healthiest.	Healthy.

LEY.

Sr plants.	Ba plants.	Remarks.
40c.c. of the solution added.	40c.c. of the solution added.	3–8cm. growth equal.
,, ,, ,, ,,	,, ,, ,, ,,	7–15cm. growth equal.
,, ,, ,, ,,	,, ,, ,, ,,	7.5–16.5 no difference yet.
,, ,, ,, ,,	,, ,, ,, ,,	Ca plants a little better.
,, ,, ,, ,,	,, ,, ,, ,,	,, ,, ,, ,,
40c.c. of the sol. 5) added.	40c.c. of the sol. 5) added.	,, ,, ,, ,,
80c.c. of each solution added.	80c.c. of each solution added.	Difference not yet remarkable
,, ,, ,, ,,	,, ,, ,, ,,	
,, ,, ,, ,,	,, ,, ,, ,,	
,, ,, ,, ,,	,, ,, ,, ,,	
Distilled water added.	Distilled water added.	
40c.c. of each solution and some distilled water added.	40c.c. of each solution and some distilled water added.	
,, ,, ,, ,,	,, ,, ,, ,,	
80c.c. of solution 5) and some distilled water added.	80c.c. of solution 5) and some distilled water added.	
40c.c. of each solution and some distilled water added.	40c.c. of each solution and some distilled water added.	Ca and Ca + Sr plants equally well, Sr and Ba plants became very bad.
80c.c. of each solution and some distilled water added.	80c.c. of each solution and some distilled water added.	
100c.c. of each solution and some distilled water added.	100c.c. of each solution and some distilled water added.	
No growth, top of the leaves turned yellow.	No growth, chlorophyll destroyed.	Ba plants suffered very much, difference very remarkable.
100c.c. of each solution and some distilled water added.	100c.c. of each solution and some distilled water added.	
100c.c. of solution 5) and some distilled water added.	100c.c. of solution 5) and some distilled water added.	
100c.c. of each solution and some distilled water added.	100c.c. of each solution and some distilled water added.	
Top yellow, no growth.	Leaves white and almost died off.	
Pure water added.		Ba plants nearly died off, Sr plants also began to die off.
Suffered much, half of the leaves died off.		Experiment stopped.



This plate shows the injurious effects of strontium and barium on barley.

COLLOTYPE BY K. OGAWA, TOKYO.

The Chemical Composition of the Spores of Aspergillus Oryzae.

BY

K. Asō, *Nōgakushi.*

Our knowledge of the chemical composition of the spores of fungi is still very scanty, and as regards their ash, no analysis whatever has thus far been made. There are however questions of physiological interest, suggesting a comparison of the spores of fungi with the seeds of the higher plants, that have induced me to determine, as accurately as possible, the composition of the spores of Aspergillus oryzae. These spores can easily be obtained in any quantity in Japan, as the rice grains covered with the mycelium and the spores of this fungus form an article of commerce under the name of Tane-kōji or Kōji-dane.[1] I have preferred however to collect the spores myself, in order to obtain them in a pure state.

Aspergillus oryzae plays a great part in several industries in Japan, being used not only in the manufacture of Sake or rice wine,[2] but also in that of Shōyu or Soja-sauce,[3] and of Miso.[4]

This fungus contains powerful enzymes,[5] a diastase, a maltase, invertase and a peptase. Oxydases appear to be present only in traces. I extracted Tane-kōji with 30% alcohol,

(1) The composition of Kōji, *i.e.* rice grains covered with the mycelium of A. oryzae was investigated by Kellner, Mori and Nagaoka. (Bull. College of Agric., Tōkyō; vol. I. No. 5), and Tane-kōji was analysed by Satō (see the article by Okumura. This Bull. Vol. III. No. 3).

(2) Okumura, Bull. College of Agric., Tōkyō; Vol. III. No. 3.

(3) Y. Nishimura, this Bull. Vol. III. No. 3.

(4) Kellner, Mori and Nagaoka, this Bull. Vol. I. No. 6. Besides Kōji is used for manufacturing Mirin (a sweet alcoholic drink) and many other Japanese beverages and articles of food.

(5) Kellner, Mori and Nagaoka, Bull. College of Agric., Tōkyō, Vol. I. No. 5. Okumura, this Bull. Vol. III. No. 3.

Takamuku, Journal of the Tōkyō Chemical Society, Vol. XIX. No. 8.

from which the enzymes were precipitated with strong alcohol and ether. After filtering and washing with strong alcohol, they were dissolved in a small quantity of water, precipitated and washed with alcohol twice more. The enzymes thus obtained were dissolved in a small quantity of water, and to this solution, a few drops of freshly prepared guaiacum tincture and diluted hydrogen peroxide were added; only a slightly bluish colouration was obtained at first, but it became more and more distinct after a few minutes. Prof. O. Loew, lately of this college, found some years ago, that oxydase is not present, and peroxydase present only in traces, in Kōji. T. Takamuku of this college found the presence of cytase in a small quantity in Soja-kōji.

I. Preparation of Samples.

I collected the spores of the fungus in the following way: Tane-kōji was prepared with roughly milled rice without mixing any ash[1]; and when the rice grains became covered with innumerable spores, they were brought out from the cellar and the spores were separated from the rice grains and the mycelium by tapping the bottom of the culture boxes[2] inverted on a sheet of paper. By this means, most of the spores were obtained on the paper, and they were then sifted with a very fine silk sieve to remove all impurities. The sample[3] thus obtained is of course not absolutely, but only tolerably pure.[4] This sample was exposed to the air in a balance room for two weeks and kept in a bottle. It was used for organic analysis.

In the same manner, I collected a second sample, which was used for ash analysis.

A third sample, which was used for special purposes, was

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(1) Tane-kōji used in the Sake-factories is commonly prepared by mixing the ash of oak-leaves and the spores with steamed rice. On the details of the preparation, see J. Okumura's article in this Bulletin, Vol. III, No. 3.

(2) The wooden boxes are commonly called Kōji-buta.

(3) One Kōji-buta gave about three grams of the sample.

(4) I examined several portions of the sample under the microscope, but I did not find any piece of the mycelium at all.

collected from a commercial Tane-kōji[1] prepared in a Sake-factory at Osaka.[2]

II. Organic Constituents of the Spores of Aspergillus Oryzae.

The result of my analysis regarding the organic constituents of the spores of the fungus was as follows:

In 100 parts of air-dry spores,[3]

Water	42.515
Dry matter	57.485

In 100 parts of dry matter,

Total nitrogen	6.380
Crude protein	39.875
Ethereal extract	0.377
Alcoholic extract after extracting with ether	27.666
Crude fibres	8.994
Total carbohydrates (as dextrose)	20.017
Ash	5.151

1. Dry matter and water.

I determined the dry matter by drying the spores to constant weight at 100°C.

2. Nitrogenous Substances.

The total nitogen was determined by Kjeldahl's method, and the crude protein was calculated by multiplying 6.25 by the quantity of nitrogen obtained.

I determined the indigestible part of the proteids in the

(1) The commercial Tane-kōji is generally prepared by mixing some ash with the rice. I determined the proportion of ash in the third sample and obtained 6.11% of dry matter, while that in the first sample was 5.15%.

(2) The first and the second samples were prepared in the Miso-factory of Kagaya at Yotsuya in Tōkyō.

(3) The first sample.

following way: 16.69 grams of the dry spores[1] were extracted with dilute caustic soda[2] after extracting with ether and alcohol, and some dilute hydrochloric acid was added until the solution gave a faintly acid reaction.

After filtering and well washing with dilute hydrochloric acid (about 0.2%), the dark brown precipitate was mixed with 250 c.c. of gastric juice and digested in the usual way in a water bath for eighteen hours at 40°C.; then filtered and thoroughly washed with dilute hydrochloric acid. The residue was dissolved in dilute ammonia, and again precipitated by making the ammoniacal solution faintly acid with dilute hydrochloric acid; then filtered. The precipitate was collected on a weighed filter and thoroughly washing first with dilute hydrochloric acid and then with water, washed with boiling alcohol and ether. Hereupon I obtained a brown mass which was weighed after drying at 100°C. 16.69 grams of the dry spores yielded 0.59 grams of the brown mass, that is, 3.535% in the dry spore. This brown mass contained 10.8% nitrogen.

It seemed to me of interest to determine the presence of some bases, if any, in the spores. About 10 grams of the dry spores[3] were extracted with boiling water, and from this aqueous solution a precipitate was obtained with basic lead acetate. After filtering, the excess of lead in the filtrate was precipitated by sulphuretted hydrogen, and filtered. After evaporating to a small volume, adding a little sulphuric acid, phospho-tungstic acid was added to the filtrate, whereby the characteristic precipitate was obtained. The phospho-tungstic precipitate was first washed with cold water containing some sulphuric acid, and then decomposed with caustic baryta, and the filtrate evaporated after removal of the excess of baryta by means of carbonic acid. The evaporated solution[4] was treated with an ammoniacal solution of silver nitrate, whereby a white precipitate was obtained. The precipitate thus obtained was collected on a

(1) The third sample.

(2) The spores could not be digested completely unless I treated them first with alkalies, because some of them floated on the surface of the gastric juice and could not be brought into intimate contact with the juice.

(3) The third sample.

(4) I applied the biuret test for peptone, but no reaction was obtained.

filter and washed with a diluted ammoniacal solution of silver nitrate and afterwards with cold water, then dissolved in a warm nitric acid of the specific gravity of 1.1, after the addition of a little urea, and upon cooling, microscopical needles were obtained which were analogous to the silver compounds of the xanthin-bases. Though the crystals obtained were too small in quantity, yet I made some further researches. After separating the crystals by filtration, they were washed with cold water, and suspended in some water slightly acidified with hydrochloric acid, and the silver was removed with sulphuretted hydrogon. The filtrate from the silver sulphide was then neutralized with ammonia and evaporated to dryness. The residue was treated with ammonia, upon which one part was dissolved and left an insoluble residue. The latter gave the reaction of Capranica for guanin, and also produced the characteristic change of colour when treated with nitric acid and caustic soda. The ammoniacal solution was evaporated and from it some powder of a faintly yellow colour was obtained. This substance did not give the reaction of Weidel, and when evaporated with nitric acid, left a yellowish residue which turned dark reddish when treated with caustic soda and on heating. It is probable that this colouration was caused by other bases than hypoxanthin, because I did not purify the latter by repeated recrystallization.[1]

The nitric acid solution from which the needle-shaped crystals were separated, was made slightly alkaline with ammonia, whereby a very small quantity of a brownish yellow flocculent precipitate was obtained, from which the silver was removed by sulphuretted hydrogen, and filtered. The filtrate was then evaporated to dryness, and a yellowish powder was obtained, which was slightly soluble in water but insoluble in alcohol and ether. On applying Weidel's test, it gave a dark reddish colour, but not Hoppe-Seyler's reaction. The change of colour with nitric acid and caustic soda was also observed. Although the amount of the sample used was too small to afford any positive proof for the presence of xanthin bases in the spores, yet it is likely that they are contained in the spores. It appears to me that many other bases besides those of the xanthin group

(1) Compare 'On the Nitrogenous Non-albuminous Constituents of Bamboo Shoots,' by Kozai. (Bull. College of Argic., Tōkyō, Vol. I. No. 7).

are contained in the spores; but this question must be deferred to future investigation.

3. Ethereal Extract.

The ethereal extract was obtained by the extraction of dried spores[1] with absolute ether in Soxhlet's extraction apparatus. After evaporating the ether, the residue was weighed in the usual way. The ethereal solution was colourless, but the residue was slightly yellow. In this extract, there were contained cholesterin and lecithin besides the common fats. About 17 grams of the dry spores[2] were extracted with ether, and after evaporation, the residue was saponified with alcoholic potash, and the alcohol evaporated. The residue thus obtained was treated with water and then put into a cylinder. Some ether was poured into this cylinder, and after shaking repeatedly, the etherial solution was drawn off with a pipette. The evaporation-residue of this ethereal solution was dissolved with some warm alcohol and left for some time. Some tabular crytals were observed in the alcoholic solution under the microscope. These crystals were dissolved in chloroform, and to it some concentrated sulphuric acid was added, whereby a red colouration was produced, and upon adding some water a green precipitate[3] appeared.

The presence of lecithin was confirmed in the following manner: several grams of spores[4] were separately extracted with ether and alcohol, and the two were mixed. After heating the evaporation-residue with a mixture of sodium carbonate and some potassium nitrate, the ash was dissolved in nitric acid and the presence of phosphoric acid in the solution was tested with ammonium molybdate.

4. Alcoholic Extract.

After extracting with ether, the spores[5] were extracted

(1) The first sample.
(2) The third sample.
(3) E. Gérard, Jahresber. f. Agric. Chem. XIX. 1896. S. 274.
(4) The second sample.
(5) The first sample.

with alcohol (98%) by means of a reverted cooler, and after evaporating, the residue was dried to constant weight at 100° C, and weighed. The alcoholic solution was neutral[1] and yellow or brownish yellow according to the quantity of the spores used. The evaporation-residue was brownish and had a pungent smell and a sweet taste. On cooling, innumerable needle-shaped crystals formed in bushes or bundles from the hot alcoholic solution. This substance left no ash upon ignition and was sweet, without smell, soluble in water and hot alcohol, very slightly so in cold alcohol and insoluble in ether. I determined the melting point of the crystals in the capillary tube and obtained 163.5° C on the average.[2] Besides, the lustre of the somewhat purified crystals was brilliant. Hence, there could be no doubt that this substance was mannitol. I determined roughly its proportion in the alcoholic extract by weighing the crystals somewhat purified by recrystallization, and obtained 44.752%. Besides mannit, there were contained trehalose,[3] and some resinous matters.

5. Crude fibres.

The quantity of the crude fibres was determined according to Gabriel's method by deducting the ash from the raw fibres found.

6. Carbohydrates.

The spores[4] were mixed with water and steamed in a digester for three hours in just the same manner as for the determination of starch, and then filtered.

The filtrate did not reduce Fehling's solution, formed no osazone, and upon boiling with concentrated sulphuric acid no dark colouration appeared. Hence, I concluded that this extract did not contain glucose, maltose or cane sugar. The extract was next boiled for about three hours with dilute hydrochloric acid (about 2.5%), and after neutralizing it with

(1) I tested with litmus paper.
(2) Of course, these crystals were not absolutely pure.
(3) About this I shall speak soon afterwards.
(4) The first sample.

sodium hydrate and filtering, the quantity of sugar was determined as dextrose by Allihn's method, and I obtained 20.017% of it in the dry matter.

To see how much sugar is contained in the alcoholic extract, I extracted the spores with alcohol (93%) and the evaporation-residue was dissolved in water, and after boiling this for about three hours with dilute hydrochloric acid (about 2.5%), the quantity of sugar was determined by the above-mentioned method. Here I obtained 6.225% sugar as dextrose in the dry matter.

The difference between these two quantities perhaps corresponds to that of the carbohydrates, which are soluble in hot water under some pressure and insoluble in boiling alcohol. Cramer[1] determined carbohydrates in the spores of Penicillium glaucum under the name of starch by inverting the carbohydrates with dilute sulphuric acid, but he did not investigate their properties. Marschall[2] determined starch in the mycelium of some fungi by converting it into glucose with an acid, but he could not obtain any positive proof of its presence. It appeared to me of some interest to investigate the carbohydrates in the spores of Aspergillus oryzae. I tested the spores as well as the mycelium of the fungus with iodine solution under the microscope; no blue, but the characteristic dark brown colour for glycogen was observed. It is then clear that the spores as well as the mycellium contained no starch, but glycogen. Indeed, starch has never been found in fungi, while glycogen is spread widely among them.

When the alcoholic extract was left for some days, some tabular crystals were obtained besides the needle-shaped crystals of mannit. They were rhombic prisms, colourless and transparent, carbonized on heating and left no ash on ignition. It was sweet, without smell, insoluble in ether and chloroform, nearly so in cold alcohol, soluble in hot alcohol, easily so in water, melted at 100° C, even in a strongly boiling water bath,[3] and solidified to a glassy mass on cooling. Its aqueous solution was neutral and could reduce Fehling's solution only after

(1) Centralbl. f. Bak. II. Abtheilung, Bd. I.

(2) Archiv f. Hygiene Bd. XXVIII. 1896.

(3) The temperature in this was about 99.° 8 C.

boiling with dilute hydrochloric acid. It produced oxalic acid upon boiling with strong nitric acid. There can hardly be any doubt that this substance was trehalose (mycose). The quantity of dextrose obtained after boiling the alcoholic extract might be equivalent to that of glucose, which was split from trehalose.

III. Mineral Constituents of the Spores of Aspergillus Oryzae.

1. Total Ash.

The ash of the spores was estimated after incineration and determination of the carbon and carbonic acid contained therein, which were deducted from the raw ash. The result is shown in the following table:

	First sample.	Second sample.	Third sample.
Total ash in dry matter.	5.151%	4.844%	6.112%

2. Mineral Constituents.

The ash-analysis[1] was carried out by the usual method for vegetable ash with the following result:

In 100 parts of the ash,

K_2O	45.964
Na_2O	4.131
CaO	1.038
MgO	4.364
Fe_2O_3	4.916
P_2O_5	39.640
SO_3	2.000
SiO_2	0.409

The test for chlorine was made and its presence in the ash distinctly proved.

(1) The ash of the second sample was here used.

DISCUSSION OF RESULTS.

A. THE ORGANIC CONSTITUENTS.

1. WATER.

The high percentage of water is a striking feature; it indicates the presence of a very hygroscopic substance, which in other kinds of spores is absent. Thus, Reinke found that the spores of Aethalium septicum contain only 7.13% water in the air-dry state. According to Cramer, Penicillium spores contain a very high percentage of dry matter, and give up all the water on drying, which they again take up in moist air. Planta found that a fresh sample of the pollen-grains of hazelnut gave up 4.21% of water on drying over sulphuric acid, and then 4.98% more on drying at 100° C, making a total of 9.19%.

2. CARBOHYDRATES.

Glycogen is very widely spread as reserve material[1] in the higher as well as the lower fungi, forming as much as 30% of the dry matter of beer-yeast. Starch is never formed in spores. Further, trehalose and mannit have been found in a number of fungi, either together or separately.[2] It is of interest to observe the differences in the chemical activities of the higher plants and fungi: thus, the starch of green plants is replaced by glycogen in the fungi and the cane sugar of phanerogams is here replaced by trehalose. The chief difference between trehalose ($C_{12}H_{22}O_{11} + 2H_2O$) and cane sugar ($C_{12}H_{22}O_{11}$) is that the former is split by hydrolysis into two molecules of glucose, and the latter into glucose and fructose; trehalose is also less easily invertible than cane sugar.[3] Remarkable in this respect is the high percentage of cane sugar in the pollen-grains of pine (11.24%) and of hazelunt (14.70%), as found by Planta.[4] Of special interest is

(1) Pfeffer: Pflanzenphysiologie, I Bd. 1897. S. 474.

(2) According to Müntz, Penicillium glaucum, Agaricus campestris &c. contain always mannitol, but no trehalose, while Agaricus muscaris produce trehalose, but no mannit. (The same book.)

(3) Zeit. Physiol. Chem., 1894., Winterstein: Zur Kenntniss der Trehalose.

(4) Landw. Versuchs-Stat. 1885. XXXI. and 1886 XXXII.

the frequent occurence of mannitol in the fungi. It serves doubtless in the germination of the spores, and is probably, in the first stage of oxidation, transformed into a hexose (mannose, glucose or fructose).

It seems to me that the crude fibres of the spores contain some chitin-like substance, the study of which will be deferred to future.

3. Nitrogenous Substance.

The percentage of crude protein was high, that of the mycelium of Aspergillus niger being 30.4%, as found by Marschall,[1] and of the pollen-grains of hazelnut 31.63% of the dry matter, according to Planta. In general, fructification-organs contain more nitrogen than others. Stützer[2] found much nuclein in the mycelium of fungi, there being 40.75 parts of nuclein-nitrogen in 100 parts of the total nitrogen. Perhaps, the brown mass which remained as an indigestible residue of the spores of Aspergillus oryzae, may be a mixture of some colouring matters and nucleo-proteids. I tested for the presence of phosphorus, sulphur and iron in the ash of the brown mass and proved their presence, phosphorus and iron being especially predominant. Hence this substance has a close relation to the haematogen studied recently by Stoklasa.[3]

The bases of the xanthin group as described before, may perhaps be partly derived from nuclein by a partial decomposition of it on heating with water.

4. Fatty Matters.

The amount of fatty matters including some lecithin and cholesterin in fungi fluctuates between wide limits, viz. 0.2% in Agaricus and 35% in Claviceps. The following table gives some figures as regards the contents of fat.

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(1) The average quantity of crude protein in the mycelium of the three fungi investigated by him was 38.000%.

(2) Zeit. Physiol. Chem., VI.

(3) Compt. rend., 1898, 128.

Fats (ethereal extract) in dry matter,

Spores of Aspergillus oryzae.	Spores of Penicillium glaucum. (a)	Pollen-grains of hazelnut, (b)	Pollen-grains of pine. (b)	Barley. (c)	Seeds of rape. (c)
0.377%	7.34%	4.20%	10.60%	2.90%	48.20%

(a) According to Cramer, (b) according to Planta, (c) according to Wolff's Chemico-Agric. Tables.

5. Alcoholic Extract.

As far as my knowledge goes, the percentage of the substances soluble in alcohol is higher in the spores than in the mycelium of fungi, as the following table shows:

In 100 parts dry matter,

	Spores of Aspergillus oryzae.	Spores of Penicillium glaucum.	Mycelium of Aspergillus niger.	Mycelium of Penicillium glaucum.	Mycelium of Mucor stolonifer.
Alcoholic extract.	27.67	30.46	18.50	11.80	11.80

It is probable that such substances as mannitol are accumulated in the spores as reserve materials, which are soluble in alcohol.

Finally, I give in the following table some figures showing relations regarding to the composition of the spores, the pollen-grains and the seeds of some plants:

Penicillium-spores were analysed by Cramer, its mycelium by Marschall and the pollen-grains by Planta, as mentioned before.

In 100 parts of dry matter,	Spores of		Pollen-grains of hazelnut.	Soy-bean.*
	Aspergillus oryzae.	Penicillium glaucum.		
Crude protein.....	39.88	28.44	31.63	37.11
N-free substances.	54.97	69.65	64.36	57.33
Ash	5.15	1.91	4.01	5.56

B. THE MINERAL CONSTITUENTS.

1. Total ash.

Though the quantity of ash in the spores of fungi depends partly upon the medium in which they were cultivated, yet it seems to me singular that such a great difference as is shown in the following table should be found between the ash of Aspergillus oryzae and of Penicillium glaucum.

	Spores of		Mycelium of fungi.
	Aspergillus. In average.	Penicillium.	In the average.
Ash in dry matter,	5.37%	1.91%	6.37%

It will prove interesting to analyse many other kinds of spores and deduce some general conclusion on this point.

2. Ash Constituents.

Marschall made a qualitative analysis of the ash of the mycelium of fungi and found iron, phosphoric acid, chlorine, sodium and potassium etc ; besides some other ingredients which were not remarkable. I determined the ash ingredients of the spores of Aspergillus oryzae quantitatively and found all common ingredients except manganese.(1) Very interesting was the high percentage of oxide of iron found in this ash ; it is probably present in the spores as a nuclein compound ; thus haematogen

* This is the average composition of many sorts of soy-beans.

(1) I made a qualitative test for this.

in the bulb of Allium cepa obtained by Stoklasa contained 1.68% of iron. According to Molisch iron is an essential constituent of fungi; but others only admit that it is useful. Traces of iron, sufficient for a large amount of mycelium, are often contained in the nutriments furnished to the fungi, which naturally take it up. K. Yabe[1] of this college states that he observed, during his studies on the developement of this Aspergillus under different conditions, that only in those solutions which contained some iron, spores were developed; which shows, in accordance with the view of Molisch, the importance of this element for the fungus. The percentage of oxide of iron in the common rice grains (not whitened) in Japan is 1.63 in the ash;[2] and according to the recent investigation by Bunge,[3] rice grains contain 1–2 milligrams of iron in 100 parts of dry matter. Aspergillus-spores contained relatively more iron than rice grains. Doubtless, this iron must have been derived from the rice grains.

The sulphuric acid in the ash is derived perhaps exclusively from the sulphur of the proteids, by oxidation during the incineration process. The presence of silica and lime in the ash is perhaps just as accidental as that of chlorine and soda. These four substances are not necessary for the developement of the lower fungi. Nor do the more complex members of the higher fungi probably require silica or soda; but as to lime, we can not yet say anything definite as to whether it is required by such complicated fungi as Phallus, Agaricus, Morchella.

Of great interest is the high percentage of potash and phosphoric acid; and on this point, there is a close analogy with the seeds of phanerogams. The following table shows the comparison of K_2O and P_2O_5 in the ash of the seeds of higher plants and that of the spores of the fungus:

(1) Bull. of Agric. College. Vol. III. No. 3.
(2) This Bull. Vol. I. No. 12., Kellner and Nagaoka.
(3) Zeit. Physiol. Chem., 1898, 25.

In 100 parts of dry matter.	Winter wheat. (1)	Not whitened paddy rice. (1)	Oats. (1)	Soy-bean. (1)	Not whitened rice. (2)	Spores of Aspergillus.
Ash.	1.68	0.87	2.67	2.83	1.47	4.844
K_2O	0.52	0.20	0.48	1.26	0.33	2.227
P_2O_5	0.79	0.46	0.68	1.04	0.71	1,920
In 100 parts of ash.						
K_2O	30.95	22.99	17.93	44.52	22.47	45.964
P_2O_5	47.02	52.87	25.47	36.75	48.31	39.640

The particularly striking relation between the spores and beans is here evident, but as to its significance we can not make any assertion without further investigations.

Of special interest is, further, the presence of 4.364% of magnesia. This base plays evidently an important rôle in the assimilation of phosphoric acid. Wherever proteids are formed with accompanying development, and phosphates are present, there is magnesia always found. From magnesium-phosphate, phosphoric acid can more easily be assimilated than from any other phosphates, as the dissociation (hydrolysis) of magnesium salts is easily accomplished. It may be safely assumed that, about as much magnesia as was found here, is contained in the spores of the related Penicillium glaucum. Indeed, we may say, no seed or spore without magnesium!

Nevertheless, it is a fact that the germination of the Penicillium-spores takes place in solutions containing, as organic nutriment, ammonium acetate alone, only when traces of magnesium salts are present. It seems, that only a good nutrition brings on those changes in the spores, which make the magnesium phosphate soluble and available for the protoplasm.

(1) According to Wolf's Chemico-Agric. Tables.

(2) Kellner and Nagaoka: Bull. College of Agric. Vol. I. No. 12.

Though no ash anayslis of the spores of Aethalium have yet been made, its plasmodium has been analysed by Reinke. According to him the ash contained 27.7% of calcium carbonate, 6.49% of phosphoric acid, 1.42% of potash, 0.13% of iron oxide and 0.71% of magnesia.

In conclusion, I must express much thanks to Dr. O. Loew, formerly professor in this college, for giving me the present subject for investigation and for many valuable suggestions during its progress.

明治三十三年二月廿五日印刷
明治三十三年二月廿八日發行

編輯兼發行者　農科大學

印刷者　佐久間衡治
東京市牛込區市ヶ谷加賀町一丁目十二番地

印刷所　株式會社　秀英舍第一工場
東京市牛込區市ヶ谷加賀町一丁目十二番地

Die Unterscheidungsmerkmale der wichtigeren in Japan wachsenden Laubhölzer.

VON

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(Tafeln VIII—XVI.)

EINLEITUNG.

Forstleute und holzverbrauchende Techniker sollen die Hölzer richtig bestimmen können. Dies kann aber nur geschehen, wenn sie die Unterscheidungsmerkmale der Hölzer genau kennen, wie solche von Dr. R. Hartig,* oder Dr. F. Schwarz† für die in Deutschland wachsenden Hölzer festgestellt worden sind. Die Laubhölzer, welche in Deutschland vorwiegend angebaut und benutzt werden, bestehen aus etwas über 50 Holzarten. Dagegen finden wir in Japan, welches ein für die Waldgewächse so günstiges Klima besitzt, wenigstens 200 Laubhölzer. Je grösser die Anzahl der vorkommenden Holzarten ist, um so schwieriger aber auch wichtiger ist es natürlich die Hölzer zu bestimmen, um die betreffenden Holzarten in Hinsicht ihrer Beschaffenheit am nützlichsten verwenden zu können.

Aus dem obigen Grunde habe ich auch die Bestimmung der wichtigeren, in Japan wachsenden Laubhölzer vorgenommen. Wenn ich auch dabei fast ganz dieselben Gesichtpunkte in's Auge gefasst habe, wie Dr. R. Hartig oder Dr. F. Schwarz es gethan hat, so habe ich für die Klassification der Hölzer nach den Merkmalen einen ganz eigenen Weg eingeschlagen, da für die Bestimmung der so zahlreichen Hölzer, wie wir sie hier haben, Hartig's oder Schwarz's einfache Klassification nicht mehr genügt.

* Die anatomischen Unterscheidungsmerkmale der wichtigeren in Deutschland wachsenden Hölzer von Dr. R. Hartig. München 1890.

† Forstliche Botanik von F. Schwarz. Berlin 1892.

Ehe ich auf die Beschreibung der Unterscheidungsmerkmale der einzelnen Hölzer eingehe, will ich die hauptsächlichsten Punkte, welche ich dabei berücksichtigt habe, kurz vorausschicken.

Die Unterscheidungsmerkmale der Hölzer sollen für ein bestimmtes Holz ganz charakteristisch, einfach und leicht wahrnehmbar sein, und durch Wachsthumsverhältnisse, Alter, etc. keine Veränderung erleiden. Wenn aber solche Unterscheidungsmerkmale nicht zu finden sind, wie z. B. es bei den zu derselben Gattung gehörenden Hölzern gewöhnlich der Fall ist, dann muss man auf einige durch Alter und Wachsthum hervorgerufene Veränderungen Rücksicht nehmen. Zu den unveränderlichen Merkmalen zähle ich überhaupt die Stellung und Stärke der Gefässe, die Zahl und Breite der Markstrahlen der Hölzer, und die leicht sichtbaren Parenchymlinien. Zu den veränderlichen Merkmalen aber Farbe und Rinde der Hölzer, dann Glätte und Glanz derselben und endlich Zellgänge und anderes. Ich habe natürlich vor allem meine Aufmerksamkeit auf den ersten Punkt gerichtet, und nur im nothwendigsten Falle auf den zweiten.

Die Stellung der Gefässe auf dem Querschnitt der Hölzer ist ganz characteristisch für bestimmte Holzarten. Die Gefässe können einzeln vertheilt, oder zu Linien oder Gruppen, oder zu einer Figur vereinigt sein, und diese Vertheilung und Vereinigung kann locker oder dicht erfolgen.

Die Grösse der Gefässe ist, wenn auch kleine Abweichungen nicht ausgeschlossen sind, für die Holzarten eine ganz bestimmte. Sie können einmal so stark sein, dass man sie auf dem Querschnitt der Hölzer als grosse Löcher wahrnehmen kann, ein anders Mal aber so klein, dass man sie selbst mit Lupe nur schwer erkennen kann. Um die Bestimmung der Gefässgrösse bei der Unterscheidung der Hölzer zu erleichtern, habe ich dieselben in 5 Skalen getheilt, und je ein Holz, dessen grösste Gefässe man als Grössenmassstab annehmen kann, dazu ausgewählt, so dass man durch den Vergleich desselben mit irgend einer andern Holzart die Gefässstärke bestimmen und beschreiben kann. So haben wir:

für die 1ste Klasse Melia japonica Don. für sehr weite Gefässe.
,, ,, 2te ,, Fraxinus Sieboldiana Bl. für weite Gefässe.
,, ,, 3te ,, Machilus Thunbergii S. et Z. für feine Gefässe.

für die 4te Klasse Styrax japonica S. et Z. für sehr feine Gefässe.

für die 5te Klass Euonymus europaea L. vor. Hamiltoniana Maxim. für äusserst feine Gefässe.

Die feinen Gefässe sind auf dem Querschnitt macroscopisch noch als deutliche Löcher, die sehr feinen auf dem Querschnitt sehr schwer, aber auf dem Längsschnitt gut als vertiefte Linien, und die äusserst feinen aber weder auf dem Querschnitt noch auf dem Längsschnitt sichtbar.

Ebenso ist die Zahl und Breite der Markstrahlen für die Hölzer ganz bestimmt. Sie können bald zahlreich, bald spärlich auftreten, und von gleicher oder verschiedener Breite sein. Was die Breite derselben anbetrifft, so sind sie zweckmässig in 5 Skalen, wie die Gefässe, zu theilen und je ein Holz, dessen Markstrahlenbreite als Massstab dient, ist auszuwählen und zu beschreiben. So haben wir:

für die 1ste Klasse Alnus japonica S. et Z. für sehr breite Markstrahlen.

für die 2te Klasse Fagus sylvatica L. var. Sieboldi Maxim. für breite Markstrahlen.

für die 3te Klasse Zelkowa acuminata Pl. für feine Markstrahlen.

für die 4te Klasse Cinnamomum camphora Nees. für sehr feine Markstrahlen.

für die 5te Klasse Aesculus turbinata Bl. für äussert feine Markstrahlen.

Man kann die feinen Markstrahlen auf dem Querschnitt ganz deutlich sehen, die sehr feinen sind macroscopisch allerdings erkennbar, jedoch mit Lupe erst deutlich sichtbar, die äusserst feinen dagegen sind macroscopisch nur als radiale Strichelung oder gar nicht mehr wahrnehmbar.

Eine gewisse Farbe ist nur einem bestimmten Holze eigenthümlich und bildet ein sicheres Unterscheidungsmerkmal, so dass man es nicht mit einen anderen verwechseln kann, aber meist nur das Kernholz zeigt diese wichtigste Farbe, welche erst in gewissen Jahren nach der Holzbildung eintritt. Für das Splintholz, welches bei den meisten Hölzern nur gelblichweiss ist, hat die Farbe keine Bedeutung mehr. Die Holzfarbe ist sehr mannigfaltig; eine ganz richtige Beschreibung derselben

ist fast unmöglich, man kann die einzelnen Farben nur aus Erfahrung richtig unterscheiden lernen.

Die Rinde der Hölzer ist mit dem Alter und Wachsthum veränderlich. Der Baum, welcher im Mittel-oder hohen Alter eine Borke bildet, hat in der Jugend meist nur eine glatte Rinde, welche von der nicht borkebildenden kaum zu unterscheiden ist. Doch ist sie bei gewissen Hölzern ein ganz bemerkbares Unterscheidungs-Hilfsmittel wenn keine anderen Merkmale zur Verfügung stehen.

Zellgänge, macroscopisch sichtbare Parenchymlinien und anderes sind mehr oder weniger nützlich bei der Unterscheidung der Hölzer. Zellgänge treten bei gewissen Holzarten fast regelmässig, Parenchymlinien bei gewissen Hölzern jedoch in allen Fällen auf.

Zum Schluss möge hier noch bemerkt sein, dass die Beschreibung der Unterscheidungsmerkmale auf das nothwendigste beschränkt, und das unwesentlichste weggelassen ist, damit die hauptsächlichsten Merkmale mehr hervortreten.

I. Die Hölzer, bei welchen die Gefässe innerhalb eines Jahrringes an der Innengrenze durch eine andere Vertheilung als den übrigen Holztheilen ausgezeichnet sind (sogenannte ringporige Hölzer).

A. DIE GEFASSE AUSSERHALB DES PORENKREISES, D.I. DES INNEREN GEFÄSSREICHEREN HOLZTHEILES IN EINEM JAHRRINGE, SIND GLEICHMÄSSIG VERTHEILT, ODER DOCH ZUWEILEN NUR BEI DEN BREITEN JAHRRINGEN IN DER NAHE DER ÄUSSEREN JAHRRINGSGRENZE ZU KURZEN PERIPHERISCHEN ODER MEHR SCHRÄGEN LINIEN VEREINIGT.

a) Die Markstrahlen fein.

1 Die Gefässe im Porenkreise sehr weit.

α. Die Gefässe an der Aussengrenze des Jahrringes sind fast nie zu Strichen vereinigt.

1. CEDRELA CHINENSIS JUSS. (Chanchin.)

(Fig. 1. 95.)

Farbe des schmalen Splintes gelbweiss, des Kernes schön bräunlichroth, glänzend. Der Porenkreis ziemlich scharf begrenzt aber nicht sehr breit. Die Jahrringe schmal. Die einzelnen, oder eine ganze lange Reihe der peripherisch neben einander stehenden Gefässe sind mit einem schwarzen Secrete ausgefüllt. Das Holz mässig schwer, mässig hart, sehr leicht spaltbar. Die Rinde bildet eine braungraue flachrissige Borke.

2. SPONDIAS SP.? (Kaname.)

(Fig. 2. 96.)

Farbe des Splintes gelbweiss, des Kernes gelblichroth. Die Stellung der Gefässe ist der des Vorigen ähnlich, sie sind aber offen. Das Holz ist häufig mit schwärzlichen Stellen versehen. Es ist mässig schwer, mässig hart, leicht spaltbar. Die gehobelte Fläche des Holzes ist nicht glatt wie die des vorigen. Die Rinde ist eine dünne dunkelrothe flachrissige Schuppenborke.

3. ALBIZZIA JULIBRISSIN BOIV. (Nemu-no-ki.)

(Fig. 3.)

Farbe des Splintes gelblichweiss, des Kernes eigenthümlich gelbgraubraun, glänzend. Der Porenkreis sehr breit, nicht scharf begrenzt, geht allmählich in den Gefässärmeren Theil über, indem die Gefässe an Stärke und Zahl abnehmen. Die Gefässe offen, klar. Das Holz leicht, weich, leicht spaltbar, die gehobelte Fläche desselben sehr glatt. Die Rinde glatt, aschgrau, glänzend.

β. Die Gefässe an der Aussengrenze des Jahrringes sind fast immer zu Strichen, wenn auch nur kurzen vereinigt.

4. MELIA JAPONICA DON. (Sendan.)

(Fig. 4.)

Farbe des sehr schmalen Splintes gelbweiss, des Kernes hellgelbbraun. Der Porenkreis sehr breit, allmählich in den gefässärmeren Holztheil übergehend. Die Jahrringe sehr breit, nicht selten erreichen sie 15 mm und mehr. Die einzelnen

Gefässe sind häufig mit einem schwarzen Secrete ausgefüllt. Das Holz mässig hart. mässig schwer, leicht spaltbar. Die Rinde mit einer dunkelbraunen längsrissigen Borke.

5. HOVENIA DULCIS THUNB. (Kemponashi.)

(Fig. 5.)

Farbe des Splintes schön gelblichweiss, des Kernes gelblichbraun bis röthlichbraun. Der Porenkreis, ziemlich scharf begrenzt. Die Gefässe offen und klar. In der Nähe der Aussengrenze des Jahrringes sind die Gefässe, welche sehr fein sind, öfters nicht zu Strichen vereinigt, doch stehen dieselben immer nahe zusammen. Das Holz schwer, hart, leicht spaltbar. Die Rinde wird zur dunkelgrauen kleinschuppigen Borke.

6. EHRETIA ACUMINATA R. BR. (Chisha-no-ki.)

(Fig. 6.)

Farbe des sehr schmalen Splintes gelblichweiss, des Kernes gelbweiss, nicht viel verschieden unter einander. Der Porenkreis sehr scharf begrenzt, sehr häufig nur ein Gefäss breit. Die Gefässe ausserhalb des Porenkreises sehr fein und nicht klar offen. Der peripherische Lauf der Jahrringe unregelmässig. Das Holz mässigschwer, hart, sehr schwer spaltbar. Die Rinde hellgraubraun, kleinschuppig.

2. Die Gefässe im Porenkreise weit.

α. Die Gefässe an der Aussengrenze des Jahrringes sind fast nie zu Strichen vereinigt.

7. RHUS VERNICIFERA D.C. (Urushi.); RHUS TRICOCARPA MIQ. (Yama-urushi)

(Fig. 7. Urushi.)

Farbe des sehr schmalen Splintes glänzendweiss, des Kernes schön grünlich gelb. Der Porenkreis nicht scharf begrenzt, aber breit. Die Gefässe im Porenkreise nicht klar, die im Herbstholztheile sehr fein, meist nicht offen, macrocopisch schwer sichtbar. Die Markstrahlen zahlreich, gleichmässig fein. Das Holz leicht, weich, leicht spaltbar. Die Rinde anfangs glänzend aschgrau, wird später flachrissig.

8. RHUS SILVESTRIS S. ET Z. (Yamahaze), RHUS SUCCEDANEA L. (Haze).

Die Farbe des Holzes und die Stellung der Gefässe dem Vorigen sehr ähnlich, nur der Splint sehr breit. Die Gefässe im Porenkreise des Yamahaze sind oft so fein, dass er auch zu der nächsten Gruppe gezählt werden kann. Das Holz wie das des Vorigen. Die Rinde auch ähnlich, aber mit zahlreicheren Lenticellen.

9. RHUS SP. (Yamahaze ?)

(Fig. 8.)

Farbe des ziemlich breiten Splintes gelblichweiss, des Kernes röthlichbraun. Der Porenkreis scharf begrenzt. Die Gefässe ausserhalb des Porenkreises offen, deutlich, oft zu zwei bis drei gruppirt. Die Markstrahlen scharf, gut sichtbar, sparsamer als bei den vorigen beiden. Das Holz schwer, mässig hart, leicht spaltbar. Die Rinde wird eine harte, rissige, dunkelbraune Borke.

β. Die Gefässe an der Aussengrenze des Jahrringes sind fast immer zu Strichen, wenn auch nur kurzen, vereinigt.

10. RHUS SEMIALATA MURR. (Nurude.)

(Fig. 9.)

Farbe des schmalen Splintes schmutzigweiss, des Kernes eigenthümlich bräunlichgrau, zuweilen etwas gelblich. Der Porenkreis, breit, nicht scharf begrenzt. Die peripherisch ungeordneten Gefässe sind fein und scharf. Die Markstrahlen ohne Glanz, und nicht scharf. Das Holz weich, leicht, schwer spaltbar, die gehobelte Fläche ist nicht glatt. Die Rinde glänzend grau, glatt, mit deutlichen rostbraunen Lenticellen.

11. PICRASMA QUASSIOIDES BENN. (Nigaki.)

(Fig. 10.)

Der Splint gelbweiss, der Kern röthlich gelb, ungleichmässig gefärbt, schön glänzend. Die peripherischen Linien in der Nähe der Aussengrenze des Jahrringes sind verschieden lang, erscheinen hellgelb. Der Porenkreis ziemlich scharf begrenzt. Die Breite des Jahrringes ist verschieden breit und an den

verschieden Seiten ungleichdick. Die Markstrahlen hellgelb gefärbt, glänzend, weit sparsamer und stärker als bei den Rhusarten. Das Holz mässig hart, mässig schwer, schwer spaltbar und schmeckt bitter in Folge eines darin enthaltenen Stoffes. Die Rinde glatt, dunkel, etwas röthlich.

12. BROUSSONETIA PAPYRIFERA VENT. (Kaji-no-ki.)

Farbe des sehr schmalen Splintes gelblichweiss, des Kernes hellgelbbraun. Der Porenkreis breit, ohne scharfe Begrenzung. Im Gegensatz zu den anderen ringporigen Hölzern sind die grössten Gefässe nicht dicht an die Innengrenze gestellt, sondern weiter davon entfernt. Die Gefässe an der Aussengrenze bilden häufig deutliche wellenförmige Linien. Das Holz leicht, weich, schwer spaltbar. Die Rinde glatt, aschbraun.

13. BROUSSONETIA KASINOKI SIEB. (Kōzo.)

In allen Punkten dem vorigen ähnlich. Die Gefässe im Porenkreise etwas feiner und undeutlich. Der Porenkreis auch immer etwas schmaler. Die Rinde wie bei der Vorigen.

3. Die Gefässe in Porenkreise fein.
Die Gefässe ausserhalb des Porenkreises gleichmässig zerstreut.

14. ELAEAGNUS MACROPHYLLA THUNB. (Natsugumi.)

Farbe des Splintes gelblich, des Kernes schmutzig gelb. Der Porenkreis scharf begrenzt. Die Gefässe im Porenkreise sind nicht selten mit einem schwarzen Secrete ausgefüllt, diejenigen ausserhalb des Porenkreises zahlreich, nicht offen, macroscopisch nicht gut sichtbar. Die feinen und feineren Markstrahlen treten neben einander auf, sind deutlich, etwas glänzend. Der Lauf des Jahrringes unregelmässig. Das Holz zeigt öfters schmutzigdunkel gefärbte Stellen. Es ist schwer, hart, schwer spaltbar. Die Rinde bildet sich eine dunkle kleinschuppige Tafelborke.

15. PRUNUS MUME S. ET Z. (Ume.)
(Fig. 11.)

Farbe des schmalen Splintes bräunlich, des Kernes roth-

braun. Der Porenkreis häufig sehr schmal, auf dem Querschnitt schwer erkennbar. Die Markstrahlen fast gleichmässig stark und stehen dicht neben einander. Der Lauf der Jahrringsgrenze unregelmässig. Das Holz schwer, hart und schwer spaltbar. Die Rinde ist von glänzenden dunkelrothen Periderm bekleidet, und wird später rissig.

16. PRUNUS COMMUNIS HUDS. (Sumomo); PRUNUS PERSICA S. ET Z. VAR. VULGARIS MAX. (Momo.)

Schliesst sich in allen Punkten dem vorigen an. Der Splint aber breiter; die Gefässe im Porenkreise grösser; die Markstrahlen deutlicher. Der Periderm aschgrau, glänzend.

17. ACANTHOPANAX SCIADOPHYLLOIDES FR. ET SAV. (Koshiabura.)

(Fig. 12.)

Farbe des breiten Splintes hellgrauweiss, schön glänzend, des Kernes gelblichgrau. Das Holz zeigt sehr oft schmutzig grüngrauliche Stellen. Der Porenkreis, der auf dem Querschnitt schwer sichtbar ist, sehr scharf begrenzt, nur ein Gefäss breit. Die Markstrahlen gleichmässig fein und scharf. Das Holz ist weich, leicht, leicht spaltbar. Die gehobelte Fläche des Holzes nicht glatt. Die Rinde dunkel, färbt sich durch Flechtenwuchs grüngrau oder grünweisslich.

18. DENDROPANAX JAPONICUM SEEM. (Kakuremino.)

Dem Vorigen sehr ähnlich. Die Gefässe ausserhalb des Porenkreises sind weniger zahlreich und nur mit Lupe gut wahrnehmbar.

19. EDGEWORTHIA CHRYSANTHA LINDL. (Mitsumata.)

Farbe des breiten Splintes grünlichweiss, des Kernes hellgraubräunlich. Der Porenkreis sehr schmal, undeutlich. Die Markstrahlen sind verschieden stark, feine mit feineren vermischt, sehr undeutlich. Das Holz weich und sehr leicht, aber schwer spaltbar. Die Rinde hellgelbbraun mit seichten Längsfurchen versehen.

b) Die Markstrahlen sehr fein.

1. Die Gefässe im Porenkreise weit. Die Gefässe an der Aussergrenze des Jahrringes häufig zu Strichen vereinigt.

20. FRAXINUS SIEBOLDIANA BL. (Shioji.)

(Fig. 13.)

Farbe des sehr breiten Splintes gelblichweiss, des Kernes hellbräunlich. Der Porenkreis ziemlich scharf begrenzt. Die feineren Gefässe ausserhalb des Porenkreises häufig zu zweien gruppirt. Die peripherische Linie in der Nähe des Aussengrenzes der Jahrringe ist nur bei den breiten Jahrringen sichtbar. Das Holz mässig hart, mässig schwer, leicht spaltbar. Die Rinde wird eine hellgelbbraune nicht tiefrissige Borke.

21. FRAXINUS MANDSHURICA RUPR. (Yachidamo.)

Dem Vorigen sehr ähnlich, aber die Farbe des Kernholzes dunkler und das Holz härter.

22. CATALPA KAEMPFERI S. ET Z. (Ki-sasage.)

(Fig. 14.)

Farbe des sehr schmalen Splintes graubräunlich, des Kernes graubraun, kein scharfer Unterschied. In dem Porenkreise stehen die Gefässe dicht zusammen, so dass der ganze Kreis schwammig erscheint. Der Lauf des Jahrringes oft unregelmässig. Die Gefässe ausserhalb des Porenkreises sparsam, aber deutlich als Löcher sichtbar. Das Holz zeigt häufig gelblich gefärbte Stellen. Es ist weich, leicht und leicht spaltbar. Die Rinde bildet eine dunkelbraune längsrissige Borke.

23. ROBINIA PSEUDACACIA L. (Inu-akashia.)

Farbe des sehr schmalen Splintes gelblichweiss, des Kernes gelbbraun. Die Gefässe des schmalen Porenkreises sind durch Füllzellen verstopft und erscheinen nur als gelbe helle Punkte; die ausserhalb des Porenkreises peripherisch angeordnet und sehr fein. Das Holz schwer, hart und leicht spaltbar. Die Rinde hellbraungelb, tiefrissig.

2. Die Gefässe im Porenkreise fein.

24. FRAXINUS BUNGEANA D.C. VAR. PUBINERVIS WG. (Toneriko.)

(Fig. 15.)

Farbe des sehr breiten Splintes hellgelblichweiss, des Kernes hellgelbbräunlich bis hellbraun. Der Porenkreis scharf begrenzt aber verschieden breit. Bei einem schmalringigen Holze ist er in der Regel ein Gefäss breit und die Gefässe fein, bei einem breitringigen aber mehrere Gefässe breit und die Gefässe häufig weit.

In dem lelzteren Falle kann man dieses Holz zur Fraxinus mandschurica gruppiren. Die Gefässse ausserhalb des Porenkreises meist zu 2 oder 3, selten 4 bis 5 verbunden, und zwar in der Nähe der Jahrringsgrenze bis zur peripherischen Linie. Das Holz schwer, hart und leicht spaltbar. Die Rinde glatt, hellgrau. Flechtenwuchs färbt sie grünlichgrau.

25. LIGUSTRUM IBOTA SIEB. (Ibota-no-ki.)

Farbe des Splintes schmutzig weiss bis weiss, des Kernes graubräunlich. Die Gefässe des Porenkreises sind auf dem Querschnitt ohne Luppe nicht mehr gut sichtbar; diejenigen ausserhalb des Porenkreises äusserst fein, so dass man sie selbst auf dem Längsschnitt nicht mehr erkennen kann. Das Holz schwer, hart und schwer spaltbar. Die Rinde wird eine hellgraue flachrissige Schuppenborke mit stark entwickelten Steinzellnestern.

c) Die Markstrahlen äusserst fein.

Die Gefässe im Porenkreise weit.

26. MALLOTUS JAPONICUS MUEL. ARG. (Akamegashiwa.)

(Fig. 16.)

Farbe des Splintes gelblichweiss, des Kernes gelbweiss, der Unterschied bei der Holzschichten ist unbedeutend. Der Porenkreis, welcher breit und nicht scharf begrenzt ist, geht allmählich in die feinporige Holzschicht über. Die Gefässe im

Porenkreise offen und stehen nicht dicht zusammen, diejenigen ausserhalb des Porenkreises vereinigen sich zu 2 bis 4 in radialer Richtung und nehmen gegen die Aussengrenze des Jahrringes an Stärke ab. Das Holz leicht, weich, schwer spaltbar. Die Rinde bildet kleine Borke, hellgraubraun mit seichten Furchen. Flechtenwuchs färbt sie gewöhnlich grünlichweissgrau.

27. ALEURITES CORDATA MUEL. ARG. (Aburagiri.)

Farbe des breiten Splintes hellbräunlich, des Kernes röthlich hellbraun. Die Markstrahlen sind röthlich gefärbt, so dass man auf dem glatt gehobelten Querschnitt ihren Lauf als radiale Strichelung wahrnehmen kann. Die Gefässe im Porenkreise, welcher nicht scharf begrenzt ist, offen, aber nicht ganz klar. Die feineren Gefässe ausserhalb des Porenkreises stehen meist einzeln und nicht dicht zusammen. Das Holz leicht, mässig hart, leicht spaltbar. Die Rinde glatt, graubraun.

B. DIE GEFÄSSE AUSSERHALB DES PORENKREISES SIND ZU PERIPHERISCHEN, ZUWEILEN ETWAS VERZWEIGTEN WELLENLINIEN VEREINIGT.

a) Die Markstrahlen breit.

1. Die Gefässe im Porenkreise weit.

28. STERCULIA PLATANIFOLIA L. (Ao-giri.)

Farbe des sehr schmalen Splintes gelblichweiss, des Kernes mattgrün bis gräulichgrün. Der Porenkreis ziemlich breit und ziemlich scharf begrenzt. Die Wellenlinien, in welchen die Gefässe nicht sichtbar, sind ziemlich dick, deutlich, nicht lang, verlaufen parallel. Das Holz weich, leicht, leicht spaltbar. Die Rinde glatt, grün.

29. CELTIS SINENSIS PERS. (Enoki.)

(Fig. 17.)

Farbe des sehr breiten Splintes grünlichweiss, des Kernes schmutzig grau. Der Porenkreis ist sehr breit, geht allmählich

nach Aussen in die kleineren Gefässe über, bis endlich an ihre Stelle die langen, meist gerade und parallel verlaufenden wellenförmigen Porenreihen treten. Die Gefässe der Wellenlinie sind fein aber offen und ganz klar. Die Markstrahlen sind nicht so breit wie die des Vorigen, aber zahlreicher. Die Jahrringe sind breit, erreichen nicht selten 25 mm und mehr. Das Holz weich, leicht, schwer spaltbar. Die Rinde dunkelaschgrau.

2. Die Gefässe im Porenkreise fein.

30. BERBERIS THUNBERGII D.C. (Megi.)

(Fig. 18.)

Farbe des Holzes intensiv gelb, kein Kern. Die Gefässe des Porenkreises sehr fein und nur ein Gefäss breit. Die Wellenlinien ausserhalb des Porenkreises sehr fein, kurz und nicht parallel verlaufend. Sie sind ohne Lupe nicht mehr sichtbar. Das Holz schwer, hart und schwer spaltbar. Die Rinde wird eine dunkelgraue längsrissige korkreiche Borke.

31. TAMARIX JUNIPERINA BGE. (Gyoriu.)

Farbe des schmalen Splintes gelbweiss, des Kernes blutroth. Der Porenkreis nicht scharf begrenzt und breit. Die Gefässe im demselben zahlreich, stehen dicht zusammen, diejenigen in den Wellenlinien fein, aber gut als Löcher sichtbar. Die Wellenlinien verlaufen parallel und nicht dicht neben einander. Das Holz weich, leicht und schwer spaltbar. Die Rinde bildet eine dunkle längsrissige Borke.

b) Die Markstrahlen fein.

Die Gefässe im Porenkreise weit.

32. ZELKOWA ACUMINATA PL. (Keyaki.)

(Fig. 19.)

Farbe des sehr breiten Splintes gelblichweiss, des Kernes hellgelblich braun bis braun. Die Gefässe im Porenkreise, welcher bei dem schmalen Jahrringe immer ein Gefäss breit, bei dem breiten aber noch durch ein oder zwei feinere Gefässe

erweitert wird, sind offen und klar. Die Wellenlinien fein aber deutlich, verschieden lang, gleichmässig parallel. Das Holz schwer, hart aber leicht spaltbar. Die Rinde anfangs glatt, grünlichgrau, später lösen sich dicke grosse Borkeschuppen ab.

33. ACANTHOPANAX RICINIFOLIUM S. ET Z. (Hari-giri.)

(Fig. 20.)

Farbe des Splintes hellgelblichweiss, des Kernes hellgräulichweiss, stark glänzend. Der Porenkreis ist immer, ohne Unterschied der Jahrringsbreite, ein Gefäss breit. Die Gefässe im Porenkreise sind fein, offen und sehr klar. Die Wellenlinien gestalten sich wie die des Vorigen, ihr Lauf ist aber gröber. Die Markstrahlen sind feiner als die des Vorigen und der Abstand derselben ungleichmässig. Das Holz mässig hart, mässig schwer, leicht spaltbar. Die Rinde bildet eine dunkelgraubraune tiefrissige Borke.

34. ULMUS CAMPESTRIS SM. VAR. LAEVIS PLANCH.

(Haru-nire.)

(Fig. 21.)

Farbe des breiten Splintes gräulichweiss, des Kernes weisslichgrau. Der Porenkreis ist gewöhnlich 2 Gefässe breit, aber immer durch einige feinere Gefässe erweitert. Die Wellenlinien sind dick und meist dicht neben einander, daher undeutlich. Die Markstrahlen sind röthlich gefärbt, aber nicht scharf. Im Vergleich mit dem vorigen Holze ist ihre Höhe, welche auf dem Tangentialschnitt sichtbar ist, etwa doppelt so hoch. Das Holz mässig hart, mässig schwer, leicht spaltbar, ohne Glanz. Die gehobelte Fläche des Holzes fühlt sich mit Fingern berührt sehr rauh an. Die Rinde bildet eine hellgraubraune flachrissige Schuppenborke.

35. ULMUS MONTANA VAR. LACINIATA TRAUTTV. (Ohyō.)

Farbe des Splintes gelblichweiss, des Kernes hellbraun bis dunkelbraun. Der Porenkreis des breiten Jahrringes ist 2 oder 3 Gefässe breit, und sehr scharf begrenzt. Die Wellenlinien fein und kurz, häufig nur 2 oder 3 Gefässchen lang. Die Mark-

strahlen sind röthlich gefärbt. Das Holz sehr hart, schwer, leicht spaltbar. Die Rinde löst sich in grosse dunkle Plattenschuppen ab.

36. Ulmus parvifolia Jacq. (Aki-nire.)

(Fig. 22.)

Farbe des Splintes hellgelb, des Kernes gelblichbraun. Die Gefässe im Porenkreise, der hellgelb erscheint, sind feiner und zahlreicher, und die Wellenlinien länger als bei dem Vorigen. Die Markstrahlen sind gelblich gefärbt. Das Holz schwer, sehr hart, schwer spaltbar. Die Rinde wird eine dunkle kleinschuppige Tafelborke mit zahlreichen rostbrauen Lenticellen.

37. Cladrastis amurensis B. et H. var. floribunda Maxim. (Inu-enju.)

Farbe des sehr schmalen Splintes gelblichweiss, des Kernes schwärzlichbraun. Die Stellung der Gefässe ist der des Vorigen sehr ähnlich, nur die Wellenlinien sind feiner und der Porenkreis etwas breiter. Das Holz schwer, hart, leicht spaltbar, die gehobelte Fläche des Holzes sehr glatt. Die Rinde ist von dunkelgrünlich grauem geschichtetem Periderm bekleidet, die sich in Pusteln aufreisst.

38. Sophora japonica L. (Enju.)

Farbe des ebenso schmalen Splintes gelblichweiss, des Kernes ist der des Vorigen ähnlich, aber heller. Die Stellung des Porenkreises und der Wellenlinien ähnelt dem Ohyō in hohem Grade nur mit dem Unterschied, dass die Wellenlinien etwas breiter sind. Die Markstrahlen sind nicht rothgefärbt, auffallender als bei dem Ohyō. Dieses ist ein wichtiges Unterscheidungsmerkmal der beiden Hölzer. Das Holz schwer, hart, leicht spaltbar, die gehobelte Fläche des Holzes nicht sehr glatt. Die Rinde bildet eine dunkelgraue langrissige Borke.

39. SOPHORA PLATYCARPA MAXIM. (Fujiki.)

(Fig. 23.)

Farbe des breiten Splintes hellgelblich, des Kernes schön gelb. Der Porenkreis ist wie bei dem Vorigen. Die Gefässe ausserhalb des Porenkreises bilden nur in der Nähe der Jahrringsgrenze eine Wellenlinie, die dicker ist als die des Vorigen. Die Markstrahlen sind scharf, und klar. Das Holz mässig hart, mässig schwer, leicht spaltbar. Die Rinde glatt gelbgrau.

40. PHELLODENDRON AMURENSE RUPR. (Kiwada.)

(Fig. 24.)

Farbe des sehr schmalen Splintes grüngraulichweiss, des Kernes gelbgrüngrau, schön glänzend, sehr oft dunkelgrün oder dunkelbraungrün. Der Porenkreis ziemlich scharf begrenzt, aber die Gefässe im demselben meist nicht klar offen. Die Wellenlinie kurz, häufig nur 2 oder 3 Gefässchen breit. Die Markstrahlen fein, aber scharf. Das Holz weich, leicht, sehr leicht spaltbar. Die Rinde dunkelgelbgrau, sehr korkreich, tiefrissig.

41. GLEDITSCHIA JAPONICA MIQ. (Saikachi.)

Farbe des breiten Splintes gelbweiss, des Kernes schön rosaroth. Die Gefässe im Porenkreise, der breit aber ziemlich scharf begrenzt ist, sind fast gleichgross mit den ausserhalb des Porenkreises einzeln vorhandenen. Die Gefässe sind alle offen. Die Wellenlinien sieht man erst in der Nähe der Aussengrenze des Jahrringes; sie verlaufen mehr schräg. Die Markstrahlen verschieden stark. Die Jahrringe sehr breit, erreichen öfters 15 mm und mehr. Das Holz mässig hart, mässig schwer, sehr schwer spaltbar. Die Rinde grau, lange Zeit glatt, erst später entsteht eine platte Borke.

42. MORUS ALBA L. VAR. STYLOSA BUR. (Yama-guwa.)

Farbe des sehr schmalen Splintes gelblichweiss, des Kernes gelblich bis gelbbraun, glänzend. Die Gefässe im Porenkreise sind verschieden gross, meist fein, zuweilen weit. Die Breite

des Porenkreises auch verschieden, bald ein oder zwei Gefässe breit, bald drei bis fünf breit. Die Wellenlinien sind in der Regel fein und kurz, zuweilen aber dick und lang. Einzelne der Gefässe sind durch ein gelbes Secret verstopft. Die Markstrahlen gleichmässig fein, aber nicht selten kommen sie mit etwas dickeren gemischt vor. Das Holz hart, schwer, schwer spaltbar. Die Rinde hellbraun, längsrissig.

43. AILANTHUS GLANDULOSA DESF. (Shinju.)

Farbe des Splintes gelblich, des Kernes gelblichweiss, schön glänzend. Der Porenkreis ist bei einem schmalen Jahrringe ein oder zwei Gefässe breit, bei einem breiten aber 4 oder 5. Die feinen, offenen Gefässe sind nur in der Nähe der Aussengrenze des Jahrringes zu Wellenlinien vereinigt. Die Markstrahlen sind glänzend, deutlich, es kommen feine und theilweise gröbere vermischt vor. Das Holz mässig hart, mässig schwer, leicht spaltbar. Die Rinde glatt, dunkelgrau, später reisst sie sich etwas auf.

44. PAULOWNIA TOMENTOSA H. BN. (Kiri.)

(Fig. 25.)

Farbe des sehr schmalen Splintes weisslichgrau ins bräunliche spielend, die des Kernes etwas röthlicher, beide Zonen schwer erkennbar. Der Porenkreis ist nicht scharf begrenzt. Die Wellenlinien breit und kurz. Alle Gefässe sind nicht klar offen. Die Markstrahlen gleichmässig fein, ziemlich scharf. Die Jahrringe sind bei schnellwüchsigem Holze sehr breit, nicht selten über 20 mm, aber von geringerer Qualität. Das Holz sehr leicht, sehr weich aber schwer spaltbar. Die Rinde hellgraubraun.

45. CLERODENDRON TRICOTOMUM THUNB. (Kusagi.)

Farbe des breiten Splintes gelblichweiss, des Kernes hellgelb. Der Porenkreis ist meist ein Gefäss breit und die Gefässe sind fein und stehen nicht dicht zusammen. Im übrigen Theile sind die Gefässe zu kurzen oder langen peripherischen fast geraden Linien angeordnet. Diese Linien in der Nähe der Aussengrenze des Jahrringes sind sehr fein. Das Holz mässig

hart, mässig schwer, leicht spaltbar. Die Rinde aufänglich glatt, dunkelgrau, später entsteht eine kleinschuppige Tafelborke.

c) Die Markstrahlen sehr fein.

46. APHANANTHE ASPERA PLANCH. (Mukunoki.)

Farbe des Splintes hellgrau, des Kernes dunkelgrau. Der Porenkreis ist sehr breit, besteht aus locker nebeneinander stehenden, feinen aber offenen Gefässen und ist nicht scharf begrenzt. Die Wellenlinien ausserhalb des Porenkreises sind bald kurz bald lang, die längeren verlaufen fast gerade und parallel. Die Markstrahlen sind gleichmässig scharf, fein und ziemlich deutlich. Das Holz hart, schwer, sehr schwer spaltbar. Die Rinde anfangs bräunlich grau, mit ziemlich groben Furchen versehen, erst später bildet sich eine Schuppenborke.

47. SAPINDUS MUKUROSI GAERTN. (Mukuroji.)

(Fig. 26.)

Farbe des breiten Splintes gelblichweiss, des Kernes hellgelb mit etwas braun. Der Porenkreis ziemlich scharf begrenzt. Die Gefässe im Porenkreise sind weit, mit einem gelblichen Secrete ausgefüllt. Die Wellenlinien, welche meist lang und fast gerade verlaufen, sind dick. Das Holz zeigt häufig dunkelgraue oder schwärzliche Stellen. Es ist mässig hart, mässig schwer, leicht spaltbar. Die Rinde glatt, dunkelgrüngrau.

C. DIE GEFASSE AUSSERHALB DES PORENKREISES SIND IN EINER RADIAL VERLAUFENDEN, OFT AUCH VERZWEIGTEN LINIE VEREINIGT.

a) Einzelne Markstrahlen sehr breit, andere aber äusserst fein.

48. QUERCUS SERRATA THUNB. (Kunugi.)

(Fig. 27.)

Farbe des breiten Splintes graulich weiss, des Kernes

dunkelbräunlich mit etwas roth. Der Porenkreis ist meist ein Gefäss breit, das häufig von einem feineren begleitet ist. Die Gefässe im Porenkreise sind weit oder oft sehr weit, meist offen und stehen nicht dicht zusammen, diejenigen in den Wellenlinien, obgleich sehr fein, doch zum Theil noch als Löcher sichtbar. Die feinen Linien von Parenchym sind zahlreich, verlaufen peripherisch und parallel. Die sehr breiten Markstrahlen sind zahlreich und etwas glänzend und die Höhe derselben, die man auf dem Tangentialschnitt deutlich sieht, schwankt von 2 bis 20 mm. Das Holz sehr hart, sehr schwer und leicht spaltbar. Die Rinde bildet eine dunkelbraune, versteinte tiefrissige Borke, aber keineswegs grobrissig.

49. QUERCUS VARIABILIS BL. (Abemaki.)

Das Holz ist dem Vorigen sehr ähnlich, Farbe des Kernes aber mehr röthlich und die Markstrahlen sind meist niedriger. Das Holz ist grob, von schlechterer Qualität. Die Rinde ist von sehr dicken Korkkrusten bedeckt.

50. QUERCUS DENTATA THUNB. (Kashiwa.)

(Fig. 28.)

Farbe des breiten Splintes grauweiss, des Kernes dunkelgrau bräunlich. Der Porenkreis ist meist zwei Gefässe breit und die Gefässe im Porenkreise sind etwas kleiner als die des Vorigen und stehen dichter zusammen. Das Holz steht im Werthe zwischen Abemaki und Kunugi. Die Rinde bildet eine rothbraune Borke, welche tiefrissig wie die des Kunugi, aber weniger versteint ist.

b) Einzelne Markstrahlen breit, andere aber äusserst fein.

51. QUERCUS GLANDULIFERA BL. (Konara.)

(Fig. 29.)

Farbe des Splintes grauweiss, des Kernes hellgraubraun mit einem Stich ins grüne. Der Porenkreis ist meist nur ein Gefäss breit, häufig aber von einem, selten mehreren kleinen

Gefässen begleitet. Die sehr feinen Gefässe in den Radiallinien sind ohne Lupe nur sehr schwer als Löcher sichtbar. Die breiten Markstrahlen zahlreich, die Höhe derselben ist bedeutend grösser als die der Kunugi, nicht selten erreicht sie 40 mm und mehr. Die peripherischen Linien von Parenchym ziemlich gut sichtbar. Das Holz sehr hart, sehr schwer, leicht spaltbar. Die Rinde anfangs glatt, aschgrau, glänzend, später bildet sich eine nicht tiefrissige Borke.

52. QUERCUS GROSSERRATA BL. (Ō-nara.)

(Fig. 97. 98.)

Dem Vorigen sehr ähnlich. Der Splint ist sehr breit, die Farbe des Kernes hellgelblich braun. Der Porenkreis ist in der Regel zwei Gefässe breit; häufig noch durch ein oder zwei begleitende Gefässe erweitert und nur in den schmalen Jahrringen ein Gefäss breit. Die Rinde ist eine dunkle grob-und tiefrissige Borke, oder eine flachrissige.*

c) Die Markstrahlen sehr fein.

53. PLATYCARYA STROBILACEA S. ET Z. (Nobu-no-ki.)

(Fig. 30.)

Farbe des breiten Splintes gelblichweiss, des Kernes dunkelgelbbraun. Der Porenkreis breit, geht allmählich in die gefässärmere Holzschicht über. Die Gefässe ausserhalb des Porenkreises vereinigen sich zu schwanzförmig verzweigten Linien und sind nicht mehr als Löcher sichtbar, erscheinen nur grauweisslich. Das Holz mässig hart, mässig schwer, leicht spaltbar. Die Rinde bildet eine röthlich graubraune, nicht tiefrissige Borke.

d) Die Markstrahlen äusserst fein.

54. CASTANEA VULGARIS LAM. VAR. JAPONICA D.C. (Kuri.)

(Fig. 31.)

Farbe des sehr schmalen Splintes schmutzig graugelb, des

* Dies Holz kann wahrscheinlich eine andere sehr verwandte Holzart sein.

Kernes hellbraun ins schwärzliche spielend. Der Porenkreis ist 2 bis 3 Gefässe breit, meist ohne scharfe Begrenzung. Die Gefässe im Porenkreise sehr weit und die schwanzförmig meist in radialer Richtung verlaufende Linie fein aber zum Theil noch als Löcherchen sichtbar. Das Holz mässig hart, mässig schwer, leicht spaltbar. Die Rinde anfangs glatt, dunkelrothbraun, später flachrissig.

II. Die Hölzer, bei welchen die Gefässe im ganzen Jahrringe gleichförmig zerstreut sind, (sogenannte zerstreutporige Hölzer).

A. EIN THEIL DER MARKSTRAHLEN SEHR BREIT, ANDERE ABER AUSSERST FEIN.

55. ALNUS JAPONICA S. ET Z. (Hannoki.)

(Fig. 32. 99.)

Farbe des frischen Holzes weiss, des trockenen aber bräunlich roth, ohne eigenthümliches Kernholz. Die breiten Markstrahlen sind zahlreich, nicht scharf begrenzt, ohne Glanz, desshalb undeutlich. Die Jahrringsgrenze markirt sich durch eine feine undeutliche Linie, welche an den Seiten der breiten Markstrahlen etwas nach aussen ausgebuchtet und innerhalb derselben nach einwärts gebogen ist. Die Gefässe sehr fein, mit unbewaffnetem Auge kaum sichtbar. Zellgänge sehr selten. Das Holz weich, leicht, sehr leicht spaltbar. Die Rinde bildet frühzeitig eine dunkle längsrissige Borke.

56. ALNUS INCANA WILLD. VAR. GLAUCA AIT. (Yamahannoki.)

(Fig. 33. 100.)

Farbe des Holzes wie die des Vorigen. Die breiten Markstrahlen weniger zahlreich als bei dem Vorigen, aber schärfer ausgeprägt, und die Jahrringsgrenze deutlicher, weniger ausgebuchtet. Zellgänge auch selten. Die Rinde ist glatt bis zum hohem Alter, reisst später etwas auf.

57. ALNUS VIRIDIS D.C. VAR SIBIRICA RGL.

(Miyama-hannoki.)

Farbe des Holzes wie die des Vorigen. Die breiten Markstrahlen zahlreich wie bei dem vorigen, aber weit feiner und schärfer begrenzt. Die Herbstholzzone, die dichter ist als die Frühlingsholzzone, breit und die Jahrringsgrenze stark ausgebuchtet. Die Rinde grau, bleibt glatt.

58. ALNUS GLUTINOSA WILLD. (Kawara-hannoki.)

Farbe des Holzes bleibt an der Luft lange Zeit weiss, wird endlich bräunlich roth. Die Markstrahlen sind dem Vorigen ähnlich, aber glänzend, und an Zahl weit weniger. Die Jahrringsgrenze ist deutlich, wenig ausgebuchtet. Das Holz ist härter und dichter als das der vorigen Alnusarten. Die Rinde dunkel, wird später schuppig.

B. EIN THEIL DER MARKSTRAHLEN BREIT.

a) Die breiten Markstrahlen sind sehr zahlreich, nicht glänzend und verlaufen etwas krummlinig. Zwischen denselben sind die feineren vertreten, aber die feinsten fehlen.

59. ARDISIA SIEBOLDI MIQ. (Mokutachibana.)

(Fig. 34.)

Farbe des Holzes graugelb, kein Kern. Die Gefässe sind fein und mit einem bräunlichen Secrete verstopft. Die Markstrahlen sind nicht scharf begrenzt. Das Holz mässig hart, mässig schwer, schwer spaltbar. Die Rinde dunkel.

60. MYRSINE CAPITELLATA WALL. (Hichi-no-ki.)

Das Holz ist dem Vorigen ähnlich, aber die Gefässe ärmer, offen und klar. Die Jahrringsgrenze ist zwischen den Markstrahlen etwas ausgebuchtet. Die Rinde wie die der vorigen.

61. AUCUBA JAPONICA THUNB. (Aoki.)

Farbe des sehr schmalen Splintes gräulichweiss, des Kernes schwarzbraun. Die Gefässe äusserst fein, in der Innengrenze des Jahrringes sind sie zu peripherischen Linien, in den übrigen Holztheilen aber bis zu 3 oder 4 verbunden. Die Gefässe sind nicht als Löcher sichtbar. Die Markstrahlen sind scharf begrenzt. Das Holz schwer, hart, leicht spaltbar. Die Rinde anfangs grün, glatt, wird später korkig.

b) Die breiten atlasglänzenden Markstrahlen sind haüfig und verlaufen gerade. Zwischen denselben sind die feineren und feinsten vertreten.

62. FAGUS SYLVATICA L. VAR. SIEBOLDI MAXIM. (Buna.)

(Fig. 35. 101.)

Das Holz ist hellröthlich mit etwas braun. Der eigentliche Kern fehlt, ein falscher, der aus pathologischen Gründen entsteht, ist häufig. Die Markstrahlen sieht man auf dem Tangentialschnitt als Spindel sehr deutlich. Die Gefässe sind in der Herbstholzzone weit sparsamer, deshalb ist das Holz dichter. Das Holz schwer, hart, leicht spaltbar. Die Rinde grüngrau, bleibt glatt.

63. FAGUS JAPONICA MAXIM. (Inu-buna.)

(Fig. 36. 102.)

Das Holz ist dem Vorigen ähnlich, aber die Farbe mehr grau und schmutzig, die gefässärmere Herbstholzzone schwach entwickelt, so dass das Holz weniger dicht ist als das des Vorigen. Die Markstrahlen sind etwas feiner und laufen meist etwas krumm. Die Rinde anfangs glatt, dunkel, sie springt aber früh flach auf.

C. DIE MEISTEN DER MARKSTRAHLEN SIND FEIN, ABER DEUTLICH UND SCHARF, SO DASS MAN DEREN VERLAUF AUF DEM QUERSCHNITT LEICHT MIT UNBEWAFFNETEM AUGE VERFOLGEN KANN.

a) Die Markstrahlen sind auf dem Tangentialschnitt als Spindel deutlich sichtbar.

1. Die Markstrahlen sind theils hoch, theils niedrig.

64. EUPTELAEA POLYANDRA S. ET Z. (Fusazakura.)

(Fig. 37. 38. 39.)

Farbe des Holzes grauweiss. Kein Kern. Die höchsten Markstrahlen sind bis zu 4 mm hoch. Die Gefässe sind fein und sehr zahlreich, im ganzen Jahrringe gleichmässig vertheilt; sie können aber nur mit Lupe erkannt werden. Die Jahrringsgrenze ist durch eine feine helle Linie gekennzeichnet. Das Holz mässig hart, schwer spaltbar. Die Rinde dunkel, mit zahlreichen braunen Lenticellen.

65. MELIOSMA PUNGENS WALL. (Yama-biwa.)

Das Holz grauroth. Kein Kern. Die höchsten Markstrahlen (bis zu 8 mm hoch) zum Theil so stark, dass sie auch an Ardicia Sieboldi erinnern, und man sie dazu gruppiren könnte. Die Gefässe sehr arm, aber ohne Lupe nicht sichtbar. Die Jahrringsgrenze ist schwer zu erkennen. Das Holz mässig schwer, hart, schwer spaltbar. Die Rinde aschgraubraun.

2. Die Markstrahlen sind niedrig und fast gleich hoch.

66. CORNUS KOUSA BUERG. (Yamabōshi.)

(Fig. 40. 41. 42.)

Das Holz schön röthlich. Kein Kern. Die Markstrahlen scharf begrenzt, glänzend. Die Gefässe sind fein, offen, gleichmässig vertheilt, mit Lupe als Löcher sichtbar. Das Holz

schwer, hart, schwer spaltbar, die gehobelte Fläche des Holzes sehr glatt. Die dunkelrothbraune Rinde löst sich frühzeitig in rundliche Plattenschuppen ab.

67. Ilex crenata Thunb. (Inu-tsuge.)

Das Holz grünlichweiss. Kein Kern. Die höchsten Markstrahlen sind bis zu 2 mm hoch und verlaufen krummlinig, ohne Glanz. Die Gefässe sparsam, sehr fein, vielfach in radiale Reihe gestellt, meist nicht offen, ohne Lupe nicht erkennbar. Die Jahrringsgrenze ist schwer zu sehen. Das Holz schwer, hart, schwer spaltbar. Die Rinde dunkelgraugrün, mit undeutlichen Lenticellen.

68. Ilex integra Thunb. (Mochi-no-ki), Ilex rotunda Thunb. (Kuroganemochi), Ilex latifolia Thunb. (Tarayō).

(Fig. 43. Mochinoki.)

Diese Hölzer sind dem des Vorigen in hohem Grade ähnlich. Die Rinde ist aber etwas verschieden von einander. Dieselbe des integra, anfangs glatt dunkelaschgrau, später etwas bräunlichgelb, des rotunda grünlichgrau, glänzend, die des latifolia aschgrau.

69. Ilex macropoda Miq. (Aohada.)

Das Holz schön grünlichweiss, kein Kern. Die Markstrahlen wie Atlas glänzend, fast gleichmässig stark, aber weniger zahlreich als bei den vorigen Holzarten. Die Gefässe sind wie die der Inutsuge vielfach in radialer Richtung angeordnet, aber feiner und zahlreicher. Die Jahrringsgrenze ist schwer erkennbar. Das Holz ist dem des Inutsuge gleich. Die Rinde glatt, dunkelgrüngrau mit stark entwickelten Steinzellnestern.

70. Ilex pedunculosa Miq. (Soyogo.)

Farbe des Holzes wie die des Vorigen. Die Markstrahlen weniger glänzend, etwas feiner. Die Gefassstellung wie die des

Vorigen. Die Rinde dunkelgrün; die Steinzellnester sehr wenig entwickelt.

71. EUSCAPHIS JAPONICA PAX. (Gonzui.)

Das Holz schmutziggrau. Kein Kern. Die Markstrahlen sind glänzend, aber an der Grenze der Jahrringe etwas undeutlich und erscheinen als ob sie dort unterbrochen wären. Die Gefässe fein, aber ärmer und noch feiner in der Herbstholzzone, welche deshalb dichter ist. Die Gefässe sind offen, aber macroscopisch schwer sichtbar. Das Holz mässig hart, mässig schwer, schwer spaltbar. Die Rinde dunkel braun mit runzeliger Oberfläche.

b) Die Markstrahlen sind auf dem Tangentialschnitt sehr schwer erkennbar.

1. Die Gefässe sind arm und fein, aber auf dem Querschnitt gut sichtbar.

α. Die Markstrahlen sind fast gleich fein.

72. MELIOSMA MYRIANTHA S. ET Z. (Awabuki.)

(Fig. 44. 45. 46.)

Farbe des Splintes hellgelbbraun, des Kernes braun. Die Gefässe sind meist zu 2 bis 4 in radialer Richtung vereinigt, offen und gelblich gerändert, mit Lupe sieht man sie klar als offene Löcher, aber mit freiem Auge nur als gelbe Punkte. Das Holz zeigt häufig unregelmässig dunkel gefärbte Stellen. Das Holz mässig hart, schwer, schwer spaltbar. Die Rinde dunkelgrau.

β. Die meisten Markstrahlen fein, dazwischen die sehr feinen bemerkbar.

73. MORUS INDICA L. (Shima-guwa.)

(Fig. 47.)

Farbe des schmalen Splints gelblichweiss, des Kernes verschieden gefärbt: hellgelb, gelbbraun bis dunkelbraun.

Die Gefässe sind sparsam, mit einem gelblichen Secrete ausgefüllt. Die einzelnen Markstrahlen sind zuweilen breit. Das Holz sehr schwer, sehr hart, schwer spaltbar.

74. Sophora spe.?

(Fig. 48.)

Farbe des Splintes hellgelblich, des Kernes hellgelb, schönglänzend. Die Gefässe sparsam, gleichmässig zerstreut, offen und klar. Die Markstrahlen fein, scharf, zahlreicher als bei dem Vorigen. Die Jahrringsgrenze markirt sich durch eine feine hellgelbe Linie. Das Holz weich, leicht, leicht spaltbar. Die Rinde gelbgrau, bleibt glatt.

75. Zanthoxylum ailanthoides S. et Z. (Karasu-zanshō.)

(Fig. 49.)

Farbe des schmalen Splintes gelbweiss, des Kernes hellgrünlichgelb. Die Gefässe werden gegen die Aussengrenze des Jahringes allmählich kleiner. Die Markstrahlen sind weniger deutlich als die des Vorigen. Wenige Zellgänge. Das Holz leicht, weich, leicht spaltbar. Die Rinde braungrau, mit starken Stacheln.

2. Die Gefässe sind so fein, dass sie auf dem Querschnitt schwer, oder fast nicht mehr, auf dem Längsschnitt dagegen als vertiefte Linien deutlich sichtbar sind.

α. Ohne Kern.

76. Acer pictum Thunb. var. Mono, Maxim.

(Itaya-kayede.)

(Fig. 50. 51. 52. 103.)

Das Holz gelblichweiss, glänzend, wird an der Luft bräunlich. Die Markstrahlen scharf, glänzend, verlaufen gerade. Die Gefässe fein, mit Lupe als offene, feine Löcherchen sichtbar. Eine Jahrringsgrenze ist durch eine feine Linie gekennzeichnet. Diese hier angegebenen Charactere sind bei den folgenden Acerarten gemein. Die Jahrringsgrenze ist ziemlich schön

gerundet, Zellgänge selten. Das Holz mässig hart, mässig schwer, leicht spaltbar. Die Rinde wird eine hellgelbbraun klein- und flachrissige Borke.

77. ACER PALMATUM THUNB. (Yamamomiji.)

(Fig. 104.)

Das Holz wie das des Vorigen. Die Jahrringsgrenze meist nicht schön gerundet. Die Rinde lange Zeit glatt, dunkelgrünlichgrau, bekommt später seichte Längsrisse.

78. ACER PURPURASCENS FR. ET SAV. (Kajikayede.)

(Fig. 105.)

Das Holz ist dem vorigen ähnlich. Die Markstrahlen sehr deutlich. Die Rinde dunkelgrau, sehr klein schuppig.

79. ACER CARPINIFOLIUM S. ET Z. (Chidori-no-ki.)

(Fig. 106.)

Das Holz ebenso. Die Markstrahlen sind sehr deutlich. Wenn das Holz an der Luft nachdunkelt, so werden die Markstrahlen auf dem Tangentialschnitt gut sichtbar; man kann das Holz dann auch zu der vorigen Gruppe zählen. Die Rinde färbt sich durch Flechtenwuchs grüngrau oder grünweisslich.

80. ACER NIKOENSE MAXIM. (Chōja-no-ki.)

(Fig. 107.)

Die Markstrahlen etwas feiner als die des Vorigen. Die Jahrringsgrenze wenig deutlich. Zellgänge selten. Die Rinde wie die des vorigen.

81. ACER ARGUTUM MAXIM. (Asanoha-kayede.)

Die Markstrahlen sehr deutlich, glänzend. Die Jahrringsgrenze nicht scharf markirt, Zellgänge sind sehr zahlreich. Die Rinde flachschuppig.

82. ACER RUFINERVE S. ET Z. (Uri-kayede.)

(Fig. 53. 108.)

Die Markstrahlen ohne besonderen Glanz, und gleichmässig fein. Die Jahrringsgrenze wellig, deutlich. Die Rinde ist schwarzgrün, mit weisslichen Verdickungen versehen, die aus harter Korkschicht bestehen.

83. ACER CRATAEGIFOLIUM S. ET Z. (Meuri-kayede.)

Dem Vorigen sehr ähnlich, nur die Rinde mehr grün.

84. ACER JAPONICUM THUNB. (Hauchiwa-kayede), ACER SIEBOLDIANUM MIQ. VAR. MICROPHYLLUM MAXIM. (Kohauchiwa-kayede.)

(Flg. 54. 109.)

Die Markstrahlen wie die des Vorigen, aber etwas glänzend. Zahlreiche Zellgänge. Die Jahrringsgrenze zeichnet sich durch eine sehr grobwellige Linie aus, ist aber nicht deutlich. Das Holz ist lockerer gebaut als die Hölzer der vorigen Acerarten. Die Rinde bildet früh längsrissige dicke Borke.

85. ACER DISTYLUM S. ET Z. (Maruba-kayede.)

(Fig. 110.)

Die Markstrahlen gleichmässig fein, ohne Glanz. Die Jahrringsgrenze ist schön gerundet, und deutlich. Zellgänge selten. Die Rinde dunkelgrau, flachrissig.

86. TILIA CORDATA MILL. VAR. JAPONICA MIQ. (Shina-noki), TILIA MIQUELIANA MAXIM. (Bodaiju.)

Das Holz röthlichweiss, ist oft mit dunkelgraulichen oder schmutzig weisslichen Stellen versehen. Die Markstrahlen sind gleichmässig fein, ohne Glanz, auf dem Taugentialschnitt mit Lupe als feine Spindel gut sichtbar. Die Gefässe sind zahlreich, gleichmässig vertheilt. Die Jahrringsgrenze ist durch eine undeutlich helle abgerundete Linie gekennzeichnet. Das Holz weich, leicht, leicht spaltbar. Die Rinde lange Zeit glatt, später flachrissig.

87. CORNUS MACROPHYLLA WALL. (Mizuki.)

(Fig. 55.)

Farbe des Holzes weiss, an der Luft schmutzig roth weisslich werdend. Die Markstrahlen fein, ziemlich deutlich, aber auf dem Tangentialschnitt mit Lupe kaum bemerkbar. Die Gefässe fein, sehr zahlreich, mit freiem Auge auf dem glattgehobelten Querschnitt noch als Löcher sichtbar. Die Jahrringsgrenze markirt sich durch eine dunkle, hie und da kleinwellig gekrümmte Linie. Das Holz zeigt häufig dunkelgefärbte Stellen. Es ist weich, leicht, leicht spaltbar. Die Rinde mattgrau mit verticalen Rissen, die hell erscheinen.

88. CORNUS IGNORATA C. KOCH. (Sawa-mizuki.)

Das Holz ist dem des Vorigen sehr ähnlich, die Farbe aber etwas röthlicher. Die Rinde in den Rissen röthlich.

β. Mit Kern.

89. STAPHYLEA BUMALDA S. ET Z. (Mitsuba-Utsugi.)

Farbe des Splintes gelbweiss, des Kernes mehr gelblich. Die Markstrahlen glänzend und deutlich, aber meist geschlossen. Das Holz hart, schwer, leicht spaltbar. Die Rinde graubraun kleinschuppig.

90. PRUNUS PSEUDO-CERASUS LINDL. VAR. SPONTANEA MAXIM (Yama-zakura), PRUNUS PSEUDO-CERASUS VAR. SIEBOLDI MAXIM (Yoshino-zakura.)

(Fig. 56. III.)

Der Splint hellbräunlich, der Kern braun. Das Holz zeigt oft unregelmässig grünlich gefärbte Stellen. Die Markstrahlen scharf, deutlich, aber ohne besonderen Glanz. Die Gefässe sind auf dem Querschnitt zum Theil macroscopisch noch als Löcher sichtbar. Sie sind vielfach zu wellenförmigen Linien verbunden. Besonders bemerkbar ist eine in der Innengrenze des Jahrringes peripherisch verlaufende Gefässreihe, welche aber nur mit Lupe deutlich wahrgenommen werden kann. Diese

Linie sieht man auch bei den folgenden Prunusarten. Zellgänge gewöhnlich selten, zuweilen aber ziemlich zahlreich. Das Holz mässig hart, mässig schwer, leicht spaltbar. Die Rinde ist von glänzend dunkelrothem, geschichtetem Periderm bekleidet.

91. PRUNUS GRAYANA MAXIM. (Uwamizu-zakura.)

(Fig. 112.)

Das Holz ist dem des Vorigen sehr ähnlich. Farbe des Kernholzes röthlichbraun. Die Gefässe sind deutlicher, Zellgänge selten. Die Rinde löst dunkelbraune dicke Borkeschuppen ab.

92. PRUNUS BUERGERIANA MIQ. (Inu-zakura.)

(Fig. 113.)

Farbe des Kernes ist hellbraun. Die Gefässe sind fein, bilden eine peripherisch ununterbrochen verlaufende feine Linie, die mit freiem Auge ziemlich gut, deren Gefässe selbst aber ohne Lupe nicht mehr sichtbar sind. Zellgänge sehr zahlreich. Das Holz ist von schlechterer Qualität. Die Rinde ist von hellgrauem glänzendem geschichtetem Periderm bekleidet.

93. PRUNUS CERASEIDOS MAXIM. (Mejiro-zakura.)

Der Splint breit. Der Kern schmutzig gelbgrünlich braun. Die sehr feinen, nur mit Lupe sichtbaren Gefässe bilden eine meist radial verlaufende, aber gewöhnlich in der Mitte einmal schief gebrochene Linie. Die Markstrahlen etwas feiner als die des Yamazakura, Zellgänge zahlreich. Das Holz ist wie das des Vorigen. Die Rinde ist der des Yamazakura ähnlich.

94. PRUNUS INCISA THUNB. (Mamezakura.)

Das Holz ist dem Vorigen gleich. Farbe des Kernes röthlich braun. Die Markstrahlen deutlich. Zellgänge sehr zahlreich. Die Rinde wie die des Vorigen.

95. MICHELIA COMPRESA MAXIM. (Ogatama-no-ki.)

Farbe des schmalen Splintes gelblich, des Kernes eigenthümlich bräunlichgelb, schön glänzend. Die Markstrahlen

sind meist in kurzen Strichen geschlossen. Die Gefässe sind vielfach in schräge Linien angeordnet, mit Lupe als Löcher deutlich sichtbar. Auf dem Querschnitt sieht man eine peripherisch verlaufende gelbe feine Linie, die mit den Jahrringen parallel ist. Das Holz mässig hart, mässig schwer, schwer spaltbar. Die Rinde glatt, dunkelaschgrau.

96. SAMBUCUS RACEMOSA L. (Niwatoko.)

Farbe des Splintes gelbweiss, des Kernes nur mehr bräunlich. Die Markstrahlen deutlich. Die Gefässe zahlreich, mit Lupe auch nicht klar sichtbar. Die Jahrringsgrenze ist durch eine hellgefärbte Linie gekennzeichnet, die aus ganz feinen Gefässen besteht. Das Holz mässig hart, leicht spaltbar. Die Rinde wird eine gelbgraue korkreiche tiefrissige Borke.

97. DEUTZIA SCABRA THUNB. (Utsugi.)

Das Holz ist dem des Vorigen ähnlich. Die Gefässe, die nur mit Lupe sichtbar, sind vielfach in peripherischer oder schräger Linie angeordnet. Es ist hart, schwer, leicht spaltbar. Die Rinde wird eine hellgelbe flachschuppige Borke.

D. DIE MEISTEN MARKSTRAHLEN SIND SEHR FEIN.

a) Die Gefässe weit, zuweilen sehr weit, sind auf dem Querschnitt deutlich als Löcher sichtbar und stehen nicht dicht zusammen.

98. DIOSPYROS KAKI L. F. (Kaki.)

(Fig. 57.)

Farbe des Splintes in frischen Zustande gelb, in der Luft grauweiss werdend, des Kernes schmutzig bräunlichschwarz. Die Verkernerung des Holzes geschicht nicht selten stellenweise. Die Gefässe weit und sehr arm, nehmen gegen die Aussengrenze des Jahrringes an Stärke und Zahl ab. Sie sind meist bis zu 4, selten mehr in radialer Richtung vereinigt, so dass man an die Hölzer mit radialen Gefässlinien gemahnt wird. Das Holz schwer, hart, schwer spaltbar. Die Rinde bildet eine dunkelbraune kleinschuppige Tafelborke.

99. DIOSPYROS LOTUS L. (Mamegaki.)

Dem Vorigen sehr ähnlich, aber die Gefässe viel feiner, das Kernholz tiefschwarz und schwerer.

100. JUGLANS SIEBOLDIANA MAXIM. (Onigurumi.)

(Fig. 58.)

Farbe des Splintes graulichweiss, des Kernes röthlich braun zuweilen schwarzbraun. Die Gefässe sind offen und klar, meist in einer schrägen Linie geordnet, und nehmen gegen die Aussengrenze des Jahrringes an Grösse stark ab. Sehr feine, schwer sichtbare Linien, die aus Parenchym bestehen, verlaufen peripherisch und parallel. Das Holz ist mässig hart, mässig schwer, leicht spaltbar. Die Rinde wird eine aschgraue tiefrissige Borke.

101. PTEROCARYA RHOIFOLIA S. ET Z. (Sawa-gurumi.)

(Fig. 59.)

Das Holz ist schön gelbweiss. Kein Kern. Die Gefässe sind, wie die des Vorigen in einer schrägen Linie geordnet. Diese Linien sind aber deutlich ausgeprägt und die Gefässe in denselben gleich gross. Die feinen aus Parenchym bestehenden peripherisch verlaufenden Linien kann man nur auf dem glatten Querschnitt mit Lupe bemerken. Das Holz mässig hart, mässig schwer, sehr leicht spaltbar. Die Rinde bildet eine dunkelbraune, ziemlich tiefrissige Borke.

102. HERNANDIA PELTATA MEISN. (Hasunohagiri.)

Farbe des Holzes ist gelblichweiss, des Kernes hellgrün bis dunkelgrün, ungleichförmig gefärbt. Das Holz zeigt gewöhnlich hellgelbe bis ins grünliche schimmernde Streifen. Die Gefässe arm und nicht klar. Das Holz schwer, sehr hart, schwer spaltbar.

103. CINNAMOMUM CAMPHORA NEES. (Kusu.)

(Fig. 60.)

Farbe des Splintes grauweiss, des Kernes bräunlichroth.

Die Gefässe stehen häufig gruppirt, wenn auch nur zu 3 bis 4. Sie sind nicht scharf begrenzt und werden gegen die Aussengrenze des Jahrringes sparsamer. Das Holz enthält eine bekannte Kamphorsubstanz, hat deshalb einen starken angenehmen Geruch, welcher sehr lange Zeit anhält. Das Holz weich, leicht, leicht spaltbar. Die Rinde ist eine graubraune flachrissige Borke.

104. MACHILUS THUNBERGII S. ET Z. (Tabu-no-ki.)

Farbe des Splintes grauweiss, des Kernes braunroth. Die Gefässe sind häufig zu 2 oder 3, selten mehr gruppirt. Sie sind ziemlich scharf begrenzt. Das Holz hat keinen Geruch. Es ist weich, leicht, schwer spaltbar. Die Rinde aufangs glatt, dunkel, wird später eine korkige hellgelbgraue Borke.

105. FICUS RETUSA VAR. NITIDA MIQ. (Gazumaru.)

(Fig. 61.)

Farbe des Holzes braun. Kein Kern. Die Gefässe sind sehr arm, stehen vereinzelt. Sehr dicke, bräunlich gefärbte parenchymatische Zonen verlaufen peripherisch und parallel, dazwischen sieht man feste aus Holzfasern bestehende Schichten. Die Jahrringsgrenze ist sehr schwer zu erkennen. Das Holz hart, schwer, schwer spaltbar. Die Rinde dunkel und glatt.

b) Die Gefässe sind auf dem Querschnitt mit unbewaffneten Augen nur schwer als Löcher, auf dem Längsschnitt aber gut als vertiefte Linien sichtbar.

106. STYRAX JAPONICA S. ET Z. (Yego-no-ki.)

(Fig. 62. 63. 64. 114.)

Farbe des frisch gefällten Holzes gelblichweiss, längere Zeit der Luft ausgesetzt wird es etwas röthlich. Kein Kern. Die Gefässe sind meist bis zu drei vereinigt. Die peripherisch verlaufenden langen Linien, welche aus Parenchym bestehen, sind auf dem Querschnitt gut sichtbar. Die Jahrringsgrenze deutlich. Das Holz hart, mässig schwer, schwer spaltbar. Die

Rinde anfänglich glatt, dunkelbraun, bildet später eine kleinschuppige Borke.

107. STYRAX OBASSIA S. ET Z. (Hakuunboku.)

(Fig. 115.)

Das Holz schliesst sich sehr nahe an das Vorige an. Die peripherisch verlaufenden Parenchymlinien sind feiner und undeutlicher. Die Rinde lange Zeit glatt bleibend.

108. IDESIA POLYCARPA MAXIM. (Ii-giri.)

Das Holz weiss, zeigt fast immer grauliche Stellen. Kein Kern. Die Gefässe sind zahlreich, mit Lupe auch nicht klar sichtbar. Die Jahrringe sind immer breiter als die des Vorigen. Die Jahrringsgrenze ist undeutlich. Das Holz weich, leicht, schwer spaltbar. Die Rinde dunkelaschgrau und glatt.

109. MAGNOLIA HYPOLEUCA S. ET Z. (Hō-no-ki.)

(Fig. 65. 116.)

Farbe des schmalen Splintes graulichweiss, des Kernes graugrün bis dunkelgrün. Die Jahrringsgrenze markirt sich durch eine feine, schön gerundete, helle Linie. Die Markstrahlen sind nur als helle feine Strichelung sichtbar. Die Gefässe zahlreich aber nicht leicht zusammen. Das Holz leicht, weich, sehr leicht spaltbar. Die Rinde dunkelgrau.

110. MAGNOLIA KOBUS D. C. (Kobushi.)

(Fig. 117.)

Dem Vorigen ähnlich. Die Farbe des Kernes hellgrün und die Jahrringsgrenze ist durch eine dunkelgrüne dichte Herbstholzzone erkennbar. Die Markstrahlen sind undeutlich. Die Rinde weisslich grau, riecht angenehm.

111. MAGNOLIA SALICIFOLIA MAXIM. (Tamushiba.)

(Fig. 118.)

Dem Vorigen ähnlich. Die Farbe des Kernholzes grünlichweiss. Die Rinde, dunkelgrau, mit grober Oberfläche.

112. ZANTHOXYLUM PIPERITUM D. C. (Sanshō), ZANTH. SCHINNIFOLIUM S. ET Z. (Inu-zanshō.)

Das Holz des piperitum intensiv gelb, des schinnifolium hellgelb. Kein Kern. Zahlreiche Zellgänge. Die Gefässe sind sparsam. Die Rinde dunkel braungrau, mit stachelartigen Korkkrusten.

113. STEWARTIA MONADELPHA S. ET Z. (Saruta.)

(Fig. 66. 119.)

Farbe des sehr breiten Splintes ist hellbraun, des Kernes bräunlichroth. Die Gefässe sind auf dem Querschnitt noch mit freiem Auge sichtbar, mit Lupe aber klar und offen. Die Jahrringsgrenze ist undeutlich. Der Lauf derselben in der axialen Richtung nicht gerade. Das Holz hart, schwer, sehr schwer spaltbar. Die Rinde hellbraun, löst sich als dünne rundliche Borkenplatte ab.

114. STEWARTIA PSEUDOCAMELLIA MAXIM. (Natsutsubaki.)

(Fig. 120.)

Das Holz steht dem des Vorigen nahe. Farbe des Kernes braun, der Splint sehr schmal. Die Gefässe sind feiner als die der Vorigen, deshalb ist das Holz dichter und schwerer. Die Jahrringsgrenze ist durch dichtere Herbstholzzone gut gekennzeichnet. Die Rinde violett braun, löst sich als ziemlich dicke Borkenplatte ab.

115. ALNUS FIRMA S. ET Z. (Yashabushi.)

Farbe des Holzes braun mit etwas roth. Kein Kern. Die Gefässe sind im Vergleiche mit dem bei den vorigen, lockerer vertheilt. Das Holz zeigt gewöhnlich schmutzigweissliche oder schwärzliche Stellen. Die Jahrringsgrenze undeutlich. Das Holz ist weich, leicht, leicht spaltbar. Die Rinde bildet eine dicke harte Schuppenborke.

116. CERCIDIPHYLLUM JAPONICUM S. ET Z. (Kutsura.)

(Fig. 67.)

Farbe des Splintes hellbräunlich, des Kernes graulich-

braun. Die Gefässe zahlreich, stehen sehr dicht zusammen, auch mit Lupe sehr schwer sichtbar. Die Jahrringsgrenze ist durch eine dunkle dichtere Herbstholzzone gekennzeichnet. Das Holz ist gleichmässig gebaut, weich, leicht, sehr leicht spaltbar. Die Rinde bildet eine dunkle nicht tiefrissige Borke.

117. MACHILUS THUNBERGII S. ET Z. VAR. JAPONICA YATABE. (Baribari.)

Farbe des Holz es ist grünlichgelb. Kein Kern. Die Gefässe stehen nicht dicht zusammen, sind aber nur mit Lupe sichtbar. Die Jahrringsgrenze markirt sich durch eine gerundete dunkle Linie. Das Holz weich, leicht, leicht spaltbar. Die Rinde hellbräunlichgrau, glatt.

118. FICUS ERECTA THUNB. (Inu-biwa.)

Dem Ficus retusa var. nitida sehr ähnlich. Die Gefässe aber weit feiner, so dass sie auf dem Querschnitt kaum mit freiem Auge erkannt werden können. Die Rinde glänzend graulichweiss.

119. LITSEA GLAUCA SIEB. (Shirodamo.)

Farbe des Holzes weisslichgrau. Kein Kern. Die Gefässe sind meist zu zwei vereinigt und gelblich gerändert, dem unbewaffnetem Auge nur als helle Punkte sichtbar, Zellgänge selten. Die Jahrringsgrenze ziemlich deutlich. Das Holz weich, leicht, schwer spaltbar. Die Rinde dunkel mit rundlichen grossen Lenticellen.

120. ACTINODAPHNE LANCIFOLIA MEISN. (Koga-no-ki.)

Farbe des splintes hellbräunlichweiss, des Kernes rothbraun. Die Gefässe stehen meist einzeln und klar sichtbar. Das Holz weich, leicht, schwer spaltbar. Die Rinde violetbraun, löst sich als rundliche Schuppenplatte ab.

121. CINNAMOMUM PEDUNCULATUM NEES. (Yabu-nikkei.)

Farbe des Holzes ist grünlichweiss, an der Luft rothgraulich werdend. Kein Kern. Die Gefässe sind weit zahlreicher und

feiner als die der beiden vorigen, und auf dem Querschnitt nur schwer als Löcher wahrnehmbar. Sie stehen im ganzen Jahrringe gleichmässig und einzeln vertheilt. Die Jahrringsgrenze ist durch eine dunkle Linie zu erkennen. Das Holz leicht, weich, schwer spaltbar. Die Rinde dunkelroth mit grossen sparsamen rost-braunen Lenticellen, sie hat einen starken Geruch.

122. LINDERA PRAECOX BL. (Aburachan.)

Farbe des Holzes dunkelgrau. Kein Kern. Die Gefässe sind auch mit Lupe schwer erkennbar. Mit dunkelbraunen Zellgängen. Das Holz schwer, hart. Die Rinde mattgrau und glatt.

123. LINDERA SERICEA BL. (Kuromoji.)

Das Holz hellgrau, besitzt einen eigenthümlichen angenehmen Geruch. Kein Kern. Zellgänge selten. Das Holz leicht, weich, leicht spaltbar. Die Rinde glatt, dunkelgrün.

124. LINDERA TRILOBA BL. (Shiromoji), LINDERA OBTUSILOBA BL. (Dankobai.)

Das Holz dem Vorigen ähnlich, besitzt aber keinen Geruch, zahlreiche Zellgänge. Die Rinde dunkelgrau.

125. LINDERA UMBELLATA THUNB. (Kanakugi.)

Farbe des Holzes röthlich. Kein Kern, zahlreiche Zellgänge.

c) Die Gefässe sind mit unbewaffnetem Auge weder auf dem Querschnitt, noch auf dem Längsschnitt sichtbar.

126. MYRICA RUBRA S. ET Z. (Yamamomo.)

(Fig. 68.)

Farbe des sehr breiten Splintes hellbräunlichroth, des Kernes dunkelroth, ohne Glanz. Die Markstrahlen sind auf dem Tangentialschnitt gut sichtbar. Die Jahrringsgrenze ist schwer zu erkennen. Das Holz hart, schwer, sehr schwer spalt-

bar. Die Rinde aufangs glatt grünlichgrau, bekommt später seichte Längsrisse.

127. TERNSTROEMIA JAPONICA THUNB. (Mokkoku.)

(Fig. 69.)

Farbe des schmalen Splintes hellroth, des Kernes tiefroth. Die Höhe der Markstrahlen, welche auf dem Tangentialschnitt ebenso gut sichtbar sind, wie die des Vorigen, ist grösser, wodurch man sie leicht von den letzteren unterscheiden kann. Das Holz schwer, hart, sehr schwer spaltbar. Die Rinde dunkel.

128. THEA JAPONICA NOIS. (Tsubaki.)

(Fig. 121.)

Farbe des Holzes hellbräunlich. Kein Kern. Die Markstrahlen sind nicht glänzend und auf dem Tangentialschnitt nicht mehr sichtbar. Die Frühjahrezone erscheint hell. Das Holz sehr schwer, sehr hart, sehr schwer spaltbar. Die Rinde glatt, hellgelbbraun.

129. THEA SAZANQUA NOIS. (Sazan-kwa.)

(Fig. 122.)

Farbe des Holzes ist bräunlichroth. Kein Kern. Die Markstrahlen sind glänzend und scharf, sonst wie der Vorigen. Die Rinde gelbbraun.

130. CLETHRA BARBINERVIS S. ET Z. (Riyōbu.)

(Fig. 70)

Farbe des Holzes ist dem Thea japonica ähnlich. Kern fehlt. Die Gefässe, mit Lupe betrachtet, sind auf dem Querschnitt als sehr feine Löcher klar sichtbar, auf dem Längsschnitt deutlicher, so dass man sie von den beiden vorigen unterscheiden kann. Das Holz schwer, hart, sehr schwer spaltbar. Die Rinde bildet eine graubraune Schuppenborke, die sich als rundliche Platte ablöst.

131. CYDONIA SINENSIS THOURIN. (Kwarin.)

(Fig. 123.)

Farbe des Holzes dem Vorigen ähnlich. Die Markstrahlen

sind roth gefärbt. Auf dem glatten Querschnitt sind die feinen Zellfaserschichten sichtbar. Die Rinde ist der des Vorigen ähnlich, aber violetbraun.

132. LYONIA OVALIFOLIA DON. (Kashioshimi.)

(Fig. 124.)

Farbe des Holzes ist dem Thea japonica ähnlich. Die Markstrahlen sind scharf, röthlich und glänzend, aber auf dem Tangentialschnitt nicht mehr mit freiem Auge sichtbar. Zellgänge selten. Das Holz hart, schwer, sehr schwer spaltbar. Die Rinde bildet eine glänzendbraune Ringelborke.

133. RHODODENDRON METTERNICHII S. ET Z. (Shakunagi.)

(Fig. 125.)

Dem Vorigen sehr ähnlich. Die Markstrahlen sind auf dem Tangetialschnitt als röthlich gefärbte Pünktchen gut sichtbar. Die Rinde bildet eine hellgraubraune kleinschuppige Borke.

134. RHODODENDRON DILATATUM MIQ. (Mitsubatsutsuji.)

Das Holz ähnelt dem des Vorigen in hohem Grad. Die Herbstholzzone stark entwickelt, färbt sich dunkel. Die Rinde wird eine dunkelrothbraune Schuppenborke.

135. POURTHIAEA VILLOSA DENE. (Ushikoroshi.)

Farbe des Holzes röthlich bis bräunlich. Kein Kern. Die Markstrahlen sind zahlreich, auf dem Tangentialschnitt aber nicht mehr erkennbar. Die Gefässe auf dem Querschnitt auch mit Lupe nicht sichtbar. Das Holz hart, schwer, sehr schwer spaltbar. Die Rinde dunkelgrau, mit vertical verlaufenden Furchen.

136. LIGUSTRUM JAPONICUM THUNB. (Nezumimochi.)

Farbe des Holzes weissgrau. Die Markstrahlen sind nur schwer sichtbar. Die Gefässe zahlreich und in der inneren Jahrringsgrenze ziemlich deutlich, mit Lupe als Löcher sichtbar, so dass man diese Art als eine Übergangsform des zerstreut-

porigen Holzes zum ringporigen ansehen kann (Siehe 25 Ligustrum 2 bota). Das Holz schwer, hart. Die Rinde dunkel.

137. VIBURNUM ODORATISSIMUM KER. (Sango-ju.)

Farbe des frischen Holzes weiss, an der Luft schmutzig bräunlich werdend. Die Gefässe sind zahlreich und im ganzen Jahrringe gleichmässig vertheilt, zum Theil mit Lupe als Löcher sichtbar. Die Markstrahlen sind röthlich gefärbt, scharf und gut sichtbar. Die Jahrringsgrenze undeutlich. Das Holz hart, schwer, sehr schwer spaltbar. Die Rinde dunkelröthlichbraun, wird später schwach schuppig.

138. VIBURNUM DILATATUM THUNB. (Gamazumi),
VIBURNUM FURCATUM BL. (Ogame-no-ki).

Farbe des Holzes in frischem Zustande gelblichweiss, an der Luft hellbraun bis braun werdend. Die Markstrahlen scharf. Die Gefässe sind auf dem Querschnitt mit Lupe noch als feine Löcher sichtbar. Die Jahrringsgrenze ziemlich deutlich. Das Holz schwer, hart. Die Rinde dunkelbraun, kleinschuppig.

E. ALLE MARKSTRAHLEN SIND ÄUSSERST FEIN, OHNE LUPE NICHT MEHR SICHTBAR.

a) Die Gefässe weit, sind auf dem Querschnitt deutlich als Löcher sichtbar und stehen nicht dicht zusammen.

139. NEPHELIUM LONGANA CAMB. (Ryūgan.)

Farbe des Holzes hellrothbraun, glänzend. Die Gefässe ungleichmässig vertheilt, und vielfach in radialer Richtung angeordnet, sie sind häufig mit einem weisslichen Secrete verstopft. Die Jahrringsgrenze undeutlich. Das Holz, schwer, hart, schwer spaltbar. Die Rinde bildet eine gelbbraune, korkige dicke Schuppenborke.

b) Die Gefässe sind mit unbewaffnetem Auge auf dem Querschnitt nur sehr schwer als Löcher, auf dem

Längsschnitt aber gut als vertiefte Linie sichtbar.

1. Die Gefässe, mit Lupe betrachtet, sind arm und klar, stehen nicht dicht zusammen.

α. Harte Hölzer.

140. Ostrya japonica Surgent. (Asada.)

(Fig. 71. 72. 73.)

Farbe des sehr breiten Splintes glänzend hellgrau, des Kernes tiefbraun. Die Gefässe, die vielfach in radialer Richtung angeordnet sind, mit Lupe anf dem Querschnitt ziemlich gut als offene Löcher sichtbar. In der Herbstholzzone, die an breitrissigem Holze ziemlich stark entwickelt und dunkel erscheint, sieht man weissliche schwanzförmige Figuren aus feinen Gefässen. Die sehr feine, aus Parenchym bestehende Linie ist auf dem Querschnitt mit unbewaffnetem Auge noch zu erkennen. Zellgänge ziemlich häufig. Das Holz hart, schwer, schwer spaltbar. Die Rinde bildet eine dunkelgelbbraune, seicht langrissige Borke.

141. Zizyphus vulgaris Lam. var. inermis Bge. (Natsume.)

Farbe des Splintes gelblichweiss, des Kernes rothbraun. Die Gefässe sind vielfach in schrägen Linien angeordnet, mit Lupe als offene Löcher klar sichtbar. Die Jahrringsgrenze markirt sich durch eine feine Linie, die man mit Lupe gut wahrnehmen kann. Das Holz sehr schwer, sehr hart, schwer spaltbar. Die Rinde wird eine tiefrissige kleinschuppige Borke.

142. Citrus trifoliata L. (Karatachi.)

Farbe des schmalen Splintes gelbweiss, des Kernes hellgrünlichgelb. Man sieht die Gefässe mit Lupe als klare offene gelbgeränderte Löcher, die in der Regel zu je zwei, selten zu drei vereinigt sind. Die feine, gelbe, eine lange Strecke peripherisch ununterbrochen verlaufende, Linie sieht man deutlich auf dem Querschnitt. Der Lauf der Jahrringe ist sehr unregelmässig. Das Holz hart, schwer, sehr schwer spaltbar. Die Rinde schwärzlichbraun.

143. LAGERSTROEMIA INDICA L. (Hyakujikko.)

Farbe des Holzes hellgrau. Der Kern fehlt. Die Stellung der Gefässe ist der des Vorigen ähnlich. Man sieht auf dem Querschnitt mit der Lupe peripherisch wellenförmig verlaufende, kurze aber ziemlich dicke Parenchymlinien. Das Holz mässig hart, schwer, sehr schwer spaltbar. Die Rinde löst hellgelbbraune, dünne, rundliche Schuppenplatte ab.

144. BETULA BHOJPATTRA WALL. VAR. TYPICA RGL. (Onoore), BETULA GLOBISPICA SHIRAI. (Jizokamba).

(Fig. 74. 126.)

Farbe des Splintes gelblichweiss, des Kernes braun. Die Gefässe sind im ganzen Jahrringe gleichmässig vertheilt und kreisrund und erscheinen nicht klar. Die Jahrringsgrenze ist durch eine undeutliche feine Linie markirt. Diese beiden Charactere sind auch den folgenden Betulaarten gemein. Das Holz sehr hart, sehr schwer, schwer spaltbar. Die Rinde bildet eine dunkelviolettrothe dicke Schuppenborke.

145. BETULA ULMIFOLIA S. ET Z. (Yoguso-minebari.)

Farbe des Holzes gelblichweiss. Kein Kern. Zellgänge selten. Das Holz hart, schwer, schwer spaltbar. Die Rinde ist von dunkelrothem geschichtetem Periderm bekleidet, das sich nicht leicht ablöst.

146. BETULA CORYLIFOLIA RGL. ET MAX. (Urajiro-kamba), BETULA GROSSA S. ET Z. (Mizume.)

Das Holz ist von dem des Vorigen kaum verschieden. Das geschichtete Periderm der Rinde ist glänzend grau, ziemlich leicht ablöslich.

β. Weiche Hölzer.

147. BETULA MAXIMOWICZIANA RGL. (Saihada.)

Das Holz zeigt den Typus dieser Gattung. Kein Kern. Zellgänge nicht zahlreich. Es ist weich, leicht, schwer spaltbar. Die Rinde mit bräunlichweissem geschichtetem Periderm.

148. BETULA ALBA L. VAR. VULGARIS D. C. (Shira-kamba.)

(Fig. 75. 127.)

Das Holz ist gelblichweiss. Kein Kern. Zellgänge zahlreich. Es ist sehr weich, sehr leicht, schwer spaltbar. Das geschichtete Periderm ist schneeweiss, sehr leicht abzulösen.

149. BETULA ALBA L. VAR. COMMUNIS RGL. (Makamba.)

Das Holz ist wie das des Vorigen. Zellgänge nicht zahlreich. Das geschichtete Periderm hellgelbbräunlich, sich sehr leicht ablösend.

150. BETULA ALBA L. VAR. CORDIFOLIA RGL. (Aka-kamba.)

Das Holz ebenso. Zellgänge zahlreich. Das geschichtete Periderm gelblichroth.

2. Die Gefässe sind, mit Lupe betrachtet, zahlreich und stehen dicht zusammen.

α. Hartes Holz.

151. SYMPLOCOS CRATAEGOIDES HAM. VAR. PALLIDA FR. ET SAV. (Shiro-tzuge.)

(Fig. 76.)

Farbe des Holzes weiss. Kein Kern. Das Holz zeigt öfters schmutzig schwärzliche Stellen. Die Gefässe offen und klar, mit freiem Auge noch gut als Löcher wahrnehmbar. Die Jahrringsgrenze ist kaum zu erkennen. Zellgänge sind ziemlich zahlreich. Das Holz hart, schwer, leicht spaltbar. Die Rinde ist eine gelbgraue Ringelborke.

β. Weiche Hölzer.

152. POPULUS TREMULA L. VAR. VILLOSA WESM. (Yamanarashi.)

(Fig. 128.)

Farbe des Holzes weisslich, etwas glänzend. Kein Kern. Die Jahrringsgrenze schön abgerundet. Zellgänge häufig, hell-

braun gefärbt. Das Holz leicht, weich, leicht spaltbar. Die Rinde anfangs glatt, grau, mit rhombischen Pusteln, wird später flachrissig.

153. POPULUS BALSAMIFERA L. VAR. SUAVEOLENS LOUD. (Dero.)

(Fig. 77. 129.)

Farbe des Holzes schön weiss, glänzend. Kein Kern. Die Rinde lange Zeit glatt bleibend, wird später langrissig.

154. SALIX CAPREA L. (Saruyanagi.)

(Fig. 78.)

Farbe des Splintes röthlichweiss, des Kernes hellroth bis braun glänzend. Zahlreiche Zellgänge. Das Holz weich, leicht, leicht spaltbar. Die Rinde bildet eine graulich braune, tiefrissige Borke.

155. SALIX URBANIANA V. SEEMENN. (Akayanagi.)

Farbe des Splintes weiss, des Kernes röthlich braun, etwas glänzend. Zellgänge häufig. Die Rinde wird eine dunkelrothe sehr tiefrissige Borke.

156. HALESIA CORYMBOSA B. ET H. (Asagara), HALESIA HISPIDA B. ET H. (Oba-asagara.)

(Fig. 79. 130.)

Farbe des sehr breiten Splintes weiss, des Kernes hellbraun, ohne Glanz. Zellgänge fehlen ganz. Die Jahrringsgrenze ist grobwellig. Das Holz weich, sehr leicht, leicht spaltbar. Die Rinde bildet eine hellbraune korkige tiefrissige Borke.

157. VIBURNUM OPULUS L. (Kamboku.)

Farbe des Holzes schön weiss, glänzend. Kein Kern und keine Zellgänge. Die Jahrringsgrenze verläuft rund. Das Holz weich, leicht, leicht spaltbar. Die Rinde wird eine dunkelbraune flachrissige Borke.

c) Die Gefässe sind mit freiem Auge weder auf dem Querschnitt, noch auf dem Längsschnitt sichtbar.

α. Harte Hölzer.

158. DISTYLIUM RACEMOSUM S. ET Z. (Isu.)

(Fig. 80. 81. 82.)

Farbe des schmalen Splintes grauroth, des Kernes eigenthümlich dunkelgrauroth, kein scharfer Unterschied. Die Gefässe sind mit starker Lupe zum Theile noch als Löcher wahrnehmbar. Die peripherisch verlaufenden Parenchymlinien mit Lupe gut sichtbar. Die Jahrringsgrenze ist sehr schwer zu erkennen. Das Holz sehr hart, sehr schwer und sehr schwer spaltbar. Die Rinde bildet eine dunkle, grobe, platte Schuppenborke.

159. DIERVILLA GRANDIFLORA S. ET Z. (Hakone-utsugi.)

Farbe des Splintes weisslichgelb, des Kernes schön rosaroth, etwas glänzend. Das Holz zeigt öfters tiefrothe Stellen. Die Jahrringsgrenze ist durch eine undeutliche helle Linie gekennzeichnet. Die Gefässe sind sehr oft mit einem Secrete ausgefüllt. Das Holz hart, schwer, leicht spaltbar. Die Rinde ist eine graubraune korkige Borke.

160. HYDRANGEA PANICULATA SIEB. (Nori-no-ki.)

Dem Vorigen ähnlich. Kernholz aber gelblichroth. Die Rinde ist eine dunkelbraune flachrissige Borke.

161. ERIOBOTRYA JAPONICA LINDL. (Biwa.)

Der sehr schmale Splint hellbraun, der Kern dunkelbraun. Die Gefässe mit Lupe nicht gut sichtbar. Die Jahrringsgrenze ist durch eine schmale helle Frühjahrszone gut zu erkennen. Das Holz sehr hart, sehr schwer und schwer spaltbar. Die Rinde bildet eine dunkle Schuppenborke, die sich als Platte ablöst.

162. PHOTINIA GLABRA THUNB. (Kanamemochi.)

Das Holz ist dem des Vorigen ähnlich, die Farbe nur heller. Zellgänge häufig. Die Rinde dunkel, flachschuppig.

163. Eurya ochnacea Szysy. (Sakaki), Eurya japonica Thunb. (Hisakaki.)

(Fig. 83. Sakaki.)

Das Holz braun, öfters mit gelbweisslichen Stellen versehen, ist sehr gleichmässig gebaut. Kein Kern. Die Jahrringsgrenze schwer erkennbar, aber immer schmal. Das Holz mässig hart, mässig schwer, schwer spaltbar. Die Rinde des ochnacea dunkelrothbraun, des japonica mehr braun.

164. Illicium Anisatum L. (Shikimi.)

Farbe des Holzes dunkelbraun. Kern fehlt. Die Markstrahlen röthlich gefärbt. Keine Zellgänge. Das Holz schwer, hart, schwer spaltbar. Die Rinde dunkelrothbraun, riecht sehr stark.

165. Andromeda japonica Thunb. (Asebi.)

Farbe des frisch gefällten Holzes schön weiss, an der Luft bräunlich werdend. Die Frühjahrszone erscheint hell. Die Markstrahlen röthlich, und verlaufen krummlinig. Das Holz hart, schwer, und schwer spaltbar. Die Rinde wird eine rothbraune Ringelborke.

166. Pirus Toringo Sieb. (Zumi.)

(Fig. 131.)

Das Holz braun. Kein Kern. Die Jahrringsgrenze ist durch eine helle schmale Frühjahrszone erkennbar. Zellgänge sehr zahlreich. Das Holz hart, schwer, sehr schwer spaltbar. Die Rinde dunkelaschgrau, flachrissig.

167. Pirus sinensis Lindl. (Nashi.)

Dem Vorigen ähnlich. Zellgänge nicht zahlreich. Das Holz schwer, hart, schwer spaltbar. Die Rinde bildet eine dunkelgraubraune kleinschuppige Tafelborke.

168. PIRUS CALLERYANA DCNE. (Konashi.)

(Fig. 132.)

Das Holz wie das des Vorigen. Kein Kern, aber öfters ein falscher Kern bemerkbar. Zahlreiche Zellgänge. Die Rinde bildet eine dunkle Plattenborke.

169. EUONYMUS EUROPAEA L. VAR. HAMILTONIANA MAXIM. (Mayumi.)

(Fig. 84. 133.)

Farbe des Holzes schön gelblich weiss. Kein Kern. Die Jahrringsgrenze nur schwer erkennbar, aber ziemlich gut gerundet. Das Holz hart, schwer, schwer spaltbar, ist homogen gebaut. Die Rinde bildet eine braungraue, korkige, langrissige Borke.

170. EUONYMUS OXYPHYLLA MIQ. (Tsuribana.)

(Fig. 134.)

In allen Punkten dem Vorigen ähnlich, aber die Rinde mattgraubraun und glatt.

171. EUONYMUS JAPONICA THUNB. (Masaki.)

Farbe des schmalen Splintes hellgelb, des Kernes hellbraun. Die Jahrringsgrenze ist durch eine feine um weniges dichtere Herbstholzzone gekennzeichnet. Das Holz ist wie das der vorigen beiden, aber von schlechterer Qualität. Die Rinde dunkel, wird später etwas rissig.

172. BUXUS SEMPERVIRENS L. (Tsuge.)

(Fig. 85.)

Farbe des Holzes hellgelb, kein Kern. Der Lauf des Jahrringes ungleichmässig und undeutlich. Es ist sehr homogen gebaut, sehr hart, sehr schwer, und sehr schwer spaltbar. Die Rinde wird eine dünne graugelbe Borke.

173. DAPHNIPHYLLUM MACROPODUM MIQ. (Yuzuriha.)

Farbe des Holzes schmutzigdunkelgrau. Kein Kern. Die

Frühjahrszone nur um weniges heller. Das Holz weich, leicht, leicht spaltbar. Die Rinde grau und glatt.

174. HAMAMELIS JAPONICA S. ET Z. (Mansaku.)

Das Holz weiss, an der Luft bräunlich werdend. Kein Kern. Zellgänge ziemlich häufig. Es ist mässig hart, schwer spaltbar. Die Rinde braungrau, glänzend. Flechtenwuchs färbt sie grünlichweiss.

175. PIRUS AUCUPARIA GAERTN. VAR. JAPONICA MAXIM. (Nanakamado.)

(Fig. 135.)

Farbe des Holzes gelblichweiss. Der Luft ausgesetzt wird es hellbraun. Die Jahrringsgrenze ist durch eine dunklere Herbstholzzone gekennzeichnet. Zellgänge zahlreich. Das Holz mässig hart. Die Rinde bräunlichaschgrau und dick. Flechtenwuchs färbt sie grünlichweiss.

176. PIRUS MIYABEI SURGENT. (Azukinashi.)

(Fig. 156.)

Farbe des Holzes ist dem Vorigen gleich. Die Markstrahlen sind aber deutlicher, so dass dieses Holz auch zu der vorigen Gruppe gezählt werden kann. Zellgänge zahlreich. Die Rinde ist dunkelgrüngrau, mit tiefbraunen zahlreichen Lenticellen, die in verticaler Richtung angeordnet sind.

177. PIRUS TSCHONOSKI MAXIM. (Miyama-nashizumi.)

Das Holz ist dem des Vorigen ähnlich. Die Rinde glänzend grünlichaschgrau mit tiefbraunen sparsamen Lenticellen.

178. PIRUS ARIA EHRH. VAR. KAMAONENSIS WALL. (Urajiro-no-ki.)

Farbe des Splintes ist in frischem Zustande gelblichweiss, an der Luft braun werdend, des Kernes rothbraun. Zellgänge zahlreich. Die Rinde grünlichaschgrau, glänzend mit rhombischen Pusteln, später entsteht eine Schuppenborke.

β. Weiches Holz.

179. AESCULUS TURBINATA BL. (Tochi.)

(Fig. 86.)

Farbe des Holzes gelblich bis röthlichweiss. Die Markstrahlen sind auf dem Radialschnitt als regelmässig parallel verlaufende, wenig krumme Linien, auf dem Tangentialschnitt als regelmässige Wellenlinien sichtbar. Das Holz weich, leicht, schwer spaltbar. Die Rinde anfangs glatt graubraun, später bildet sich eine korkige rissige flache Schuppenborke.

III. Die Hölzer, bei welchen alle Gefässe in radialer und nicht selten verzweigter Linie gruppirt sind. Diese Linie wollen wir die radiale Gefässlinie nennen.

A. DIE EINZELNEM MARKSTRAHLEN SIND SEHR BREIT, DIE MEISTEN ÄUSSERST FEIN, KAUM SICHTBAR.

180. QUERCUS ACUTA THUNB. (Akagashi.)

(Fig. 87.)

Farbe des sehr schmalen Splintes hellbraun, des Kernes dunkelrothbraun. Das Holz zeigt häufig schwärzliche Stellen. Die Gefässe fein und offen. Die radialen Gefässlinien, die meist ein oder zwei, selten drei Gefässe breit sind, nicht lang, verzweigen sich häufig. Die Jahrringsgrenze, welche zwischen den starken Markstrahlen etwas nach aussen ausgebogen sind, schwer erkennbar. Die feinen peripherisch verlaufenden Linien aus Parenchym, sind deutlich sichtbar. Diese beiden Charactere sind bei den folgenden Quercus- und Pataniaarten gemein. Das Holz ist sehr hart, sehr schwer, schwer spaltbar. Die Rinde bildet eine dunkle versteinte Borke, die sich als dicke Platte abstösst.

181. QUERCUS GILVA BL. (Ichii-gashi.)

Dem Vorigen ähnlich. Der hellbraune Splint sehr breit,

die Farbe des Kernes hell- bis bräunlichroth. Die Gefässe in den radialen Gefässlinien etwas stärker und zahlreicher und die Linien selbst bedeutend länger als bei dem vorigen. Die Gefässlinien zeichnen sich in der Regel durch eine ganze Menge Jahrringe, während sie dies bei dem obigen Quercus acuta gewöhnlich nicht thun. Das Holz zeigt häufig schärzliche Stellen. Es ist sehr hart, sehr schwer aber leicht spaltbar. Die Rinde stösst sich in dunklen nicht sehr dicken Borkenplatten ab.

182. QUERCUS VIBRAYEANA TR. ET TAV. (Shira-gashi.)

(Fig. 88.)

Farbe des sehr schmalen Splintes schmutzigweiss bis weiss, des Kernes kaum davon verschieden und nur etwas grauer. Die Gefässe sind fein und offen. Die radialen Gefässlinien sind gewöhnlich ein Gefäss breit. Die Gefässe in den ersteren werden gegen die Aussengrenze des Jahrringes allmählich kleiner. Die Jahrringsgrenze ist durch eine um weniges dichtere Herbstholzzone markirt. Die Markstrahlen sind etwas schmaler als die der vorigen und seidenglänzend. Die peripherisch verlaufenden Parenchymlinien gut sichtbar. Das Holz etwas weicher als das des Vorigen, aber ebenso schwer und leicht spaltbar. Die Rinde dunkel, versteint, lange Zeit glatt, wird später häufig ganz klein-aber dickschuppig.

183. QUERCUS MYRSINAEFOLIA BL. (Urajiro-gashi.)

Steht dem Vorigen sehr nahe. Die radialen Gefässlinien sind gewöhnlich mehere Gefässe breit und ziehen sich durch viele Jahrringe. Das Holz weiss, Kern fehlt. Die Rinde dunkelgrüngrau, glatt bleibend.

184. QUERCUS PHYLLIREOIDES A. GR. (Ubamegashi.)

Farbe des breiten Splintes grauweiss, des Kernes dunkelbraun. Die Gefässe sehr fein, mit freiem Auge ziemlich schwer sichtbar. Die radialen Gefässlinien sind meist ein oder zwei, selten mehere Gefässe breit und ziehen sich meistens durch zwei, selten mehrere Jahrringe. Die Jahrringsgrenze schwer erkennbar. Das Holz sehr schwer, sehr hart, sehr schwer spaltbar. Die Rinde bildet eine dunkle rissige Borke.

185. QUERCUS GLAUCA THUNB. (Ara-gashi.)

Das Holz ist graulichweiss, schmutzig, kein Kern, sehr häufig mit gelblichen oder schmutzig dunklen Stellen versehen. Die Gefässstellung ist der des Quercus Vibrayeana gleich. Das Holz ist aber von schlechterer Qualität. Die Rinde dunkelgrau, lange Zeit glatt bleibend, oder schon zeitig klein-aber dickschuppig werdend.

186. QUERCUS THALASSICA HCE. (Shirifuka-gashi.)

Das Holz ist dem Vorigen sehr ähnlich, steht im Werthe aber nach. Die Rinde glatt bleibend.

187. QUERCUS SESSILIFOLIA BL. (Tsukubane-gashi.)

Farbe des schmalen Splintes schmutzig weiss, des Kernes dunkelbraun. Die Gefässstellung ist dem Vibrayeana ähnlich. Das Holz steht in Werth etwas nach.

188. PASANIA CUSPIDATA OERST. (Shii.)

(Fig. 89. 90. 137.)

Farbe des breiten Splintes schmutzig hellgelbbräunlich, des Kernes hellgelbbraun, kein scharfer Unterschied. Die Gefässe sind an der Innengrenze des Jahrringes sehr weit und nehmen von Innen nach Aussen an Stärke bedeutend ab, bis sie endlich verschwindend klein werden. Die radialen Gefässlinien sind ein Gefäss breit, treten aber dicht neben einander auf. Die grossen Markstrahlen sind sehr ungleichmässig vertheilt, ohne Glanz und nicht scharf begrenzt. Nicht selten sieht man ein Holz, an welchem die breiten Markstrahlen ganz fehlen. Ob dieses Holz eine andere verwandte Species von Pasania ist oder nicht, kann ich jetzt nicht sagen. Die Jahrringsgrenze ist durch eine dichtere Herbstholzzone gekennzeichnet und zwischen den weit von einander entfernten grossen Markstrahlen nach Aussen stark ausgebogen. Das Holz zeigt öfters gelbliche Stellen. Das Holz hart, schwer, schwer spaltbar. Die Rinde ist anfangs glatt, dunkel, später entsteht eine tiefrissige Borke.

189. PASANIA GLABRA OERST. (Mateba-shii.)

(Fig. 138.)

Farbe des Holzes wie des Vorigen. Der Splint schmal. Die Gefässe feiner als die des vorigen, aber offen und klar. Die radialen Gefässlinien ziehen sich durch mehere Jahrringe und sind meist ein zuweilen zwei oder mehererc Gefässe breit. Die grossen Markstrahlen sind undeutlich wie bei dem Vorigen, aber zahlreicher und gleichmässig vertheilt. Das Holz ist härter und schwerer. Die Rinde glatt, versteint, dunkelgrüngrau, bekommt später seichte Risse.

B. DIE MARKSTRAHLEN ÄUSSERST FEIN.

190. HIBISCUS SYRIACUS L. (Mukuge.)

Das Holz graubraun, zeigt häufig dunkelgraue Stellen. Die Gefässstellung ist der des Pasania cuspidata sehr ähnlich, die radialen Gefässlinien stehen aber sehr dicht, und die Gefässe feiner. Die peripherisch verlaufenden Parenchymlinien fehlen. Die Jahrringsgrenze ist abgerundet, nur hie und da grobwellig. Das Holz weich, leicht, schwer spaltbar. Die Rinde dunkelgrau.

C. DIE BREITEN MARKSTRAHLEN ENTSTEHEN DURCH DAS ZUSAMMENTRETEN ZAHLREICHER, SCHMALER MARKSTRAHLEN ZU EINEM COMPONIRTEN, SOGENANNTEN FALSCHEN MARKSTRAHLE. ES FEHLT IHNEN AN GLANZ UND SCHARFER BEGRENZUNG.

191. CARPINUS LAXIFLORA BL. (Akashide.)

(Fig. 91. 139.)

Farbe des Holzes hellgelblichweiss. Kern fehlt. Die Markstrahlen undeutlich. Die Jahrringsgrenze, die sich durch punktierte helle Linien kennzeichnet, ist zwischen den grossen Markstrahlen stark ausgebuchtet. Zellgänge ziemlich häufig,

was bei den folgenden Corpinusarten auch der Fall ist. Die Gefässe sehr fein, ohne Lupe schwer erkennber. Die radialen Gefässlinien sind kurz. Das Holz schwer, hart, schwer spaltbar. Die Rinde glatt, mattgrau mit spaunrückiger Oberfläche.

192. CARPINUS YEDOENSIS MAXIM. (Inu-shide.)

(Fig. 140.)

Farbe des Holzes gelblichgrau. Die Gefässe fein, deutlich, so dass man sie auf dem Querschnitt mit freiem Auge noch sehen kann. Sonst wie die der Vorigen. Die Oberfläche der Rinde stark spaunrückig.

193. CARPINUS JAPONICA BL. (Kuma-shide.)

(Fig. 141.)

Farbe des Holzes und Grösse der Gefässe ähnlich dem Carpinus laciflora. Die Markstrahlen sind deutlich, ziemlich scharf begrenzt, sogar mit etwas Glanz. Die Jahrringsgrenze ist weniger ausgebuchtet. Die Rinde mit vielen grossen braunen Lenticellen.

194. CARPINUS CORDATA BL. (Sawashiba.)

(Fig. 92. 142.)

Die feinen Markstrahlen treten nicht so dicht zu falschen Markstrahlen zusammen wie bei der vorigen Art, häufig hat das Holz nur grosse Neigung solche zu bilden. Sonst ist das Holz in hohem Grade dem des Carpinus laxiflora ähnlich, Farbe des Holzes nur etwas röthlich. Die Rinde mit zahlreichen tiefbraunen grossen Lenticellen, ist nicht glatt.

195. CORYLUS HETROPHYLLA FISCH. (Hashibami.)

Farbe des Holzes röthlichweiss. Kein Kern. Die falschen Markstrahlen sind röthlich gefärbt, ziemlich scharf begrenzt und deutlich, so dass man das Zusammentreten der sehr feinen Markstrahlen zu falschen nur mit Lupe klar erkennen kann. Man sieht dieselben auf dem Tangentialschnitt ganz deutlich, während sie bei den Carpinusarten nur als undeutliche matte

Striche erkennbar sind. Die Gefässe sind äusserst fein, ohne Lupe gar nicht zu erkennen. Das Holz mässig hart, leicht spaltbar. Die Rinde bräunlichgrau, glänzend.

IV. Die Hölzer, bei welchen die Gefässe sich zu Figuren verbinden und auf dem Querschnitt nur als helle Figuren sichtbar sind. Die Markstrahlen äusserst fein.

196. OSMANTHUS AQUIFOLIUM BET. H. (Hiiragi.)

(Fig. 93.)

Das Holz gelblichweiss, zuweilen mit etwas grün. Kein Kern. Die Jahrringsgrenze markirt sich durch eine feine hellröthliche Linie, welche aber nur gut auf dem Längsschnitt erkennbar ist. Die Gefässe auch mit Lupe nicht sichtbar. Das Holz sehr hart, sehr schwer, schwer spaltbar. Die Rinde anfangs glänzend, wird später eine dunkle kleinschuppige korkige Borke.

197. OSMANTHUS FRAGRANS LOUR. (Mokusei.)

In allen Punkten dem Vorigen ähnlich, aber die Figur der Gefässverbindung ist gröber und die Gefässe mit Lupe noch als undeutliche Löcher sichtbar. Die Rinde wie die des Vorigen, wird aber früher schuppig und weniger korkig.

198. PITTOSPORUM TOBIRA AIT. (Tobera.)

Das Holz gelblichweiss mit etwas grün, aber längere Zeit der Luft ausgesetzt wird es röthlich grau. Die Linien der Figur sehr fein, verlaufen unregelmässig bald schräg, bald wellenförmig. Die Gefässe mit Lupe nicht sichtbar. Die Jahrringsgrenze ist durch eine feine helle Linie gekennzeichnet. Das Holz hart, schwer spaltbar, hat im frischen Zustande einen sehr unangenehmen Geruch. Die Rinde dunkelbrau.

199. ORIXA JAPONICA THUNB. (Ko-kusagi.)

Das Holz ist gelblichweiss. Kein Kern. Die Figur ist der

des Osmanthus aquifolium ähnlich, aber weit feiner und mit Lupe undeutlich als gedrängt zusammenstehende Gefässe sichtbar. Die Jahrringsgrenze ist durch eine feine undeutliche Linie gekennzeichnet. Das Holz hart, schwer spaltbar. Die Rinde dunkelgrau.

V. Das Holz welches in seinem secundären Holze kein Gefäss besitzt.

200. TROCHODENDRON ARALIOIDES S. ET Z. (Yamaguruma.)

(Fig. 94.)

Das Holz gelblichweiss. Kein Kern. Die weiche Frühjahrszone ist von der dichten Herbstzone sehr scharf getrennt, was ich kaum bei anderen Nadel- und Laubhölzer, welche in Japan wachsen, bemerkt habe. Die Markstrahlen sind hellrötlich gefärbt, fein, aber ganz deutlich, wie es bei den Nadelhölzern nicht der Fall ist, wodurch man dieses Holz leicht von den letzteren unterscheiden kann.

Register.

Übersicht.

I. Ringporige Hölzer.

A. Die Gefässe ausserhalb des Porenkreises, d.i. des inneren Gefässreicheren Holztheiles in einem Jahrringe, sind gleichmässig vertheilt, oder doch zuweilen nur bei den breiten Jahrringen in der Nähe der äusseren Jahrringsgrenze zu kurzen peripherischen oder mehr schrägen Linien vereinigt.

a) **Die Markstrahlen fein.**

1. Die Gefässe im Porenkreise sehr weit.

a. Die Gefässe an der Aussengrenze des Jahrringes sind fast nie zu Strichen vereinigt.

1. Cedrela chinensis Juss. (Chanchin.)
2. Spondias sp.? (Kaname.)
3. Albizzia Julibrissin Boiv. (Nemu-no-ki.)

b. Die Gefässe an der Aussengrenze des Jahrringes sind fast immer zu Strichen, wenn auch nur zu kurzen, vereinigt.

4. Melia japonica Don. (Sendan.)
5. Hovenia dulcis Thunb. (Kemponashi.)
6. Ehretia acuminata R. Br. (Chisha-no ki.)

2. Die Gefässe im Porenkreise weit.

a. Die Gefässe an der Aussengrenze des Jahrringes sind fast nie zu Strichen vereinigt.

7. Rhus vernicifera DC. (Urushi.); Rhus tricocarpa Miq. (Yama-urushi.)
8. Rhus silvestris S. et Z. (Yamahaze.) Rhus succedanea L. (Haze.)
9. Rhus sp. (Yamahaze?)

b. Die Gefässe an der Aussengrenze des Jahrringes sind fast immer zu Strichen, wenn auch nur kurzen, vereinigt.

10. Rhus semialata Murr. (Nurude.)
11. Picrasma quassioides Benn. (Nigaki.)
12. Broussonetia papyrifera Vent. (Kaji-no-ki.)
13. Broussonetia Kasinoki Sieb. (Kōzo.)

3. Die Gefässe in Porenkreise fein.

Die Gefässe ausserhalb des Porenkreises gleichmässig zerstreut.

14. Elaeagnus macrophylla Thunb. (Natsugumi.)
15. Prunus Mume S. et Z. (Ume.)
16. Prunus communis Huds. (Sumomo.); Prunus Persica S. et Z. var. vulgaris Max. (Momo.)
17. Acanthopanax sciadophylloides Fr. et Sav. (Ko-hiabura.)
18. Dendropanax japonicum Seem. (Kakuremino.)
19. Edgeworthia chrysantha Lindl. (Mitsumata.)

b) **Die Markstrahlen sehr fein.**

1. Die Gefässe im Porenkreise weit. Die Gefässe an der Aussengrenze des Jahrringes häufig zu Strichen vereinigt.

20. Fraxinus Sieboldiana Bl. (Shioji.)
21. Fraxinus mandshurica Rupr. (Yachidamo.)
22. Catalpa Kaempferi S. et Z. (Ki-sasage.)
23. Robinia pseudacacia L. (Inu-akashia.)

2. Die Gefässe im Porenkreise fein.

24. Fraxinus Bungeana DC. var. pubinervis Wg. (Toneriko.)
25. Ligustrum Ibota Sieb. (Ibota-no-ki.)

c.) **Die Markstrahlen äusserst fein.**

Die Gefässe im Porenkreise weit.

26. Mallotus japonicus Muell. Arg. (Akamegashiwa.)
27. Aleurites cordata Muell. Arg. (Aburagiri.)

B. Die Gefässe ausserhalb des Porenkreises sind zu peripherischen, zuweilen etwas verzweigten Wellenlinien vereinigt.

a.) **Die Markstrahlen breit.**

1. Die Gefässe im Porenkreise weit.

28. Sterculia platanifolia L. (Ao-giri.)
29. Celtis sinensis Pers. (Enoki.)

2. Die Gefässe im Porenkreise fein.

30. Berberis Thunbergii DC. (Megi.)
31. Tamarix juniperina Bge. (Gyoriu.)

b.) **Die Markstrahlen fein.**

Die Gefässe im Porenkreise weit.

32. Zelkowa acuminata Pl. (Keyaki.)
33. Acanthopanax ricinifolium S. et Z. (Hari-giri.)
34. Ulmus campestris Sm. var. laevis Planch. (Haru-nire.)
35. Ulmus montana Sm. var. laciniata Trautv. (Ohyō.)
36. Ulmus parvifolia Jacq. (Akinire.)
37. Cladrastis amurensis B. et H. var. floribunda Maxim. (Inu-enju.)
38. Sophora japonica L. (Enju.)
39. Sophora platycarpa Maxim. (Fujiki.)
40. Phellodendron amurense Rupr. (Kiwada.)
41. Gleditschia japonica Miq. (Saikachi.)
42. Morus alba L. var. stylosa Bur. (Yama-guwa.)
43. Ailanthus glandulosa Desf. (Shinju.)
44. Paulownia tomentosa H. Bn. (Kiri.)
45. Clerodendron tricotomum Thunb. (Kusagi.)

c.) **Die Markstrahlen sehr fein.**

46. Aphananthe aspera Planch. (Mukunoki.)
47. Sapindus Mukurosi Gaertn. (Mukuroji.)

C. Die Gefässe ausserhalb des Porenkreises sind in einer radial verlaufenden, oft auch verzweigten Linie vereinigt.

a.) **Einzelne Markstrahlen sehr breit, andere aber äusserst fein.**

48. Quercus serrata Thunb. (Kunugi.)
49. Quercus variabilis Bl. (Abemaki.)
50. Quercus dentata Thunb. (Kashiwa.)

b.) **Einzelne Markstrahlen breit, andere aber äusserst fein.**

51. Quercus glandulifera Bl. (Konara.)

52. Quercus grosseserrata Bl. (Ō-nara.)

c.) **Die Markstrahlen sehr fein.**

53. Platycarya strobilacea S. et Z. (Nobu-no ki.)

d.) **Die Markstrahlen äusserst fein.**

54. Castanea vulgaris Lam. var. japonica DC. (Kuri.)

II. Zerstreutporige Hölzer.

A. Ein Theil der Markstrahlen sehr breit, anderer aber äusserst fein

55. Alnus japonica S. et Z. (Hannoki.)
56. Alnus incana Willd. var. glauca Ait. (Yamahannoki.)
57. Alnus viridis DC. var sibirica Rgl. (Miyama-hannoki.)
58. Alnus glutinosa Willd. (Kawara-hannoki.)

B. Ein Theil der Markstrahlen breit.

a.) **Die breiten Markstrahlen sind sehr zahlreich, nicht glänzend und verlaufen etwas krummlinig. Zwischen denselben sind die feineren vertreten, aber die feinsten fehlen.**

59. Ardisia Sieboldi Miq. (Mokutachibana.)
60. Myrsine capitellata Wall. (Hichi-no-ki.)
61. Aucuba japonica Thunb. (Aoki.)

b.) **Die breiten atlasglänzenden Markstlahlen sind häufig und veilaufen gerade. Zweischen denselben sind die feineren und feisten vertreten.**

62. Fagus sylvatica L. var. Sieboldi Maxim. (Buna.)
63. Fagus japonica Maxim. (Inu-buna.)

C. Die meisten der Markstrahlen sind fein, aber deutlich und scharf, so dass man deren Verlauf auf dem Querschnitt leicht mit unbewaffnetem Auge verfolgen kann.

a.) **Die Markstrahlen sind auf dem Tangentialschnitt als Spindel deutlich sichtbar.**

1. Die Markstrahlen sind theils hoch, theils niedrig.

64. Euptelaea polyandra S. et Z. (Fusazakura.)
65. Meliosma pungens Wall. (Yama-biwa.)

2. Die Markstrahlen sind niedrig und fast gleich hoch.

66. Cornus Kousa Buerg. (Yamaboshi.)
67. Ilex crenata Thunb. (Inu-tsuge.)
68. Ilex integra Thunb. (Mochi-no-ki); Ilex rotunda Thunb. (Kuroganemochi.) Ilex latifolia Thunb. (Tarayō.)
69. Ilex macropoda Miq. (Aohada.)
70. Ilex pedunculosa Miq. (Soyogo.)
71. Euscaphis japonica Pax. (Gonzui.)

b. **Die Markstrahlen sind auf dem Tangentialschnitt sehr schwer erkennbar.**

1. Die Gefässe sind arm und klein, aber auf dem Querschnitt gut sichtbar.

a. Die Markstrahlen sind fast gleich breit.

72. Millettia japonica Sieb. et Z. (Awa-fuji.)
Die meisten Markstrahlen fein, dazwischen die sehr feinen bemerkbar.
73. Morus indica L. (Shima-guwa.)
74. Sophora sp.
75. Xanthoxylum Bunthoides Sieb. et Z. (Kuro-zanshō.)

2. Die Gefässe sind so fein, dass sie auf dem Querschnitt schwer oder fast nicht mehr, auf dem Längsschnitt dagegen als vertieft liegen deutlich sichtbar sind.

a. Ohne Kern.

76. Acer japonicum Thunb. var. Matsumurae. (Itaya-kaede.)
77. Acer palmatum Thunb. (Yamamomiji.)
78. Acer purpurascens Fr. et Sav. (Kajikaede.)
79. Acer carpinifolium Sieb. et Z. (Chōsen-ekki.)
80. Acer nikoense Maxim. (Chōjū-no-ki.)
81. Acer argutum Maxim. (Aomcha-kaede.)
82. Acer rufinerve Sieb. et Z. (Uri-kaede.)
83. Acer crataegifolium Sieb. et Z. (Meuri-kaede.)
84. Acer japonicum Thunb. (Hauchiwa-kaede.) Acer Sieboldianum Miq. var. microphyllum Maxim. (Kohauchiwa-kaede.)
85. Acer distylum Sieb. et Z. (Maruba-kaede.)
86. Tilia cordata Mill. var. japonica Maxim. (Shina-no-ki.). Tilia Miqueliana Maxim. (Bodaiju.)
87. Cornus macrophylla Wall. (Mi-ki.)
88. Cornus Igaponica C. Koch. (Sawa-mizuki.)

b. Mit Kern.

89. Staphylea Bumalda Sieb. et Z. (Mitsuba-tsutsuji.)
90. Prunus Pseudo-Cerasus Lindl. var. spontanea Maxim. (Yama-zakura.); Prunus Pseudo-Cerasus var. Sieboldi Maxim. (Yoshino-zakura.
91. Prunus Grayana Maxim. (Uwamizu-zakura.)
92. Prunus Buergeriana Miq. (Inu-zakura.)
93. Prunus Cerasoides Maxim. (Mejiro-zakura.)
94. Prunus persica Benth. (Momo-zakura.)
95. Michelia compressa Maxim. (Ogatamanoki.)
96. Sambucus racemosa L. (Niwatoko.)
97. Deutzia scabra Thunb. (Uzugi.)

D. Die meisten Markstrahlen sind sehr fein.

a. **Die Gefässe weit, zuweilen sehr weit, sind auf dem Querschnitt deutlich als Löcher sichtbar und stehen nicht dicht zusammen.**

98. Diospyros Kaki L. f. (Kaki.)
99. Diospyros Lotus L. (Mamegaki.)

100. Juglans Sieboldiana Maxim. (Onigurumi.)
101. Pterocarya rhoifolia S. et Z. (Sawa-gurumi.)
102. Hernandia peltata Meisn. (Hasunohagiri.)
103. Cinnamomum Camphora Nees. (Kusu.)
104. Machilus Thunbergii S. et Z. (Tabu-no-ki.)
105. Ficus retusa L. var. nitida Miq. (Gazumaru.)

b.) **Die Gefässe sind auf dem Querschnitt mit unbewaffnetem Auge nur schwer als Löcher, auf dem Längsschnitt aber gut als vertiefte Linien sichtbar.**

106. Styrax japonica S. et Z. (Yego-no-ki.)
107. Styrax Obassia S. et Z. (Hakuumboku.)
108. Idesia polycarpa Maxim. (Ii-giri.)
109. Magnolia hypoleuca S. et Z. (Hō-no-ki.)
110. Magnolia Kobus DC. (Kobushi.)
111. Magnolia salicifolia Maxim. (Tamushiba.)
112. Zanthoxylum piperitum DC. (Sanshō); Zanthoxylum schiniifolium S. et Z. (Inuzanshō.)
113. Stewartia monadelpha S. et Z. (Saruta.)
114. Stewartia pseudocamellia Maxim. (Natsutsubaki.)
115. Alnus firma S. et Z. (Yashabushi.)
116. Cercidiphyllum japonicum S. et Z. (Katsura.)
117. Machilus Thunbergii S. et Z. var japonica Yatabe. (Baribari.)
118. Ficus erecta Thunb. (Inu-biwa.)
119. Litsea glauca Sieb. (Shirodamo.)
120. Actinopaphne lancifolia Meisn. (Kago-no-ki.)
121. Cinnamomum pedunculatum Nees. (Yabu-nikkei.)
122. Lindera praecox Bl. (Aburachan.)
123. Lindera sericea Bl. (Kuromoji.)
124. Lindera triloba Bl. (Shiromoji); Lindera obtusiloba Bl. (Dankobai.)
125. Lindera umbellata Thunb. (Kanakugi.)

c.) **Die Gefässe sind mit undewaffnetem Auge weder auf den Querschnitt, noch auf dem Längsschnitt sichtbar.**

126. Myrica rubra S. et Z. (Yamamomo.)
127. Ternstroemia japonica Thunb. (Mokkoku.)
128. Thea japonica Nois. (Tsubaki.)
129 Thea Sazanqua Nois. (Sazan-kwa.)
130. Clethra barbinervis S. et Z. (Ryōbu.)
131. Cydonia sinensis Thourin. (Kwarin.)
132. Lyonia ovalifolia Don. (Kashioshimi.)
133. Rhododendron Metternichii S. et Z. (Shakunagi.)
134. Rhododendron dilatatum Miq. (Mitsubatsutsuji.)
135. Pourthiaea villosa Dene. (Ushikoroshi.)
136. Ligustrum japonicum Thunb. (Nezumimochi.)
137. Viburnum odoratissimum Ker. (Sangoju.)
138. Viburnum dilatatum Thunb. (Gamazumi.) ;Viburnum furcatum Bl. (Ogame-no-ki.)

E. Alle Markstrahlen sind äusserst fein, ohne Lupe nicht mehr sichtbar.

a.) Die Gefässe weit, sind auf dem Querschnitt deutlich als Löcher sichtbar und stehen nicht zusammen.

139. Nephelium Longana Camb. (Ryūgan.)

b.) Die Gefässe sind mit unbewaffnetem Auge auf dem Querschnitt nur sehr schwer als Löcher, auf dem Längsschnitt aber gut als vertiefte Linie sichtbar.

1. Die Gefässe, mit Lupe betrachtet, sind arm und klar, stehen nicht dicht zusammen.

α. Harte Hölzer.

140. Ostrya japonica Sargent. (Asada.)
141. Zizyphus vulgaris Lam. var. inermis Bge. (Natsume.)
142. Citrus trifoliata L. (Karatachi.)
143. Lagerstroemia indica L. (Hyakujikko.)
144. Betula Bhojpattra Wall. var. typica Rgl. (Ono-ore.); Betula globispica Shirai. (Jizo-kamba.)
145. Betula ulmifolia S. et Z. (Yoguso-minebari.)
146. Betula corylifolia Rgl. et Max. (Urajiro-kamba.); Betula grossa S. et Z. (Mizume.)

β. Weiche Hölzer.

147. Betula Maximowicziana Rgl. (Saihada.)
148. Betula alba L. var. vulgaris DC. (Shira-kamba.)
149. Betula alba L. var. communis Rgl. (Makamba.)
150. Betula alba L. var. cordifolia Rgl. (Aka-kamba.)

2. Die Gefässe sind, mit Lupe betrachtet, zahlreich und stehen dicht zusammen.

α. Hartes Holz.

151. Symplocos crataegoides Ham. var. pallida Fr. et Sav. (Shiro-tsuge.)

β. Weiche Hölzer.

152. Populus tremula L. var. villosa Wesm. (Yamanarashi.)
153. Populus balsamifera L. var. suaveolens Loud. (Dero.)
154. Salix Caprea L. (Saruyanagi.)
155. Salix Urbaniana v. Seemann. (Akayanagi.)
156. Halesia corymbosa B. et H. (Asagara); Halesia hispida B. et H. (Oba-asagara.)
157. Viburnum Opulus L. (Kamboku.)

c.) Die Gefässe sind mit freiem Auge weder auf dem Querschnitt noch auf dem Längsschnitt sichtbar.

α. Harte Hölzer.

158. Distylium racemosum S. et Z. (Isu.)
159. Diervilla grandiflora S. et Z. (Hakone-utsugi.)
160. Hydrangea paniculata Sieb. (Nori-no-ki.)
161. Eriobotrya japonica Lindl. (Biwa.)
162. Photinia glabra Thunb. (Kanamemochi.)

163. Eurya ochnacea Szysz. (Sakaki.); Eurya japonica Thunb. (Hisakaki.)
164. Illicium Anisatum L. (Shikimi.)
165. Andromeda japonica Thunb. (Asebi.)
166. Pirus Toringo Sieb. (Zumi.)
167. Pirus sinensis Lindl. (Nashi.)
168. Pirus Calleryana Dcne. (Konashi.)
169. Euonymus europaea L. var. Hamiltoniana Maxim. (Mayumi.)
170. Euonymus oxyphylla Miq. (Tsuribana.)
171. Euonymus japonica Thunb. (Masaki.)
172. Buxus sempervirens L. (Tsuge.)
173. Daphniphyllum macropodum Miq. (Yuzuriha.)
174. Hamamelis japonica S. et Z. (Mansaku.)
175. Pirus aucuparia Gaertn. var. japonica Maxim. (Nanakamado.)
176. Pirus Miyabei Sargent. (Azukinashi.)
177. Pirus Tschonoski Maxim (Miyama-nashizumi.)
178. Pirus aria Ehrh. var. kamaonensis Wall. (Urajiro-no-ki.)

β. Weiches Holz.

179. Aesculus turbinata Bl. (Tochi.)

III. Die Hölzer, bei welchen die alle Gefässe in radialer und nicht selten verzweigter Linie gruppirt sind. Diese Linie wollen wir die radiale Gefässlinie nennen.

A. Die einzelnen Markstrahlen sind sehr breit, die meisten äusserst fein, kaum sichtbar.

180. Quercus acuta Thunb. (Akagashi.)
181. Quercus gilva Bl. (Ichii-gashi.)
182. Quercus Vibrayeana Fr. et Sav. (Shira-gashi.)
183. Quercus myrsinaefolia Bl. (Urajiro-gashi.)
184. Quercus phillireoides A. Gr. (Ubamegashi.)
185. Quercus glauca Thunb. (Aragashi.)
186. Quercus thalassica Hce. (Shirifuka-gashi.)
187. Quercus sessilifolia Bl. (Tsukubane-gashi.)
188. Pasania cuspidata Oerst. (Shii.)
189. Pasania glabra Oerst. (Mateba-shii.)

B. Die Markstrahlen äusserst fein.

190. Hibiscus syriacus L. (Mukuge.)

C. Die breiten Markstrahlen entstehen durch das Zusammentreten zahlreicher, schmaler Markstrahlen zu einem componirten, sogenannten falschen Markstrahle. Es fehlt ihnen an Glanz und scharfer Begrenzung.

191. Carpinus laxiflora Bl. (Akashide.)
192. Carpinus yedoensis Maxim. (Inu-shide.)
193. Carpinus japonica Bl. (Kuma-shide.)
194. Carpinus cordata Bl. (Sawashiba.)

195. Corylus heterophylla Fisch. (Hashibami.)

IV. Die Hölzer, bei welchen die Gefässe sich zu Figuren verbinden und auf dem Querschnitt nur als helle Figuren sichtbar sind. Die Markstrahlen äusserst fein.

196. Osmanthus Aquifolium B. et H. (Hiiragi.)
197. Osmanthus fragrans Lour. (Mokusei.)
198. Pittosporum Tobira Ait. (Tobera.)
199. Orixa japonica Thunb. (Ko-kusagi.)

V. Das Holz welches in seinem secundären Holze kein Gefäss besitzt.

200. Trochodendron aralioides S. et Z. (Yamaguruma.)

TAFEL VIII.

Fig. 1. Cedrela chinensis Juss. (Chanchin.)
Fig. 2. Spondias sp ? (Kaname.)
Fig. 3. Albizzia Julibrissin Boiv. (Nemu-no-ki.)
Fig. 4. Melia japonica Don. (Sendan.)
Fig. 5. Hovenia dulcis Thunb. (Kemponashi.)
Fig. 6. Ehretia acuminata R. Br. (Chisha-no-ki.)
Fig. 7. Rhus vernicifera D.C. (Urushi.)
Fig. 8. Rhus sp. (Yamahaze ?)
Fig. 9. ,, semialata Murr. (Nurude.)
Fig. 10. Picrasma quassioides Benn. (Nigaki.)
Fig. 11. Prunus Mume S. et Z. (Ume.)
Fig. 12. Acanthopanax sciadophylloides Fr. et Sav. (Koshiabura.)
Fig. 13. Fraxinus Sieboldiana Bl. (Shioji.)
Fig. 14. Catalpa Kaempferi S. et Z. (Ki-sasage.)
Fig. 15. Fraxinus Bungeana D.C. var. pubinervis Wg. (Toneriko.)
Fig. 16. Mallotus japonicus Muel. Arg. (Akamegashiwa.)

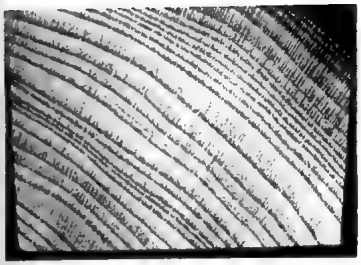


Fig. 1.

Fig. 2.

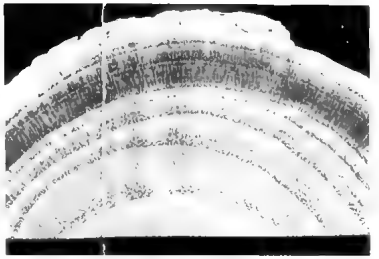
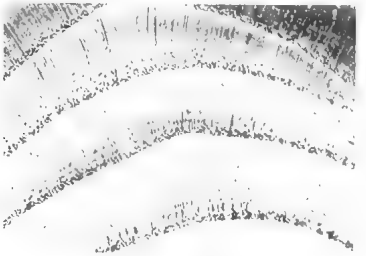


Fig. 3.



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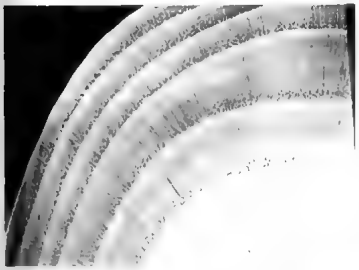

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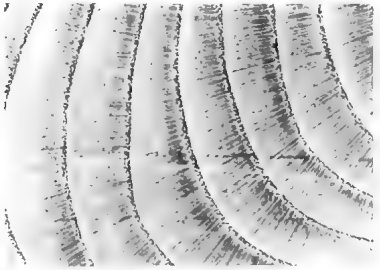

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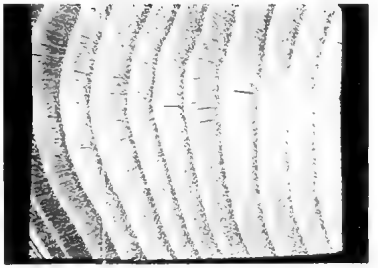
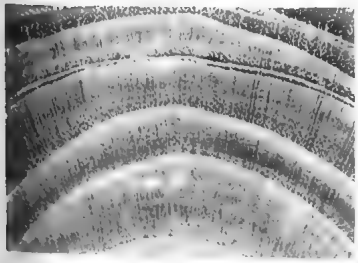


Fig. 7.

Fig. 8.



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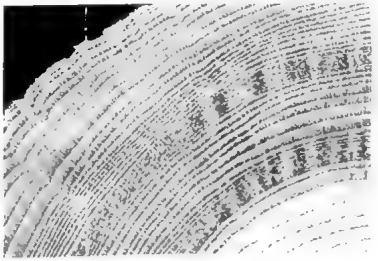

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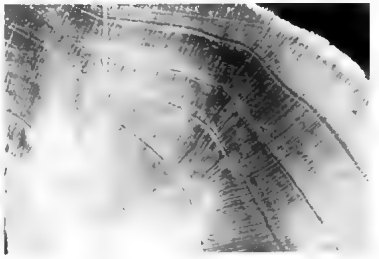

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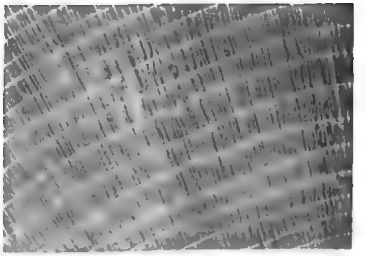

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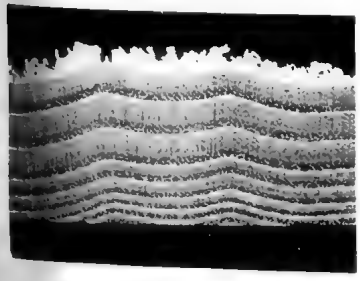

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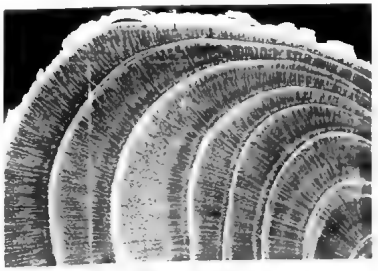

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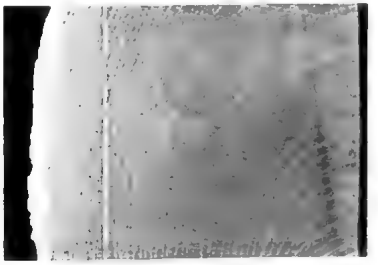

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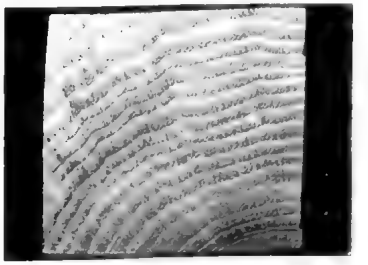


Fig. 16.

TAFEL IX.

Fig. 17. Celtis sinensis Pers. (Enoki.)
Fig. 18. Berberis Thunbergii D C. (Megi.)
Fig. 19. Zelkowa acuminata Pl. (Keyaki.)
Fig. 20. Acanthopanax ricinifolium S. et Z. (Hari-giri.)
Fig. 21. Ulmus campestris Sm. var. laevis Planch. (Haru-nire.)
Fig. 22. Ulmus palvifolia Jacq. (Aki-nire.)
Fig. 23. Sophora platycarpa Maxim. (Fujiki.)
Fig. 24. Phellodendron amurense Rupr. (Kiwada.)
Fig. 25. Paulownia tomentosa H. Bn. (Kiri.)
Fig. 26. Sapindus Mukurosi Gaertn. (Mukuroji.)
Fig. 27. Quercus serrata Thunb. (Kunugi.)
Fig. 28. ,, dentata Thunb. (Kashiwa.)
Fig. 29. ,, glandulifera Bl. (Konara.)
Fig. 30. Platycarya strobilacea S. et Z. (Nobu-no-ki.)
Fig. 31. Castanea vulgaris Lam. var. japonica D.C. (Kuri.)
Fig. 32. Alnus japonica S. et Z. (Hannoki.)

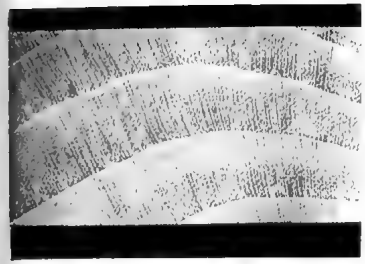
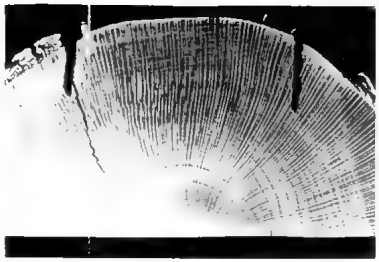


Fig. 17.



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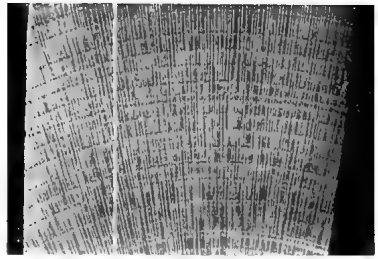

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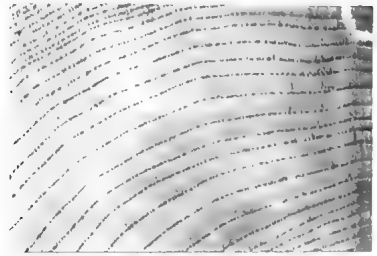

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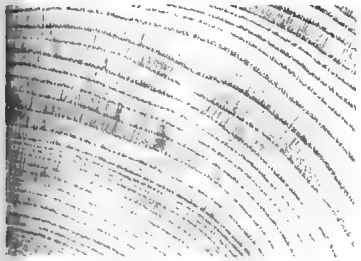

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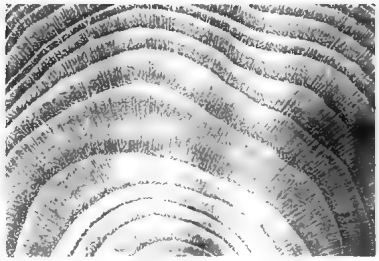

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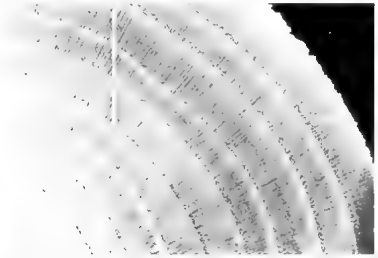
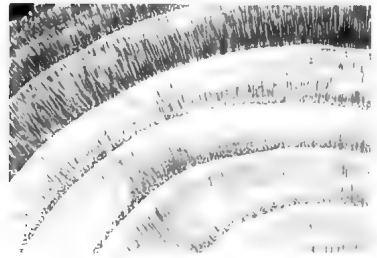


Fig. 23.



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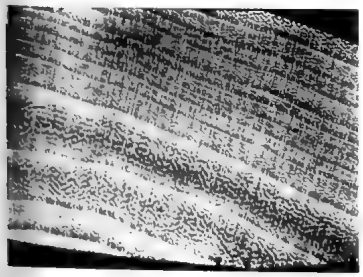


Fig. 25.

Fig. 26.

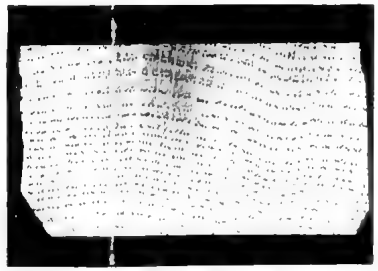
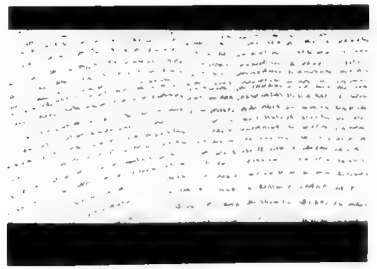


Fig. 27.



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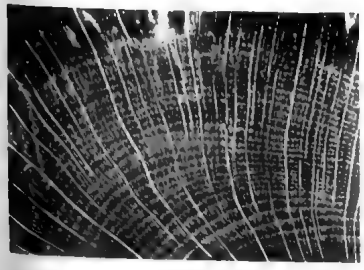


Fig. 29.

Fig. 30.

Fig. 31.

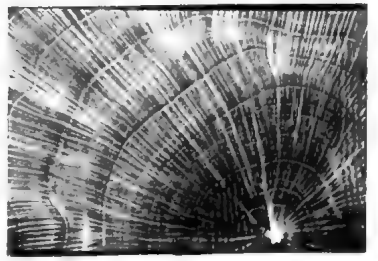
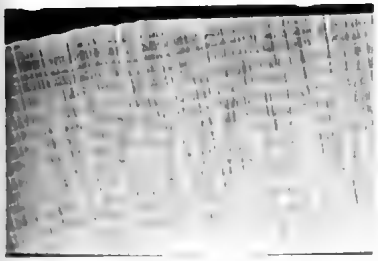


Fig. 32.

TAFEL X.

Fig. 33. Alnus incana Willd. var. glauca Ait. (Yama-hannoki.)
Fig. 34. Ardisia Sieboldi Miq. (Mokutachibana.)
Fig. 35. Fagus sylvatica L. var. Sieboldi Maxim. (Buna.)
Fig. 36. „ japonica Maxim. (Inu-buna.)
Fig. 37. Euptelaea polyandra S. et Z. (Fusazakura.)
Fig. 38. „ „ „ „
Fig. 39. „ „ „ „
Fig. 40. Cornus Kousa Buerg. (Yamabōshi.)
Fig. 41. „ „ „ „
Fig. 42. „ „ „ „
Fig. 43. Ilex integra Thunb. (Mochi-no-ki.)
Fig. 44. Meliosma myriantha S. et Z. (Awabuki.)
Fig. 45. „ „ „ „
Fig. 46. „ „ „ „
Fig. 47. Morus indica L. (Shima-guwa.)
Fig. 48. Sophora spe ?
Fig. 49. Zanthoxglum ailanthoides S. et Z. (Karasu-zanshō.)
Fig. 50. Acer pictum Thunb. var. Mono Maxim. (Itaya-kayede.)
Fig. 51. „ „ „ „ „ „ „
Fig. 52. „ „ „ „ „ „ „



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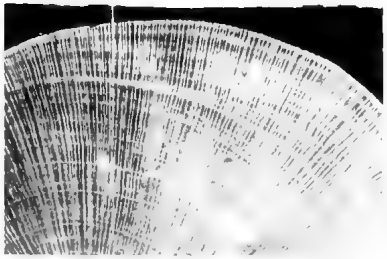

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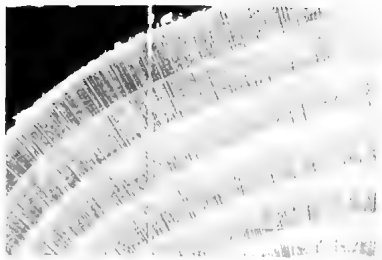

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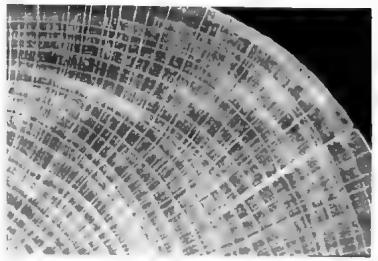

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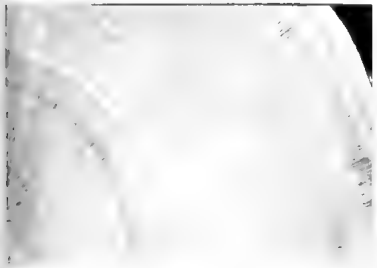

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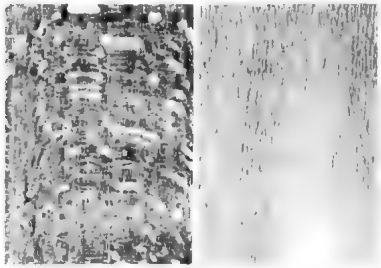
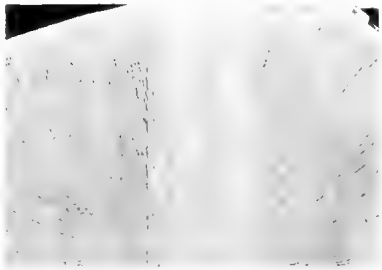


Fig. 38 Fig. [illegible].



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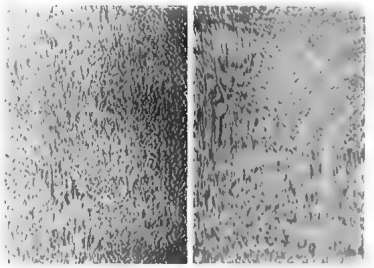

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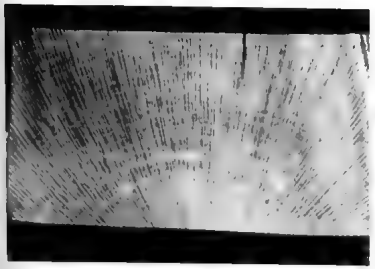

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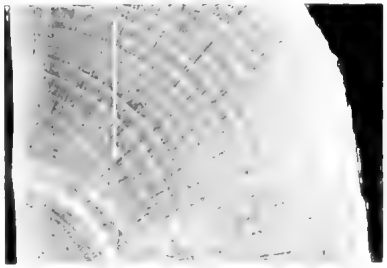


Fig. 44.

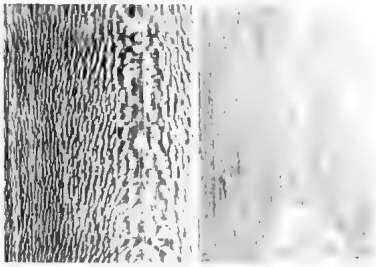


Fig. 45. Fig. [illegible].

Fig. 47.

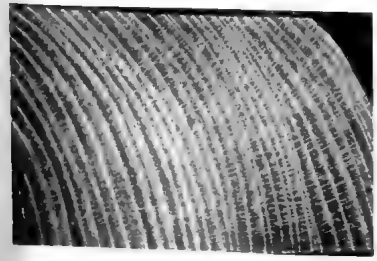


Fig. 48.

Fig. 49.

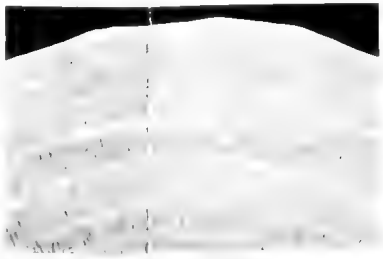


Fig. 50.

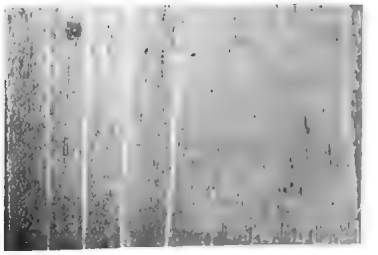
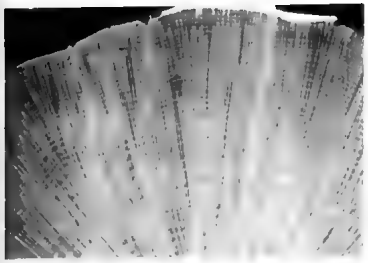


Fig. 52 Fig. [illegible].

TAFEL XI.

Fig. 53. Acer rufinerve S. et Z. (Uri-kayede.)
Fig. 54. ,, japonicum Thunb. (Hauchiwa-kayede.)
Fig. 55. Cornus macrophylla Wall. (Mizuki.)
Fig. 56. Prunus pseudo-cerasus Lindl. var. spontanea Maxim. (Yama-zakura), Prunus pseudo-cerasus var. Sieboldi Maxim. (Yoshino-zakura.)
Fig. 57. Diospyros Kaki L. f. (Kaki.)
Fig. 58. Juglans Sieboldiana Maxim. (Onigurumi.)
Fig. 59. Pterocarya rhoifolia S. et Z. (Sawa-gurumi.)
Fig. 60. Cinnamomum Camphora Nees. (Kusu.)
Fig. 61. Ficus retusa var. nitida Miq. (Gazumaru.)
Fig. 62. Styrax japonica S. et Z. (Yego-no-ki.)
Fig. 63. ,, ,, ,, ,,
Fig. 64. ,, ,, ,, ,,
Fig. 65. Magnolia hypoleuca S. et Z. (Hō-no-ki.)
Fig. 66. Stewartia monadelpha S. et Z. (Saruta.)
Fig. 67. Cercidiphyllum japonicum S. et Z. (Kutsura.)
Fig. 68. Myrica rubra S. et Z. (Yamamomo.)
Fig. 69. Ternstroemia japonica Thunb. (Mokkoku.)



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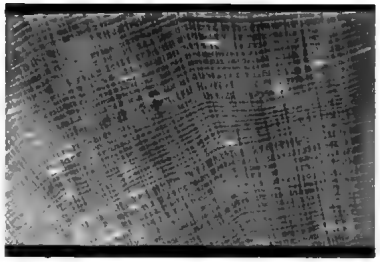

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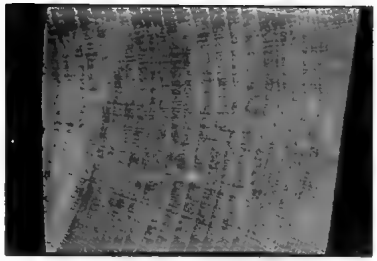

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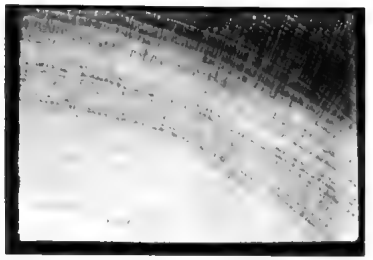

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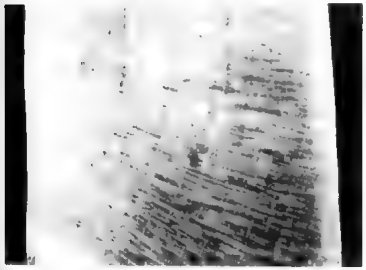

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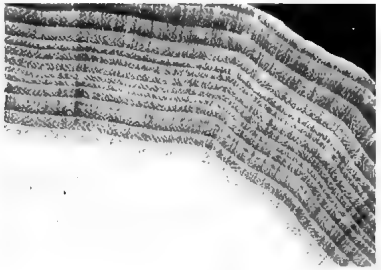
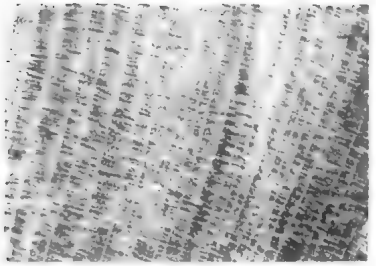


Fig. 58.

Fig. 59.



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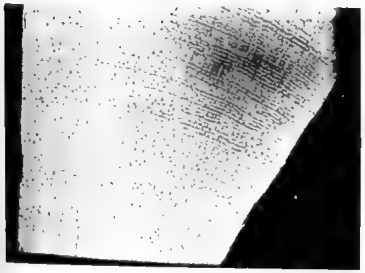

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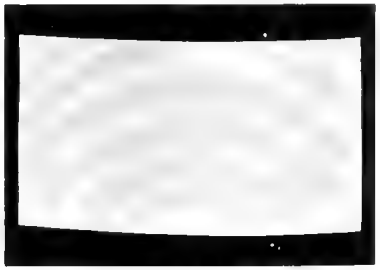


Fig. 62.

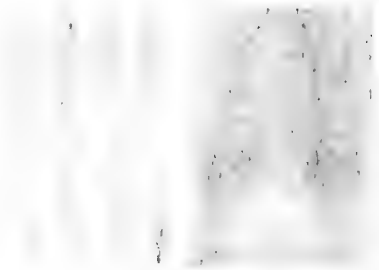


Fig. 63. Fig. 64.

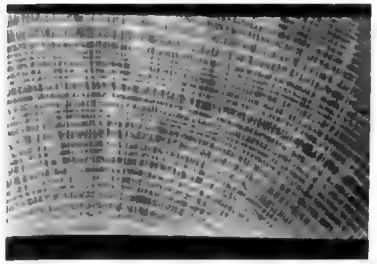


Fig. 65.

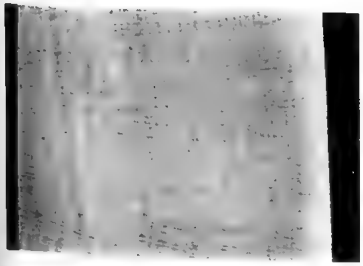
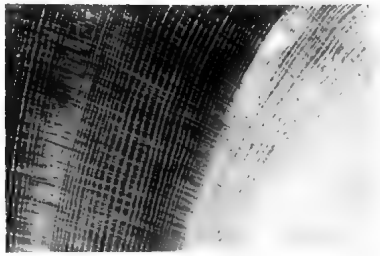


Fig. 66.



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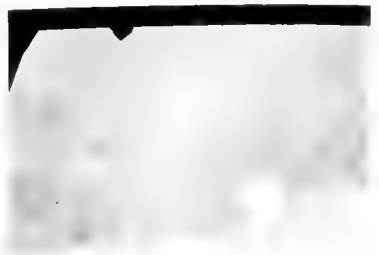


Fig. 68.

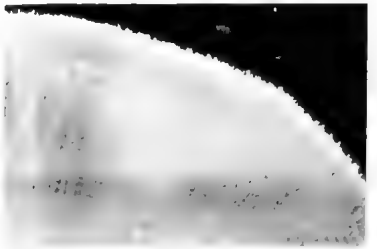


Fig. 69.

TAFEL XII.

Fig. 70. Clethra barbinervis S. et Z. (Riyōbu.)
Fig. 71. Ostrya japonica Surgent. (Asada.)
Fig. 72. ,, ,, ,, ,,
Fig. 73. ,, ,, ,, ,,
Fig. 74 Betula Bhojpattra Wall. var. typica Rgl. (Onoore), Betula glopispica Shirai. (Jizokamba.)
Fig. 75. Betula alba L. var. vulgaris D.C. (Shira-kamba.)
Fig. 76. Symplocos crataegoides Ham. var. pallida Fr. et Sav. (Shiro-tsuge.)
Fig. 77. Populus balsamifera L. var. suaveolens Loud. (Dero.)
Fig. 7[illegible] Salix Caprea L. (Saruyanagi.)
Fig. 7[illegible] Halesia corymbosa B. et H. (Asagara), Halesia hispida B. et H. (Oba-asagara.)
Fig. 80. Distylium racemosum S. et Z. (Isu.)
Fig. 81. ,, ,, ,, ,,
Fig. 82. ,, ,, ,, ,,
Fig. 83. Eurya japonica Thunb. (Hisakaki.)
Fig. 84. Euonymus europæa var, Hamiltoniana Maxim. (Mayumi.)
Fig. 85. Buxus sempervirens L. (Tsuge.)
Fig. 86. Aesculus turbinata Bl. (Tochi.)
Fig. 87 Quercus acuta Thunb. (Akagashi.)

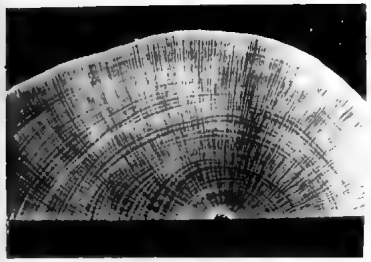


Fig. 70.

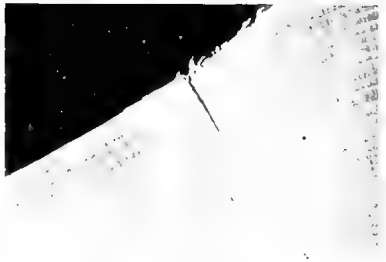


Fig. 71.

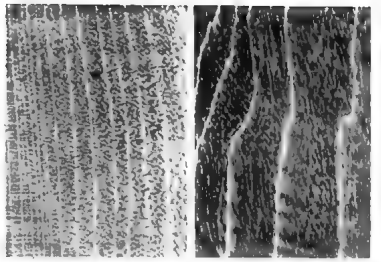


Fig. 72. Fig. 73.

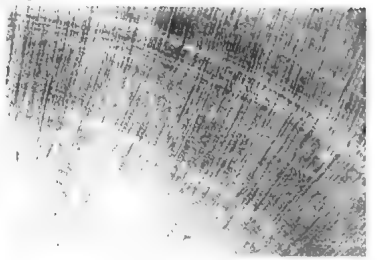
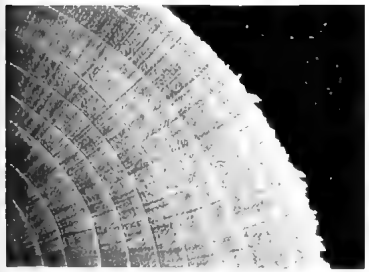


Fig. 74.



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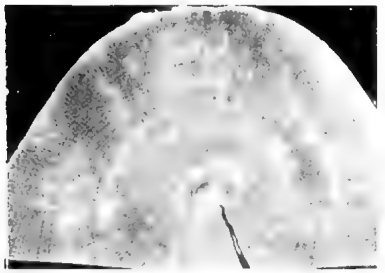

Fig. 76.

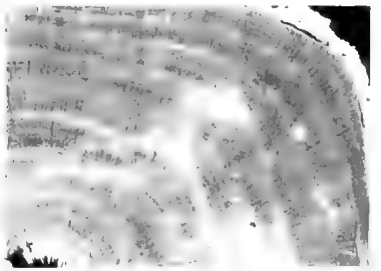

Fig. 77.

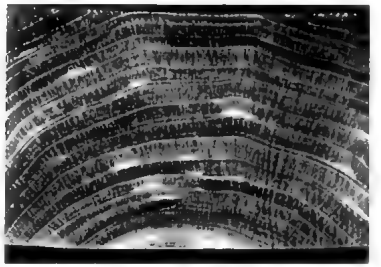

Fig. 78.

Fig. 79.

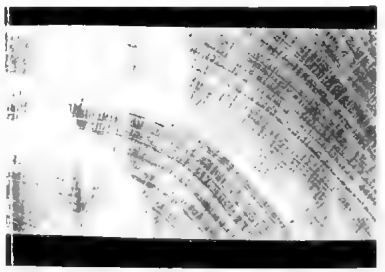

Fig. 80.

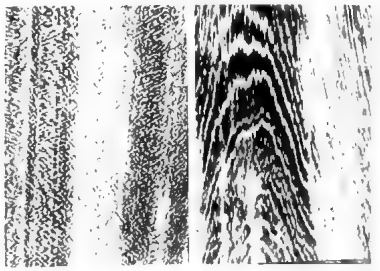

Fig. 81. Fig. 82.

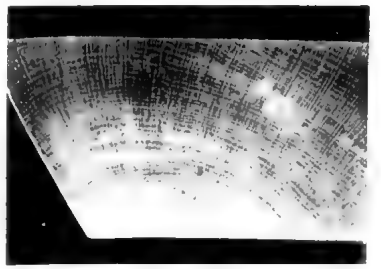

Fig. 83.

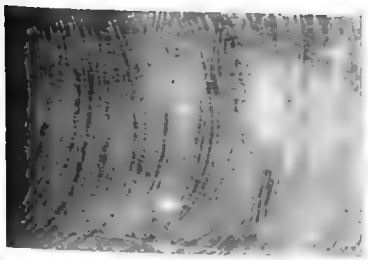

Fig. 84.

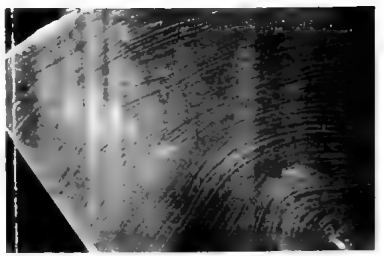

Fig. 85.

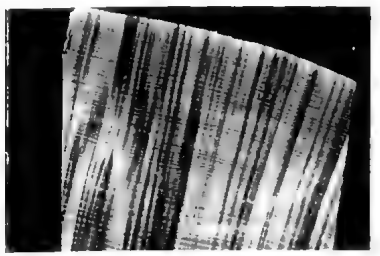

Fig. 86.

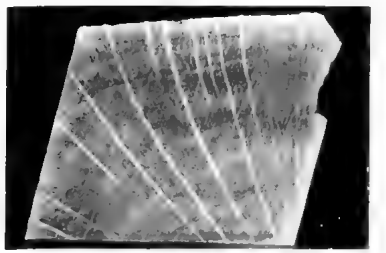

Fig. 87.

TAEEL XIII.

Fig. 88. Quercus Vibrayeana Tr. et Tav. (Shira-gashi.)
Fig. 79. Pasania cuspidata Oerst. (Shii.)
Fig. 90. „ „ „ „
Fig. 91. Carpinus laxiflora Bl. (Akashide.)
Fig. 92. „ cordata Bl. (Sawashiba.)
Fig. 93. Osmanthus Aquifolium Bet. H. (Hiiragi.)
Fig. 94. Trochodendron aralioides S. et Z. (Yamaguruma.)
Fig. 95. Cedrela chinensis Juss. (Chanchin.)
Fig. 96. Spondias sp.? (Kaname.)
Fig. 97. Quercus grosserrata Bl. (Ō-nara.)
Fig. 98. „ „ „ „
Fig. 99. Alnus japonicus S. et Z. (Hannoki.)
Fig. 100. „ incana Willd. var. glauca Ait. (Yamahannoki.)
Fig. 101. Fagus sylvatica L. var. Sieboldi Maxim. (Buna.)
Fig. 102. „ japonica Maxim. (Inu-buna.)
Fig. 103. Acer pictum Thunb. var Mono Maxim. (Itayakaede.)

Fig. 88.

Fig. 89.

Fig. 90.

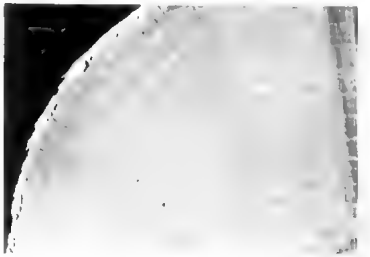

Fig. 91.

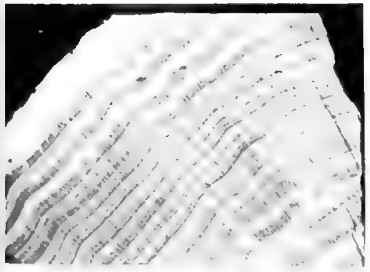

Fig. 92.

Fig. 93.

Fig. 94.

Fig. 95.

Fig. 96.

Fig. 97.

Fig. 98.

Fig. 99.

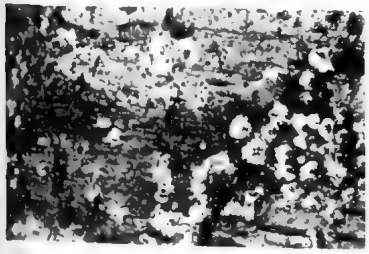

Fig. 100.

Fig. 101.

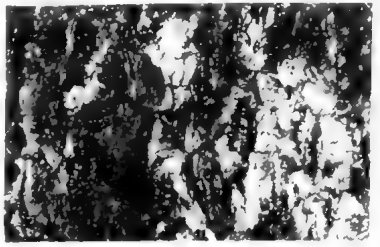

Fig. 102.

Fig. 103.

TAFEL XIV.

Fig. 104. Acer palmatum Thunb. (Yamamomiji.)
Fig. 105. ,, purpurascens Fr. et Sav. (Kajikayede.)
Fig. 106. ,, carpinifolium S. et Z. (Chidori-no-ki.)
Fig. 107. ,, nikoense Maxim. (Chōja-no-ki.)
Fig. 108. ,, rufinerve S. et Z. (Uri-kayede.)
Fig. 109. ,, Japonicum Thunb. (Hauchiwa-kayede.)
Fig. 110. ,, distylum S et Z. (Maruba-kayede.)
Fig. 111. Prunus pseudo-cerasus Lindl. var. spontanea Maxim. (Yama-zakura), Prunus pseudo-cerasus Lindl. var. Sieboldi Maxim. (Yoshino-zakura.)
Fig. 112. Prunus Grayana Maxim. (Uwamizu-zakura.)
Fig. 113. ,, Buergeriana Miq. (Inu-zakura.)
Fig. 114. Styrax japonica S. et Z. (Yego-no-ki.)
Fig. 115. ,, Obassia S. et Z. (Hakuunboku.)
Fig. 116. Magnolia hypoleuca S. et Z. (Hō-no-ki.)
Fig. 117. ,, Kobus D. C. (Kobushi.)
Fig. 118. ,, salicifolia Maxim. (Tamushiba.)
Fig. 119. Stewartia monadelpha S. et Z. (Saruta.)

Fig. 104.

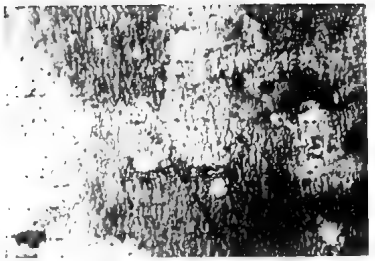

Fig. 105.

Fig. 106.

Fig. 107.

Fig. 108.

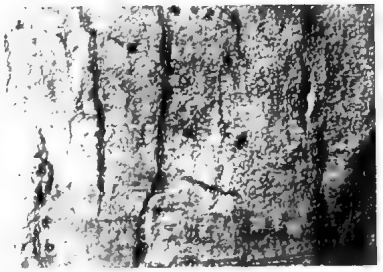

Fig. 109.

Fig. 110.

Fig. 111.

Fig. 112.

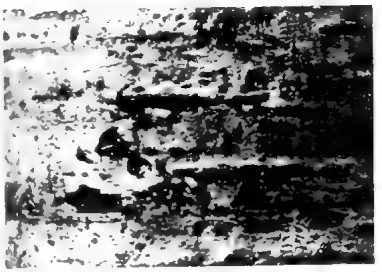

Fig. 113.

Fig. 114.

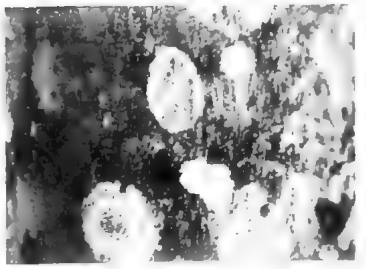

Fig. 115.

Fig. 116.

Fig. 117.

Fig. 118.

Fig. 119.

TAFEL XV.

Fig. 120. Stewartia pseudocamellia Maxim. (Natsutsubaki.)
Fig. 121. Thea japonica Nois. (Tsubaki.)
Fig. 122. ,, Sazanqua Nois. (Sazan-kwa.)
Fig. 123. Cydonia sinensis Thourin. (Kwarin.)
Fig. 124. Lyonia ovalifolia Don. (Kashioshimi.)
Fig. 125. Rhododendron Metteynichii S. et Z. (Shakunagi.)
Fig. 126. Betula Bhojpattra Wahl. var. typica Rgl. (Onoore), Betula globispica Shirai. (Zizokamba.)
Fig. 127. Betuta alba L. var. vulgaris D.C. (Shira-kamba.)
Fig. 128. Populus tremula L. var. villosa Wesm. (Yamanarashi.)
Fig. 129. Populus balsamifera L. var. suaveolens Loud. (Dero.)
Fig. 130. Halesia corymbosa B. et H. (Asagara), H. hispida B. et H. (Oba-asagara)
Fig. 131. Pirus Toringo Sieb. (Zumi.)
Fig. 132. ,, Calleryana Dcne. (Konashi.)
Fig. 133. Euonymus europaea L. var. Hamiltoniana Maxim. (Mayumi.)
Fig. 134. Euonymus oxyphylla Miq. (Tsuribana.)
Fig. 135. Pirus aucuparia Gaertn. var. japonica Maxim. (Nanakamado.)

Fig. 120.

Fig. 121.

Fig. 122.

Fig. 123.

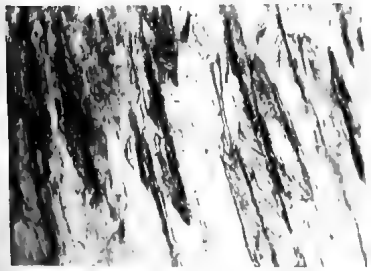

Fig. 124.

Fig. 125.

Fig. 126.

Fig. 127.

Fig. 128.

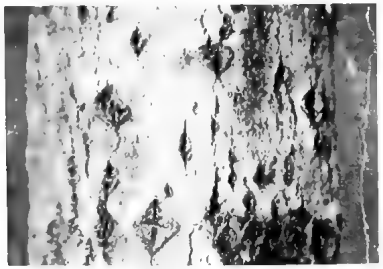

Fig. 129.

Fig. 130.

Fig. 131.

Fig. 132.

Fig. 133.

Fig. 134.

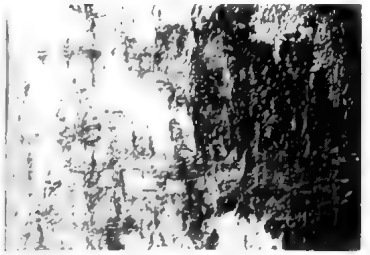

Fig. 135.

TAFEL XVI.

Fig. 136. Pirus Miyabei Surgent. (Azukinashi.)
Fig. 137. Pasania cuspidata Oerst. (Shii.)
Fig. 138. ,, glabra Oerst. (Mateba-shii.)
Fig. 139. Carpinus laxiflora Bl. (Akashide.)
Fig. 140. ,, yedoensis Maxim. (Inu-shide.)
Fig. 141. ,, japonica Bl. (Kuma-shide.)
Fig. 142. ,, cordata Bl. (Sawa-shiba.)

Fig. 136.

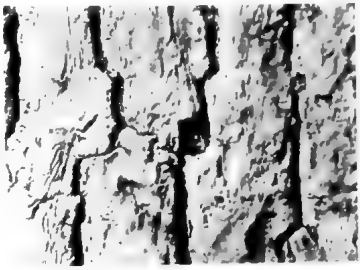

Fig. 137.

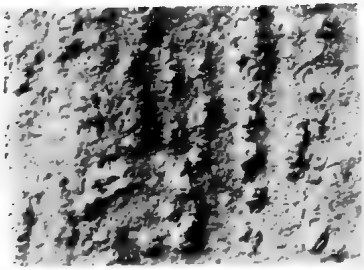

Fig. 138.

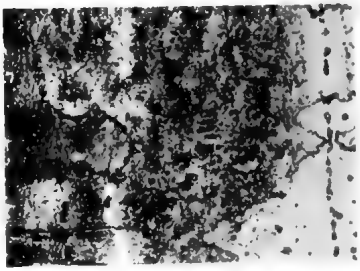

Fig. 139.

Fig. 140.

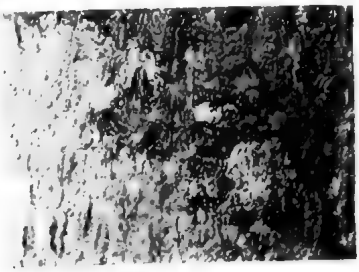

Fig. 141.

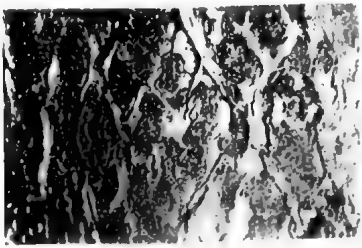

Fig. 142.

Die Gattung Tilia in Japan.

VON

Homi Shirasawa, *Ringakushi.*

Die Gattung Tilia, deren Blüten mit sehr merkwürdigen und eigenthümlich ausgebildeten Organen (Flügelblätter, Staminodien) versehen sind, besteht nur an baumartigen Gewächsen, welche als Zierbäume, sowie auch als Waldbäume geschätzt werden. Man beschreibt zunächst, als typische Formen, ueber 10 Arten auf der nördlichen Hemisphäre.

Doch ist diese Gattung bis jetzt in Japan und China oft vernachlässigt worden. Maximowicz beschrieb erst zwei chinesische Arten *T. Chinensis** und *T. paucicostata*,* und, obwohl bei uns mehr Arten als in anderen Ländern vorkommen, hat doch bisher niemand eine eingehende Bearbeitung derselben unternommen, ausser Miquel und Maximowicz.

Die erste Beschreibung über die Tiliaarten Japans lieferte Miquel, (Prol. Fl. Jap. 1865-1867), nach ihm Franchet et Savatier (Enum. Plantarum Japonicarum). Maximowicz, der lange Jahre' über die Flora Ostasiens sehr werthvolle Studien gemacht hatte, schrieb im Jahre 1880, (Mélanges Biologiques), über die Tiliaarten Japans eine Verbesserung, hauptsächlich über die T. Mandschurica Miq. Obwohl unsere Tilia-arten durch Maximowicz's Arbeit zu Klarheit gelangt sind, liegen doch, veranlasst durch das mangelhafte Material in den Händen der Fremden, mehr oder weniger Lücken vor, die für die Wissenschaft von Bedeutung sind.

Diesen Mangel zu ergänzen habe ich mich seit einigen Jahren bestrebt, genügendes Material zu sammeln, und da jetzt alles nötige vorhanden ist, um auch über die Gebiete, in welchen die obigen Arten vorkommen, klar zu werden, habe ich die vorliegende Arbeit ausgeführt. Endlich fühle ich mich verpflichtet meinen wärmsten und herzlichsten Dank Herrn Prof.

* Plantae Chinensis.

Rigakuhakushi, J. Matsumura und auch Herrn T. Makino auszusprechen, welche mir ihre freundliche und gütige Hülfe bei der voliegenden Arbeit hatten zu Theil werden lassen.

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De Candolle,	Prodromus. I.
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„	Mélanges Biologiques, 2.
	„ „ 10.
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Franchet,	Plantae Davidianae.
Ledebour u. Plant,	Flora Rossica. etc.

植物名實圖考
大和本草
草木圖說
古名錄
秘傳花鏡
閩書南產志 等

I. Die Beschreibung der einzelnen Arten.

GRUPPE A.

TILIA KIUSIANA MAKINO ET SHIRASAWA, NOV. SP.

NOM. JAP. HERANOKI, 大和本草巻之十二. 四十頁

(Taf. XVII. Fig. 1).

Blütenknospen eipyramidenförmig, abgestumpft, an der Basis abgestutzt. Blüten hängend, schwach duftend, 4½-5½ mm lang, 5-6 mm im Durchmesser, Kelch fünfblätterig, Kelchblätter eilanzettförmig, allmählich zugespitzt, ganzrandig, circa 3½ mm lang, nach innen gewölbt, am Grunde der Innenseite dicht, doch oben am Rande und aussen dünn behaart; Blumenblätter 5, an den Kelchblättern abwechselnd stehend, länger als dieselben, circa 4½ mm lang, eilanzettförmig, zugespitzt, kahl, weissgelblich gefärbt; Staubgefässe 15-20, an den Staminodien verwachsen, fünf in Bündeln, kürzer als die Staminodien, ungleich lang; Staminodien fünf, kürzer als die Blumenblätter, länglich, gekielt, gestumpft, an des Basis verschmälert, weissgelblich; Fruchtknoten fast kugelig, 1½ mm Durchmesser, behaart, fünffächerig, in jedem Fache mit zwei centralständigen Samenknospen; Griffel 1, cylinderisch, gerade, kahl; doppelt so lang als der Fruchtknoten, Narbe mit 5 Vertiefungen; Staubfäden bandförming, kahl—; Staubbeutel 2, getrennt, der eine verlängert, umgekehrt-eiförmig, der andere schmal-eiförmig, oft beide zusammengesetzt; Frucht nüsschenartig, kugelig, fünf mm Durchmesser, graubraun behaart, am Grunde undeutlich fünfrippig, kurz geschnabelt, meist einsamig; Blütenstand mit einem Flügelblatt versehen, trägt 20-36 Blüten; Blütenstiel meist widerholt dreigabelig, Flügelblätter länglich, 5-7 cm lang, 0,8—1.2 cm breit, häufig rückwärts gewendet, hellbraun gelblich, mit unregelmässig verbreiteten Nerven anastomosiert. Gemeinschaftlicher Blütenstiel 1-1½ cm lang, und Blütenstielchen ½-2 cm lang.

Knospen wechselständig, sitzend, eiförmig, etwas flach zugespitzt, kahl, die aüsseren zwei Schuppen sichtbar, im Winter udnkelröthlich.

Blätter dünn, papierartig, länglich, eiförmig, zugespitzt, am Grunde unsymetrisch schiefherzförmig oder abgestutzt, einfach unregelmässig, kurz kerbgesägt, oberseits dunkelgrün, unterseits hellgrünlich, auf der Unterseite meist sechs Nerven auf einer Seite der Ader sichtbar erhaben, Blattstiel braun, dünn behaart.

Die Rinde ist in der Jugend graubraun, glatt, weisslich gefleckt, bildet später eine Borke, langrissig und löst sich in kleinschuppigen Blättern ab.

Das Holz ist gelblichweiss, Kern und Splint ziemlich verschieden gefärbt, weich, leicht spaltbar, und die Gefässe sind im ganzen Jahrringe gleichmässig vertheilt. Das specifische Gewicht im Lufttrockenzustande ist 0,44, das Absoluttrockengewicht 0,36.

Es eignet sich nicht gut zur Feuerung und hat bis jezt keine andere Nutzverwendung gefunden, als zu hölzernen Schuhen (Geta) und Züudhölzern verarbeitet zu werden. Der Stamm ist geradschaftig mit kurzkegelförmiger Krone, erreicht 60 cm Durchmesser und 18 m Höhe; man findet aber grosse Bäume nur selten in einzelnen Orten, da sie meist schon in der Jugend abgetrieben worden.

Zur Bastfaserbereitung wird das 15-20 zährige Holz im Walde alljährlich abgefällt, es liefert bei langer Dauer der Mutterstöcke immer mehr reichliche und kräftigere Ausschläge, so kommen oft 10-15 am einem Stocke vor. Mitte Mai schabt man die Rinde von den 15-20 cm im Durchmesser und 7 m hohen Stämmen ab, legt sie zwei Wochen in Teichwasser, wäscht sie dann mit frischem Wasser bis die Bastfaser fast gereinigt ist. Die Faser ist sehr zähe und wird zur Mattenweberei, sowie zu Flechtwerkmaterial und Binden verwendet.

Dieser Baum findet sich nur auf der Kiusiu-insel (woher der Name T. Kiusiana stammt), dem südlichen Theil Japans und besonders in dem Grenzgebirge der Provinz Hiuga, Bungo, Buzen, Chikugo und Higo von 100 m bis auf 600 m über der Meeresfläche, wo er vermischt mit anderen winterkahlen Laubhölzern, am häufigsten mit Carpinus laxifera Bl., Quercus glandulifera Bl., Quercus serrata Thunb. und Castanea vulgaris Lam. var. japonica DC. vorkommt, hie und da wohl auch fast reine Bestände von geringerer Ausdehnung bildet. Er eignet

sich mehr für südliche als für nördliche Exposition, zieht aber im Allgemeinen tiefgründige, fruchtbare Berghänge vor.

Seit einigen Jahren habe ich mich bemüht die wissenschaftlichen Untersuchungsmerkmale zwischen dieser Holzart und Tilia cordata Mill. var japonica Miq. festzustellen. Im Jahre 1897 kam, nach den Anweisungen der Herrn Yoshio Tanaka, glücklicherweise das nötige Material für diese Arbeit in meine Hände. In Übereinstimmung mit meinem Collegen Herrn T. Makino nannten wir die Art T. Kiusiana, jedoch erst auf meiner diesjährigen Reise nach Kiusiu habe ich vollständige Klarheit über Standort, Verbreitung, etc. erlangt.

Tilia cordata Mill. dict. n. 1, var. Japonica Miquel, Prol. Fl. Jap. 207; Fr. et Sav., Enum. Pl. Jap. 1. P. 66.

Nom. Jap. Shinanoki.

Blüten 20-40 in einem Stande, hängend schwach duftend. Blütenknospen kurzeiförmig ; Kelchblätter und Blumenblätter 5, die ersteren ovallanzettförmig, zugespitzt, nach innen gewölbt, 3-4 mm breit, 5-6 mm lang, am Grunde filzig, am Rande dünn und an der Rückseite kleinwarzig behaart, die letzteren circa 4 mm breit, 7 mm lang, an der Basis verschmälert, gelblich; Staubgefässe kürzer als die Staminodien, 25-30, je 5-6 in Bündeln vereinigt, ungleich lang, Staubbeutel 2, meist getrennt, flach elliptisch, auf fast gleicher Höhe stehend, Staminodien verlängert, linealisch, haben ungefähr dieselbe Länge wie die Blumenblätter, Fruchtknoten kugelig, länglich 2 mm Durchmesser, behaart, fünf-fächerig. Griffel 6 mm lang, behaart. Frucht oval oder umgekehrt eiförmig, 5 mm Durchmesser, graubraun behaart, kurz geschnabelt, ohne Rippen; Schale zerbrechlich, Flügelblätter gestielt, circa 5-8 cm lang, 1-1½ cm breit, Blütenstiel 3½ cm lang, Blütenstielchen ½-1 cm lang, kahl.

Blätter mit 2-4 cm, dünnbehaarten Stielen, papierartig, ei- oder schiefherzförmig, plötzlich in eine längere schmale Spitze ausgehend, circa 3-6 cm breit, 4-7 cm lang, spitz gesägt, kahl, doch in den Winkeln der Adern und der von Grunde austrahlenden Hauptnerven dicht braun behaart, oberseits dunkelgelblichgrün, unterseits hellblaugrün.

Knospen eiförmig, zugespitzt, im Winter dunkelröthlich und

die einjährigen Zweige braunröthlich. Rinde dunkelgraubraun, Borke lang gerippt, Bastfaser sehr zähe.

Holz leicht, weich, Splint mit 7-9 Jahrringen, hellgelblich, mit den im ganzen Jahrringe fast gleichmässig zerstreuten Gefässen; in frischem Zustande hat es ein specifisches Gewicht von 50, im lufttrockenen ein Gewicht von 40 und absolut-trockenen Zustande von 33.

Diese in Japan meist gemeine Lindenart ist als Waldbaum fast in allen Provinzen verbreitet, so südlich von den Kiusiuinseln bis nördlich nach Hokkaidō, und zwar in dem südlichen Theil der Hauptinsel (Honshu) in der Höhe von 350-2500 m, im Norden von Honshū in 250-1800 m und in Hokkaidō über 100 m.

Sie verlangt mehr frischen, mässig feuchten Boden in Niederungen, Thälern oder geschützten Lagen wie seichtgründig trockenen Boden an Berghängen, und erreicht häufig eine Höhe von 20 m.

Bei der kräftigen Stockausschlagfähigkeit treibt sie zahlreiche Stämme auf einem Stocke.

Das Holz ist zur Verfertigung von Möbeln, besonders jedoch zur Bastfaserbereitung zu Seilen und Weberei geeignet. Es wird aber unter Umständen auch nur als Brennmaterial benutzt.

Bemerkung: C. S. Sargent's Forest Flora of Japan p. 20.—This (T. cordata var Japonica) is the only Linden cultivated by the Japanese, who occasionally plant it in temple gardens, especially in the interior and mountainous part of the Empire.—Es scheint mir, daher er diese Holzart mit T. Miqueliana Max. verwechselt hat.

GRUPPE B.

TILIA MAXIMOWICZIANA MIQU.

SYN. T. MIQUELIANA FOLIIS ROTUNDIORIBUS MAX., IM BRIEFE AN PROF. DR. K. MIYABE (1887)., MATSUMURA'S SHOKUBUTZUMEII P. 294. N. 3121.

NOM. JAP. ŌBABODAIJU.

(Taf. XVIII. fig. 2)

Hinsichtlich seines Habitus hat diese Art die grössten Organe (Blätter, Blüten, etc.) unter den japanischen Tilia-Arten. Blüten hängend, 10-18 an einem gemeinschaftlichen Stiele, stark duftend, circa 10-11 mm Durchmesser, 10 mm lang; Kelchblätter

5, innen gewölbt, rautenförmig, zugespitzt, an der Basis etwas breit, 9 mm lang, 5 mm breit, dick, gelblichgrün, aussen und innen behaart, am Grunde der Innenseite mit weisslich glänzender dichter Behaarung, Kronenblätter 5, rinnenförmig, gekielt, dünn, 10 mm lang, 4 mm breit, kahl, hellgelblich, Staubgefässe 65-75, ungleich lang und undeutlich in fünf Phalangen vereinigt, kürzer als die Kronenblätter; Staubfäden zweiästig, Staubbeutel verschmälert ovalförmig, und sehr verschieden in der Grösse, Staminodien fünf, an der Spitze deutlich gezähnt, stark gekielt, gleich lang mit den Kronenblättern, doch schmäler als dieselben, Fruchtknoten rundlich eiförmig, 4 mm Durchmesser, fünffächerig, in jedem Fache zweisamig, Griffel cylinderisch, kahl, zur Blütezeit 5 mm lang und später weit verlängert bis 9 mm. Narbe mit fünf Furchen, Flügelblätter sitzend, beiderseits grau, dünn behaart, zur Blütezeit hellgrünlich, circa 1½-2 cm breit 7-10 cm lang, Pedunculus .3-5 cm lang, Pedicelle 1-1½ cm lang, behaart.

Frucht rundlich ovalförmig, circa 8 mm Durchmesser, mit holziger, dicker, und grau behaarter, mehr oder weniger deutlich fünfrippiger Schale.

Blätter dick, hautartig, rundlichherzförmig, einfach, ziemlich gleichmässig, 9-12 cm breit und lang, mit 3-5 cm langen Stielen, oberseits dunkelgrün, unterseits grau behaart. Die Blätter an den fruchtbaren Zweigen und bei jungen Ausschlägen sind viel grösser, fast 25 cm breit und lang.

Die jungen Zweige, Blattstiele und Blattnerven graubraun filzig behaart. Rinde dick, dunkelvioletgrau, lange Jahre glatt, Borke weisslichgrau, seicht, langrippig, Bastfaser nicht so stark wie die der vorigen Arten. Holz weich, leicht spaltbar, Kern braunlich gelblich weiss, Splint mehr hell gefärbt; specifisches Gewicht in frischem Zustande ist 0,52, in lufttrockenem 5,35 und in absolut trockenem 5,31.

Das Gebiet, in dem diese Art vorkommt, ist Mitteljapan nördlich bis Hokkaido. Ich habe vor einigen Jahren diesen Baum in Thal des Tone-Flusses in der Provinz Kōtsuke, und am Fusse des Berges Hakkōda in der Provinz Mutsu, und auch in der Provinz Rikuchu gefunden, wo er in einer Höhe von 250-500 m über der Meeresfläche mit Alnus incana, Willd. var. glauca Ait. Populas tremula le. var. villosa Wesm. Quercus grosseserrata Bl. ets. einen gemischten Bestand bildet. Die weit ausgebreiteten

starken Aeste, die dicken unterseits weisslichgrau behaarten Blätter und die gelbgrünlichen grossen Flügelblätter lassen uns leicht sein Vorhandensein erkennen. Er liebt mehr tiefgründige Thäler als trockene Berghänge, wo er häufig 70 cm Durchmesser und 18 m Höhe erreicht. In Hokkaido, nach Prof. Dr. K. Miyabe und Prof. J. Matsumura, kommt er auf oben erwähnten Boden mit der voher erwähnten Tilia-Art (T. cordata var. japonica), mit Quercus grosseserrata, Bl. Cercidiphyllum japonicum S. et Z. Ulmus montana Sm. var. laciniata Trautv., Magnolia hypoleuca S. et Z. und Magnolia Kobus DC. vermischt vor, und erreicht 1 m Durchmesser.

Zur Verwendung des Holzes findet man keine charakteristischen schätzbaren Eigenschaften, und wird es nur als Brennmaterial benutzt.

Aus der Rinde wird Bastfaser für Seilenmaterial bereitet, sie ist aber minder zähe als dieselbe der vorigen Arten.

C. S. Sargent beschrieb diese Holzart unter dem Namen T. Miqueliana, Forest Flora of Japan p. 19. (1894).

TILIA MIQUELIANA MAXIM, MÉL BIOL. X. p. 584.
SYN. T. MANDSCHURICA MIQ. PROL. 206;
FR. ET SAV. ENUM. PL. JAP. I. p. 67.,
NON RUPR. ET MAXIM.
NOM. JAP. BODAIJU.

Blüten stark duftend, 10-22 auf einem regelmässig wiederholt dreigabeligen Stande, 8 mm Durchmesser und 8 mm in Höhe, Blütenknospen halboval, am Grunde fünf, tief gefurcht, Kelchblätter 5, oben gelblichgrün, unten in der Basis grün, 7 mm lang 3 mm breit, am Rande und Grunde fein behaart, Kronenblätter 5-zählig, länger als die Kelchblätter, hellgelblich, Staubgefässe 60-75, zu fünf Bündeln vereinigt, an der Spitze zweiästig und fast gleichlang, Staubbeutel ovalförmig in der Mitte ziemlich verschmälert, Staminodien schmäler und kürzer als Kronenblätter, Furchtknoten halbelliptisch, fünf-fächerig, fünffach gefurcht, und rippig, Griffel cylindrisch, zur Blütezeit kürzer als Staubgefässe und weiter nach vorne hervortretend, Narbe mehr oder weniger tief fünfrissig, Flügelblätter gestielt, 2 cm breit, 5-9 cm lang, gelblichgrün, Blütenstiele circa $4\frac{1}{2}$ cm läng, Stielchen circa 1 cm lang, grauweisslich, weich-haarig.

Frucht fast kugelig, ziemlich lang, 8 mm Durchmesser, dünn behaart, mit 5 Rippen, die Schale sehr hart.

Blätter dicklich, breit deltaförmig, zugespitzt, am Grunde unsymmetrisch, herzförmig oder abgestutzt, fast gleichmässig gezähnt, 6 cm breit, und gleich lang, auf der oberen Seite glänzend dunkelgrün, auf der unteren Seite grau, Blattstiel und einjährige Zweige kleinwarzig behaart. Blätter an den jungen Ausschlägen auffallend gross, fast 15 cm breit und 22 cm lang.

Knospen kugelig, zugespitzt, etwas flach, im Winter gelblichgrün gefärbt. Junge Zweige grün, ältere grau, Borke lang gerippt.

Die Bastfaser hat weniger Zähigkeit als die der vorigen Gruppe.

Holz weich, Kern hellbraungelblich weiss, Splint gelbweiss, die Gefäss englumig, spec. Lufttrocken-Gewicht 0,63 und Absoluttrocken-Gewicht 0,54. Rinde dunkelvioletgrau, lange Zeit glatt, später treten Längsrisse auf.

Diese erst nach Miquel im Jahre 1867 als in Japan einheimisch beschriebene Holzart, welche überall in Mitteljapan verbreitet ist, wächst sehr üppig, und erreicht 12 m Höhe mit locker austretenden Aesten. Besonders in den Tempelhainen des Buddha wird sie angepflanzt, wo sie als ein heiliger Baum betrachtet wird. Sie ist in Japan unter dem Namen Bodaiju (菩提樹 Ficus religiosa) bekannt, ist jedoch eine andere Art als die gleichnamige, welche in Indien vorkommt.

Hinsichtlich ihres Habitus, Wachstums und ihres häufigen Vorkommens, schien es Miquel eine japanische Art zu sein, die jedoch nicht von Anfang an in Japan einheimisch war, sondern erst vor 710 Jahren durch einen buddhistischen Priester aus China eingeführt wurde.

II. Gruppierung der japanischen Tilia-arten.

Für eine Gattung, welche zahlreiche Arten umfasst, ist es notwendig die Untergattungen oder Sectionen festzustellen, welche alle untereinander ähnliche und in allen wesentlichen Merkmalen übereinstimmende Arten einschliessen. Die Gattung Tilia, die schon beschrieben worden ist, umfasst mehr als

15 Arten, welche nur in der nördlichen Hemisphäre vorkommen.

In Bezug auf ihre Blütenbildung ist das Vorhandensein oder Nichtvorhandensein der Staminodien ein entscheidendes Merkmal, wodurch sie in zwei Untergattungen oder Sectionen eingetheilt werden kann.

Alle japanischen Tilia-arten weisen jedoch Staminodien auf, also bilden diese für uns kein Unterscheidungsmerkmal. Obwohl die japanischen Tilia-arten in der Entwickelung der Blätterbildungen 4 selbständige Arten darstellen, glaube ich doch, das sie in directer Verwandschaft mit denselben Arten des Continentes Ostasiens stehen. Desshalb habe ich in meiner Arbeit unsere Tilia-Arten in zwei Gruppen classificiert, deren typische Formen *T. cordata* und *T. Mandschurica* auch im Continente vorkommen. In nachfolgendem sind die wesentlich characteristischen Eigenschaften der beiden Gruppen aufgeführt.

GRUPPE A.

(T. cordata)

Blüten

Klein, schwach duftend, zahlreich, 20-40 in einem Stande.

Blumenblätter ungekielt, die Spitze derselben und Staminodien fast ganzrandig.

Staubgefässe 15-30; Staubbeutel elliptisch.

Früchte

Fruchtschale dünn, zerbrechlich, fast rippenlos, geschnabelt.

Knospen

Dunkelröthlich, kahl.

Blätter

Dünn, papierartig, schmal, kahl, hellgefärbt.

Zweige

Schlank, dunkelröthlich, kahl.

GRUPPE B.

(T. Mandschurica)

Blüten

Gross, stark duftend, wenig, 10-20 in einem Stande.

Blumenblätter

Gekielt, die Spitze derselben und Staminodien gezähnt.

Staubgefässe 60-75, Staubbeutel länglich oval.

Frucht

Fruchtschale stark, mit schwachen Rippen, nicht geschnabelt.

Knospen

Grün, behaart.

Blätter

Dick, hautartig, breit, dicht behaart, dunkelgefärbt.

Zweige

Dick, grünlich, behaart.

Zur ersten Gruppe gehören Tilia cordata var. japonica und Tilia Kiusiana, und die zweite Gruppe umfasst Tilia Maximowicziana und Tilia Miqueliana.

III. Das Verbreitungsgebiet der beiden Gruppen.

1. DIE TILIA CORDATA GRUPPE.

Die typische Art diese Gruppe T. cordata ist in ganz Europa, namentlich in Mittel-und Nordeuropa, und im Ural, Kaukasischen Gebirge vorhanden; in Sibirien tritt sie, nach Maximowicz, zuerst im südlichen Theil des nördlichen Amur auf und kommt anfangs nur auf Flachland in lichten Gehölzern in der Gesellschaft von Populus tremula vor, ist an dem mittleren Amur sehr häufig an den Rändern der Laubwälder anzutreffen, und scheint hier eine grosse Entwicklung zu erreichen, ist jedoch auch am unteren Amur und längs der Ussuri ein gewöhnlicher Waldbaum. Von hier aus zieht sich die Art an der Küste von Korea entlang nach Südosten bis sie endlich Japan erreicht, wo sie in einer Form der Varietät (var. japonica) auftritt, und fast in ganzem Lande verbreitet ist. Hier nimmt sie eine Veränderung des Habitus an, und zwar ist die Entwickelungsgeschichte der Staminodien in ihren Blüten sehr interessant. In Europa, sowie in Sibirien entwickeln ihre Blüten keine Staminodien, während sie in Korea nur selten, doch in Japan ganz vollständig ausgebildet sind. Es muss jedoch erwähnt werden, dass die Staminodienbildungen der T. cordata var. japonica

und T. Kiusiana in gewisser Beziehung zu einander stehen. T. Kiusiana entwickelt zwar breitere und vollständigere Staminodien als T. cordata var. japonica, und die Zahl der Staubgefässe ist bedeutend kleiner, jedoch scheint es mir, dass T. cordata var. japonica vielleicht ein Verbindungsglied zwischen der in Sibirien und Europa vorkommenden Art (T. cordata) und der T. Kiusiana sein dürfte; denn es ist die Beobachtung gemacht worden, dass die Staminodienbildung dieser Tilia-Gruppe sich je mehr wir nach Osten gehen, immer mehr entwickelt, bis sie in T. Kiusiana, welche hier in Japan, in einem beschränkten Gebiete vorkommt, ihre höchste Entwickelung erreicht.

2. DIE TILIA MANDSCHURICA GRUPPE.

Tilia Mandschurica Maxim. stellt eine gute Representantin dieser Gruppe vor. Nach Miximowicz ist ihre Heimat Mandschuria, sowie das Amur-und Ussuri-gebiet und zwar wächst sie an Berghängen oder Waldrändern in dieser Gegend häufig mit mehreren Stämmen auf einem Stocke and mit weit ausgestreckten Aesten bis 40′ Höhe und 6″ Dicke.

Tilia Maximowicziana kommt in nördlichen Theil des Japanischen Reiches vor, besonders in Hokkaido wächst er höchst üppig. Die in der Blütenbildung der T. Maximowicziana sehr ähnliche T. Miqueliana, welche ich noch nicht in Freien vorkommenden Zustande beobachtet habe, wächst nach alten chinesischen Beschreibungen im ostlichen mittlerem Theile Chinas und erreicht eine grosse Höhe.

In Bezug hierauf scheint es mir, dass das eigentliche Gebiet dieser Gruppe auf die nördlich kühlere Region beschränkt sei, und dass die in Hokkaido vorkommende Art (T. Maxim.) eine Fortsetzung der des Continents (T. Mands.) darstellt.

Die Blätter der T. Maximowiczana haben eine grosse Aehnlichkeit mit denen der T. Mandschurica, und ihre Blüten und Früchte sind fast ebenso wie die der T. Miqueliana, also ist die T. Maximowicziana eine Zwischenform der beiden Arten, und so möchte ich sie im Gegensatz zu Maximowicz's Benennung, T. Miqueliana Max. fol. volundioribus, T. Maximowicziana benennen.

Tokio, Sept. 1899.

Figuren-erklärung.

TAF. XVII.

(TILIA KIUSIANA.)

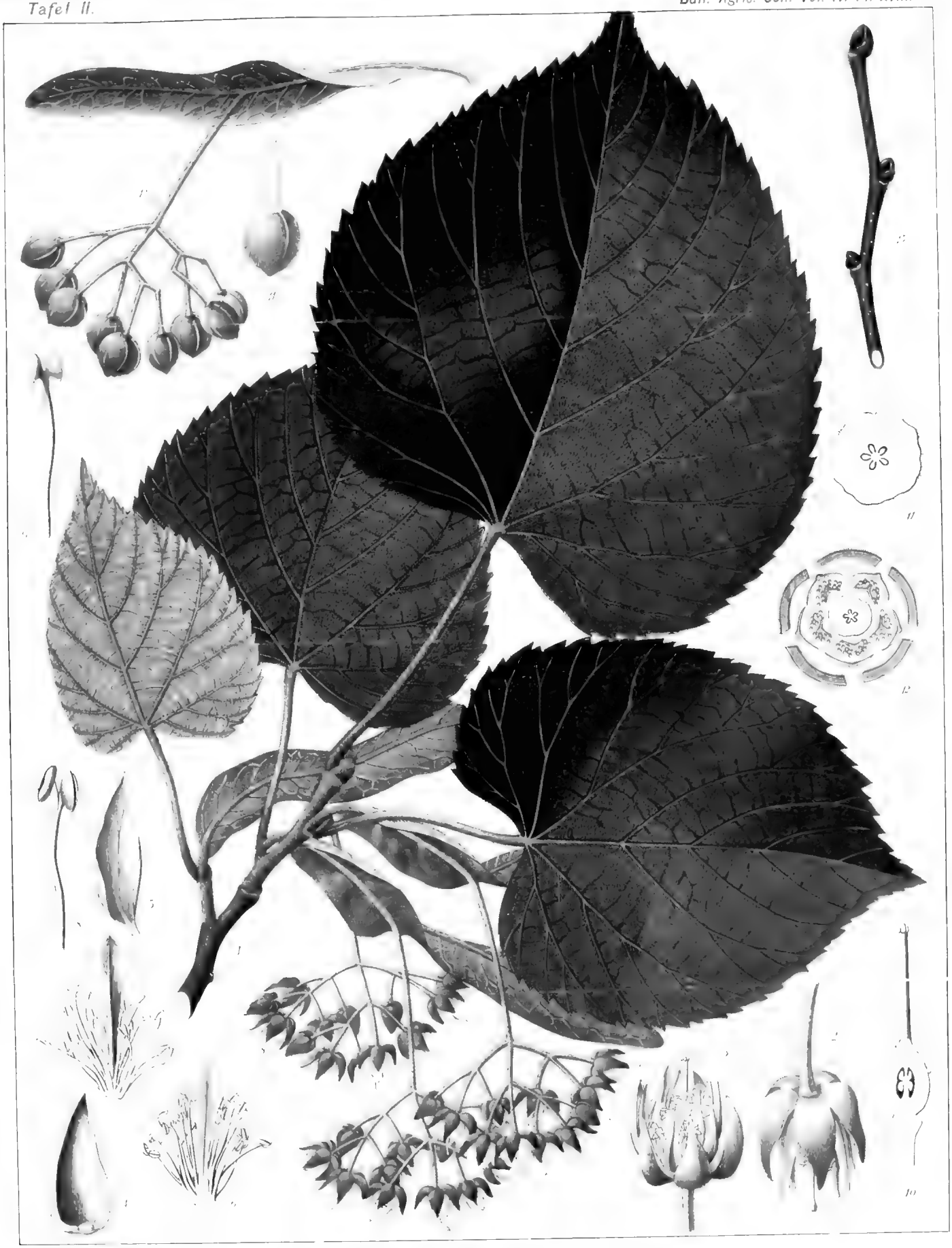
Fig. 1. Zweige mit Blütenständen in der natür. Gr.
„ 2. Blütenknospe.
„ 3, 4. Aufblühende Blüten.
„ 5. Kelchblatt von innen gesehen.
„ 6. Kronenblatt.
„ 7. Staminodus von innen gesehen.
„ 8. Derselbe von aussen gesehen.
„ 9, 10. Staubgefässe.
„ 11. Längsschnitt des Fruchtknotens.
„ 12. Querschnitt desselben.
„ 13. Querschnitt der Blüte.
„ 14. Zweige mit reifen Früchten, im natür. Gr.
„ 15. Frucht, zweifach vergrössert.
„ 16. Längsschnitt der Frucht.
„ 17. 18. Zweige im Winterkahlzustande.

TAF. XVIII.

(T. Maximowicziana.)

Fig. 1. Zweige mit drei Blütenständen.
„ 2. 3. Aufblühende Blüten.
„ 4. Kelchblatt von innen gesehen.
„ 5. Kronenblatt von innen gesehen.
„ 6. Staminodie von innen gesehen.
„ 7. Dieselbe von aussen.
„ 8, 9. Staubgefässe.
„ 10. Querschnitt des Fruchtknotens.
„ 11. Längschnitt desselben.
„ 12. Querschnitt der Blüte.
„ 13. Früchte in einen Stande, natür. Gr.
„ 14. Frucht, doppelt vergrössert.
„ 15. Zweige im Winterzustande.

T. Yoshino et Shirasawa



Vitis Maximowiczjana Shirasawa

明治三十三年七月十七日印刷
明治三十三年七月二十日發行

編輯兼發行者　農科大學

印刷者　佐久間衡治
東京市牛込區市ヶ谷加賀町一丁目十二番地

印刷所　株式會社　秀英舍第一工場
東京市牛込區市ヶ谷加賀町一丁目十二番地

Report of Investigations on the Mulberry-Dwarf Troubles—a Disease widely spread in Japan.

BY

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INTRODUCTION.

Since more than ten years there has been observed a disease of the mulberry, known as "Ishikubyô or shikuyōbyo"* attacking the leaves and branches, and checking growth and finally causing the death of the whole plant. When this disease once appears in a farm it spreads gradually, and finally attacking the entire farm, causes an immense loss to silkworm raisers by destroying the crop of the mulberry almost entirely. And although several persons have been engaged in seeking for a method of combatting the disease, none has as yet been successful, the disease in the meantime becoming more and more rampant, and calling forth an earnest demand for remedy on the part of the interested farmers. At last the Japanese government took the matter in hand, and a bill was passed through the Diet providing an annual expenditure of several thousand yen after April, 1897 for an investigation of the question. A board of specialists was appointed, consisting of the following gentlemen besides the present writer: Prof. C. Sasaki, Mr. J. Omori, Mr. I. Honda, Mr. N. Ichikawa, Mr. H. Nomura. Since then we have inspected the actual condition of the attacked farms in various localities and have instituted a systematic investigation of the problem from different points of view on an experiment farm at Nishigahara (a suburb of Tokyo) of about 275,000 sq. ft. in extent. In the mean while Mr. Nomura withdrew from the board, and Mr. Ichikawa

* Here translated "Mulberry-Dwarf Troubles."

having left for Europe, Prof. M. Miyoshi and Mr. K. Fujii were appointed to fill the vacancies. Mr. Ichikawa having recently returned was again added to the board.

From the beginning the writer took up the chemical side of the question. And as a preliminary step, comparative chemical analyses of healthy and attacked plants were made to ascertain the differential character of the latter, and as the writer believes himself to have been fortunate enough to discover the cause which induces the disease, he has decided to publish the result in order that others engaged in seeking for a remedy may avail themselves of it. The present paper is reproduced with the permission of the board from a report originally presented to it, and contains only the results obtained by myself. It must, however, be stated that most of the field experiments cited in this paper were conducted by Mr. Ichikawa for several years, either alone or in conjunction with the other members of the board. I must also note my indebtedness to other members of the board for supplying me with informations regarding the parasites, by which my view has directly or indirectly been strengthened.

There is no means of ascertainning the first origin of the disease in question, but it is probably not older than twenty or thirty years. As stated before, the disease has become especially malignant since over ten years ; and up to the present time, it has been reported from nearly all parts of the country. The damage has been particularly great in Ōita, Fukuoka, Kumamoto, Tottori, Shimane, Aichi, Shizuoka, Yamanashi, Tokyo, Saitama, Fukushima, and Gumma, and in some localities entire farms have been attacked by the disease, and the owners have been compelled to give up the cultivation of the mulberry. Localities where silkworm culture has recently come into practice appear to have a special propensity to the disease, and the newly started industry is very apt to receive a serious check from it. The entire annual damage for the whole country would doubtless amount to several millions of yen ; no wonder that the public has been awakened to it.

The different varieties of the mulberry are unequally liable to the disease. Generally speaking, those varieties which are esteemed for the quality of the leaves, large crop, and rapid growth have a greater propensity to the disease, while those which are charactarized by hard leaves, less sap, and slow

growth are comparatively free from it. Such varieties as Takasuke, Tsuruta, Hosoye, and Ichihei belong to the first category, Takasuke taking the lead, while Jūmonji and Rosô belong to the second. Moreover, the capacity for resistance towards the disease is subject to variation within the same variety, according to the treatment under which a plant has been brought up: plants that have deen induced to excessive growth with abundant soluble manures being more liable to attack, while those that have grown on lean soil among mountains or have not been manured are free from it. The quality of the soil also has a part in it, the disease appearing more commonly in those soils which are specially adapted for the growth of the mulberry. There is hardly an exception to this almost universal rule.

Before proceeding to the details of the investigation, a few words must be devoted to the manner in which the mulberry crop is gathered. In former times the plants were entirely left to their natural growth; but towards the end of the sixties the so-called cutting method was started and has now generally come in vogue. This consists in cutting the stem or shoots near the level of the ground in about the third year of the life of the plant, and letting new shoots come out from the stump. The season for cutting is usually from the earlier or later part of May to the earlier part of June, that is to say, at the time of the fullest development of the leaves. By August or September the new shoots reach the height of five or six feet, and these are again cut down in May of the next year. This way of treatment secures a large crop and lessens the injury from insects and fungi. Portability is also an advantage, though secondary.

There are several ways of cutting, but they may be reduced to three, which may be designated "low cutting," "medium cutting," and "high cutting." In the first, the stems are cut down immediately above the level of the ground, and in some cases the stumps are even covered with earth; in the second, the stumps are left about one foot high, while in the third, they are five to six feet. The relative advantage of these different ways of cutting depends upon local conditions, but it is a noteworthy fact that cases of the disease are especially numerous in plants subjected to low and medium cutting, while those subjected to high cutting are rarely attacked. Moreover, in those

localities where, owing to deep snow and economical circumstances, the old custom of leaving the plants to their natural growth is still adhered to, the disease is entirely unknown. This fact deserves especial emphasis, as having a close bearing on the true cause of the disease. Further, it deserves mention here that although if the shoots are cut in spring before the expansion of the leaves or in autumn after the fall of the leaves, the plants remain perfectly healthy, yet if the cutting takes place in the period of luxuriant growth, and especially in the so-called "late cutting" (i.e. in the latter part of July or in August), they are particularly liable to attack.

The first sign of the disease usually appears on new shoots springing from the stump. Namely, when these have reached the height of one foot or so the upper leaves either begin to shrivel or manifest other signs of debility, and as the shoots continue to lengthen, all the new leaves developed from them betray the same character. The diseased leaves may turn yellowish or remain dirty green, or may be indistinguishable in color from the healthy leaves, merely shrivelling up. In acute cases the leaves may all shrivel up in one year, but more usually only a few leaves near the top of the shoots first betray their debility, whence the disease spreads with each successive cutting, until in the course of a few years the entire plant is attacked or even dies off. The branches of the attacked plants remain usually slender, and the twigs and leaves are very numerous. Sometimes also, the branches lose their strength and become procumbent. In fact the diagnosis is somewhat variable, sometimes consisting in the paucity of the sap, as if the plants were grown in dry soils, or sometimes in the arrest of growth, as if the plants were still young; but the unfailing signs are the imperfect development and shrivelling of the leaves and the slenderness and dwarfed condition of the branches. Moreover, when a plant is once attacked recovery is possible only after two or three years of complete protection from the cutting.

Parasites have been hunted for in the leaves and branches of the diseased plants, but none have been found that can be regarded as having a causal connection with the disease. The roots of the diseased plants are usually in a decayed condition, and in more advanced cases even roots of a finger's thickness are completely rotten; and hence this decay of the roots has been

pointed out by some as the cause of the disease, and ascribed to mirco-organisms. But as observations were extended it became apparent that this decay of the roots is not the cause of the disease, but only a secondary phenomenon, due to want of nourishment; and we see that in the first stages of the affection, the roots do not show any perceptible difference from those of healthy plants, and only when the disease becomes more advanced do the roots decay. If the decay of the roots be the cause of the disease and due to micro-organisms, then it is hard to understand why the disease is almost confined to plants subjected to cutting, while those that are left to their natural growth are entirely free from it. The explanation appears to me to be that the decay of the roots is a result of the disease just as the imperfect growth of the leaves, and that both are effects of some underlying cause.

I shall not give a detailed diagnosis of the disease, and the reader is referred to the accompanying photographic reproductions. Numerous figures are also given in the chapter on the comparative analyses of healthy and affected plants, which have been made particularly exact and are thought sufficient to bring out the characteristics of the disease.

Previous to the appointment of the board before mentioned, several persons have been engaged on the same problem, but it is thought unnecessary to detail the results obtained by them. We must, however, acknowledge our indebtedness to the results obtained by Messrs. Ichikawa and Hori in the Agricultural Experiment Station of Aichi Prefecture, which are largely cited in the present paper. An extended inspection in different localities has convinced me that the present disease is confined to those plants which have been subjected to cutting; hence I was led to inquire into the effect of cutting on the plant. I found that during the growing period the reserve materials in the barks of the roots and stems are all transported to the growing parts and reach the minimum, and therefore if the plants are cut down in this period the new shoots would be forced to depend on an exceedingly small quantity of reserve materials. Hence the leaves would be only imperfectly developed, and the reserve materials would be exhausted before the plants can absorb and assimilate adequate nourishment from the soil and the atmosphere. Hence I concluded that the primary cause of the disease is to be sought in the practice now in vogue of subjecting the mulberry to re-

peated cuttings. This view has been tested by numerous experiments, and no doubt remains in my mind of its correctness. Moreover, experiments have shown that the same disease can also be induced by excessive plucking of the leaves, which would evidently have the same physiological effect as repeated cuttings of the stem. There is therefore no doubt that the present disease is caused by nothing else but the deficiency of reserve material. This, I believe, is proved by the analyses and experiments detailed in the following pages.

Chapter I. Chemical Composition of Healthy and Diseased Plants.

It is the object of this chapter to compare the chemical composition of healthy and diseased plants and to find out the characteristic differences between them. The samples used for analysis were all taken from the mulberry farm of the Tokyo Sericultural Experiment Station at Nishigahara.

1. TSURUTA. MAY 6, 1898.

	Healthy.	Diseased.
Number of stems	11	61
Number of leaves	627	1880
Average length of stem	96.1 cm.	45.
Total fresh weight of stems	656.6 g.	919.0.
Average fresh weight of 1 stem	59.7	15.1.
Total fresh weight of leaves	143.25	122.50.
Average fresh weight of 1 leaf	0.229	0.065.
Total dry weight of leaves	25.90	25.83.
Average dry weight of 1 leaf	0.041	0.04.
Moisture in fresh leaves	81.20	78.14.
Dry matter in fresh leaves	18.80	21.86.
Number of leaves on 1 stem	57.	31.
Dry matter in leaves of 1 stem	2.355	0.423.

In 100 parts of dry matter,

	Healthy leaves.	Diseased leaves.
Crude proteids	47.94	34.56
Crude fats	1.92	3.10

	Healthy leaves.	Diseased leaves.
Crude fibres Nitrogen-free extracts.	41.27	54.20
Crude ash (free from carbon and sand)	7.87	8.14
Total nitrogen.	**7.67**	**5.53**
Albuminoid nitrogen	**5.15**	**4.07**
Non-albuminoid nitrogen	**2.52**	**1.46**

In 100 parts of ash (free from carbon and sand),

	Healthy leaves.	Diseased leaves.
SiO_2	3.71	3.37
SO_3	6.23	3.85
P_2O_5	14.34	13.55
K_2O	35.20	30.53
CaO	15.20	18.90
MgO	8.22	10.46

The above results show that the diseased leaves contain less nitrogen but more fats and nitrogen-free extracts. In the ash ingredients, we find less sulphates and potash but more lime and magnesia in the diseased leaves.

2. TSURUTA OCTOBER 15, 1897.

	Healthy.	Diseased.
Number of leaves	100.	500.
Total fresh weight of leaves	322.	181.
Average fresh weight of 1 leaf	3.22	0.36
Total dry weight of leaves	96.35	67.68
Average dry weight of 1 leaf	0.96	0.135
Moisture in fresh leaves	70.00	62.6
Dry matter in fresh leaves	30.00	37.4

In 100 parts of dry matter,

	Healthy leaves.	Diseased leaves.
Crude proteids	26.88	23.13
Crude fats	5.80	4.40
Crude fibres	**8.74**	**6.70**
Nitrogen-free extracts	51.23	57.71
Crude ash (free from carbon and sand)	7.35	8.06

	Healthy leaves.	Diseased leaves.
Total nitrogen	**4.30**	**3.70**
Albuminoid nitrogen	**3.60**	**3.00**
Non-albuminoid nitrogen	**0.70**	**0.70**

In 100 parts of ash (free from carbon and sand),

	Healthy leaves.	Diseased leaves.
SiO_2	1.68	1.85
SO_3	2.32	2.64
P_2O_5	9.44	7.52
K_2O	27.40	14.10
CaO	22.10	38.60
MgO	9.60	12.20

In this case also the diseased leaves are exceedingly poor in nitrogen and fibres, and the ash contains less potash and phosphoric acid but more lime and magnesia.

3. HOSOYE. MAY 6, 1898.

	Healthy.	Diseased,
Number of stems	6	60.
Number of leaves	1345	3025.
Average length of 1 stem	195 cm.	82.5
Total fresh weight of stems	1231	1131.
Average fresh weight of 1 stem	205.0	18.85
Total fresh weight of leaves	1598.0	147.0
Average fresh weight of 1 leaf	0.111	0.049
Total dry weight of leaves	32.224	31.149
Average dry weight of 1 leaf	0.024	0.010
Moisture in fresh leaves	79.84	78.81
Dry matter in fresh leaves	20.16	21.19
Number of leaves on 1 stem	22.4	50.
Dry matter of leaves on 1 stem	5.371	0.519

In 100 parts of dry matter,

	Healthy leaves.	Diseased leaves.
Crude proteids	41.00	38.00
Crude fats	2.26	1.51
Crude fibres Nitrogen-free extracts	49.18	52.18

	Healthy leaves.	Diseased leaves.
Crude ash (free from carbon and sand)	7.56	8.16
Total nitrogen	**6.56**	**6.08**
Albuminoid nitrogen	**4.08**	**3.94**
Non-albuminoid nitrogen	**2.48**	**2.14**

In 100 parts of ash (free from carbon and sand),

	Healthy leaves.	Diseased leaves.
SiO_2	1.07	2.50
SO_3	5.15	4.27
P_2O_5	12.68	14.99
K_2O	36.30	32.00
CaO	17.23	15.75
MgO	8.45	7.26

This result is essentially similar to the preceding; but the difference between the healthy and diseased leaves is not so remarkable, the analysed leaves being still very young and the disease not much advanced.

4. Hosoye. October 15, 1897

	Healthy	Diseased
Number of shoots (one stock)	28	73.
Number of leaves	236	930.
Average length of 1 shoot	39 cm.	33.
Total fresh weight of shoots	62	66.
Average fresh weight of 1 shoot	2.22	0.90
Total dry weight of shoots	7.84	9.87
Average dry weight of 1 shoot	0.280	0.135
Moisture in shoots	87.35	85.05
Dry matter in shoots	12.65	14.95
Total fresh weight of leaves	87.0	117.0
Average fresh weight of 1 leaf	0.304	0.126
Total dry weight of leaves	18.348	30.835
Average dry weight of 1 leaf	0.066	0.033
Moisture in fresh leaves	78.90	73.64
Dry matter in fresh leaves	21.10	26.36
Number of leaves on 1 shoot	10.	13.
Dry matter in leaves of 1 shoot	0.695	0.422

The plants were cut down in early summer and once more August 30. The new shoots had reached the height of 30–40 cm on October 15, when they were cut down and dried for analysis. The shoots coming from unhealthy plants already showed distinct signs of the disease.

In 100 parts of dry matter,

	Healthy.		Diseased.	
	Leaves.	Shoots.	Leaves.	Shoots.
Crude proteids	33.00	19.75	23.13	20.56
Crude fats	6.19	2.35	4.96	3.70
Crude fibres	10.53	65.40	7.26	66.84
Nitrogen-free extracts	39.52		56.83	
Crude ash (free from carbon and sand)	11.76	12.50	7.82	8.90
Total nitrogen	5.28	3.16	3.70	3.29
Albuminoid nitrogen	3.80	1.40	2.70	1.30
Non-albuminoid nitrogen	1.48	1.76	1.00	1.99

In 100 parts of ash (free from carbon and sand),

	Healthy.		Diseased.	
	Leaves.	Stems.	Leaves.	Stems.
SiO_2	4.87	1.02	4.00	1.90
SO_3	3.25	6.00	3.60	7.10
P_2O_5	8.56	8.10	11.80	10.90
K_2O	24.35	29.70	22.20	29.50
CaO	27.80	19.10	30.00	24.40
MgO	8.50	14.10	7.00	15.00

We see from the above tables that the diseased leaves are considerably poorer in nitrogen, fibres and ash. The diseased stems, on the contrary, are apparently rich in nitrogen; but this does not mean any absolute richness in nitrogen, because the development of fibres in the diseased stems was very bad, in consequence of which the persentage of nitrogen was much increased. If we compare the absolute quantities of nitrogen, then, of course, we should get a remarkable difference. We observe also a great difference between the ash contents of the healthy and diseased plants. These facts indicate that diseased

plants contained a far smaller quantity of reserve materials in the roots, and that the new shoots coming out after the cutting suffered from the deficiency of nutriment, and consequently became diseased.

5. YOTSUME. MAY 22, 1898.

	Healthy	Diseased.
Number of stems	4.	35.
Number of leaves	553.	1387.
Average length of stems	66. cm.	45.
Total fresh weight of stems	117.	210.
Average fresh weight of 1 stem	29.25	6.00
Total fresh weight of leaves	100.85	98,65
Average fresh weight of 1 leaf	0.181	0.071
Total dry weight of leaves	28.612	27.024
Average dry weight of 1 leaf	0.051	0.020
Moisture in fresh leaves	71.63	72.60
Dry matter in fresh leaves	28.37	27.40
Number of leaves on 1 stem	140.	40.
Dry matter in leaves of 1 stem	7.158	0.772

In 100 parts of dry matter,

	Healthy leaves.	Diseased leaves.
Crude proteids	28.75	27.94
Crude fats	3.83	3.45
Crude fibres Nitrogen-free extracts	59.66	60 07
Crude ash (free from carbon and sand)	7.76	8.54
Total nitrogen	**4.60**	**4.47**
Albuminoid nitrogen	**4.34**	**4.15**
Non-albuminoid nitrogen	**0.26**	**0.32**

In 100 parts of ash (free from carbon and sand),

	Healthy leaves	Diseased leaves
SiO_2	3.44	4.60

	Healthy leaves.	Diseased leaves.
SO_3	3.26	3.34
P_2O_5	13.86	13.14
K_2O	31.81	28.30
CaO	25.07	23.08
MgO	11.48	11.60

In this case the difference in nitrogen is not so remarkable, the leaves being too young.

6. TAKASUKE. MAY 22, 1898.

	Healthy.	Diseased.
Number of stem	3.	21.
Number of leaves	408.	941.
Average length of 1 stem	90.	45.
Total fresh weight of stems	150.	182.
Average fresh weight of 1 stem	50.00	8.70
Total fresh weight of leaves	154.5	132.35
Average fresh weight of 1 leaf	0.376	0.141
Total dry weight of leaves	35.261	42.321
Average dry weight of 1 leaf	0.111	0.045
Moisture in fresh leaves	70.50	68.00
Dry matter in fresh leaves	29.50	32.00
Number of leaves on 1 stem	136.	45.
Dry matter in leaves of 1 stem	15.087	2.015

In 100 parts of dry matter,

	Healthy leaves.	Diseased leaves.
Crude proteids	23.75	18.44
Crude fats	3.46	3.34
Crude fibres	**9.61**	**8.60**
Nitrogen-free extracts	56.84	63.49
Crude ash (free from carbon and sand)	6.34	6.13
Total nitrogen	**3.80**	**2.95**
Albuminoid nitrogen	**3.05**	**2.79**
Non-albuminoid nitrogen	**0.75**	**0.16**

In 100 parts of ash (free from carbon and sand),

	Healthy leaves.	Diseased leaves.
SiO_2	2.64	3.77
SO_3	3.64	4.00
P_2O_5	17.90	19.02
K_2O	28.51	29.43
CaO	23.64	23.00
MgO	10.44	12.82

The diseased leaves contain here a far smaller quantity of nitrogen and fibres than in the former case; no remarkable difference in the ash ingredients is to be observed.

7. TAKASUKE. JULY 9, 1898.

	Healthy.	Diseased.
Number of shoots (one stock)	51.	68.
Number of leaves	910.	1297.
Average length of 1 shoot	30.	18.
Total fresh weight of shoots	277.75	70.35
Average fresh weight of 1 shoot	5.446	2.035
Total dry weight of shoots	44.329	16.242
Average dry weight of 1 shoot	0.869	0.239
Moisture in shoots	84.04	78.00
Dry matter in shoots	15.96	22.00
Total fresh weight of leaves	355.20	142.71
Average fresh weight of 1 leaf	0.39	0.11
Total dry weight of leaves	82.72	44.023
Average dry weight of 1 leaf	0.099	0.034
Moisture in fresh leaves	76.70	69.15
Dry matter in fresh leaves	23.30	30.85
Number of leaves on 1 shoot	18.	19.
Dry matter in leaves of 1 shoot	1.622	0.647

In 100 parts of dry matter,

	Healthy.		Diseased.	
	Leaves.	Shoots.	Leaves.	Shoots.
Crude proteids	30.50	15.38	23.13	14.44
Crude facts	2.84	0.83	3.05	2.20

	Healthy.		Diseased.	
	Leaves.	Shoots.	Leaves.	Shoots.
Crude fibres	**11.14**	**46.30**	**8.03**	**40.14**
Nitrogen-free extracts	47.26	31.54	58.90	36.93
Ash (free from carbon and sand)	8.26	5.95	6.89	6.29
Total nitrogen	**4.88**	**2.46**	**3.70**	**2.31**
Albuminoid nitrogen	**3.85**	**1.10**	**3.35**	**1.20**
Non-albuminoid nitrogen	**1.03**	**1.36**	**0.36**	**1.11**

In 100 parts of ash (free from carbon and sand),

	Healthy.		Diseased.	
	Leaves.	Shoots.	Leaves.	Shoots.
SiO_2	4.68	1.19	4.83	2.90
SO_3	2.60	4.00	2.81	4.20
P_2O_5	14.60	14.60	11.90	18.87
K_2O	21.15	38.50	18.70	31.30
CaO	27.21	22.26	32.43	28.90
MgO	10.13	13.70	14.42	16.43

As the shoots were very young, being developed after cutting at the end of May they were especially well fitted for comparison. The diseased leaves were remarkably poor in nitrogen, fibres, and ash, and among the ash ingredients, there were much less phosphoric acid and potash, and much more lime and magnesia than in the healthy leaves. This result much resembles the case of Hosoye (II), the conditions of development being the same in both. In the diseased stems we find also less fibres, but no remarkable difference is to be found in the quantity of nitrogen.

8. Takasuke. September 9, 1898.

	Healthy.	Diseased.
Number of stems	4.	4.
Number of leaves	143.	1056.
Average length of 1 stem	180.	90.
Total fresh weight of stems	403.	145.
Average fresh weight of 1 stem	101.	365.
Total dry weight of stems	118.25	44.24

	Healthy.	Diseased.
Average dry weight of 1 stem	29.56	11.06
Moisture in stems	70.66	69.49
Dry matter in stems	29.34	30.51
Total fresh weight of leaves	367.5	172.00
Average fresh weight of 1 leaf	2.57	0.162
Total dry weight of leaves	124.08	81.35
Average dry weight of 1 leaf	0.868	0.076
Moisture in fresh leaves	66.23	52.70
Dry matter in fresh leaves	33.77	47.30
Number of leaves on 1 stem	36.	266.
Dry matter in leaves of 1 stem	31.02	20.34

In 100 parts of dry matter,

	Healthy.		Diseased.	
	Leaves.	Stems.	Leaves.	Stems.
Crude proteids	22.88	5.38	20.25	10.07
Crude fats	7.34	1.10	4.34	1.57
Crude fibres	**9.59**	**54.60**	**7.11**	**50.54**
Nitrogen-free extracts	52.42	34.58	60.88	32.56
Ash (free from carbon and sand)	7.77	4.34	7.42	5.27
Total nitrogen	**3.66**	**0.86**	**3.24**	**1.61**
Albuminoid nitrogen	**3.37**	**0.54**	**2.98**	**0.98**
Non-albuminoid nitrogen	**0.29**	**0.32**	**0.26**	**0.63**

In 100 parts of ash (free from carbon and sand)

	Healthy.		Diseased.	
	Leaves.	Stems.	Leaves.	Stems.
SiO_2	1.35	0.94	1.54	1.36
SO_3	1.22	2.00	1.90	4.00
P_2O_5	6.41	8.90	6.10	8.74
K_2O	19.20	39.60	18.10	34.90
CaO	40.28	18.80	35.54	16.62
MgO	13.78	7.04	18.65	12.46

9. JŪMONJI. JULY 9, 1898.

	Healthy.	Diseased.
Number of shoots	36.	48.
Number of leaves	261.	547.
Average length of 1 shoot	16.5	12.
Total fresh weight of shoots	90.	48.7
Average fresh weight of 1 shoot	2.50	1.02
Total dry weight of shoots	10.765	5.532
Average dry weight of 1 shoot	0.299	0.115
Moisture in shoots	88.04	88.60
Dry matter in shoots	11.96	11.40
Total fresh weight of leaves	256.20	136.15
Average fresh weight of 1 leaf	0.215	0.249
Total weight of leaves	29.425	13.605
Average dry weight of 1 leaf	0.113	0.025
Moisture in fresh leaves	88.50	90.00
Dry matter in fresh leaves	11.50	10.00
Number of leaves on 1 shoot	7.	11.
Dry matter in leaves of 1 shoot	0.817	0.284

In 100 parts of dry matter,

	Healthy.		Diseased.	
	Leaves.	Shoots.	Leaves.	Shoots.
Crude proteids	36.63	21.75	32.50	26.65
Crude fats	4.26	2.20	8.08	2.54
Crude fibres	11.04	36.72	9.56	27.67
Nitrogen-free extracts	37.67	31.85	45.04	31.52
Ash (free from carbon and sand)	10.40	7.48	9.82	11.64
Total nitrogen	5.86	3.48	5.20	4.26
Albuminoid nitrogen	4.18	1.47	4.10	1.79
Non-albuminoid nitrogen	1.68	2.01	1.10	2.47

In 100 parts of ash (free from carbon, carbonic acid, and sand),

	Healthy leaves.	Diseased leaves.
SiO_2	1.90	2.10
SO_3	2.43	2.70
P_2O_5	20.40	20.60
K_2O	23.63	24.66
CaO	28.10	26.77
MgO	8.85	12.56

10. JŪMONJI. SEPTEMBER 9, 1898.

	Healthy.	Diseased.
Number of stems	3.	10.
Number of leaves	135.	374.
Average length of 1 stem	150.	75.
Total fresh weight of stems	175.5	120.
Average fresh weight of 1 stem	58.5	12.00
Total dry weight of stems	51.566	32.277
Average dry weight of 1 stem	17.188	3.328
Moisture in stems	70.60	72.27
Dry matter in stems	29.40	27.73
Total fresh weight of leaves	159.	129.
Average fresh weight of 1 leaf	1.178	0.345
Total dry weight of leaves	55.266	47.534
Average dry weight of 1 leaf	0.409	0.127
Moisture in fresh leaves	65.24	63.12
Dry matter in fresh leaves	34.76	
Number of leaves on 1 stem	45.	73.
Dry matter in leaves of 1 stem	18.422	4.753

In 100 parts of dry matter,

	Healthy.		Diseased.	
	Leaves.	Stems.	Leaves.	Stems.
Crude proteids	22.38	5.31	16.50	5.44
Crude fats	7.29	1.00	6.72	1.30
Crude fibres	9.32	59.00	9.73	56.20

	Healthy.		Diseased.	
	Leaves.	Stems.	Leaves.	Stems.
Nitrogen-free extracts	50.88	30.36	60.50	33.19
Ash (free from carbon and sand)	10.13	4.33	6.55	3.87
Total nitrogen	3.58	0.85	2.64	0.87
Albuminoid nitrogen	3.10	0.57	2.32	0.66
Non-albuminoid nitrogen	0.48	0.28	0.31	0.21

In 100 parts of ash (free from carbon and sand),

	Healthy.		Diseased.	
	Leaves.	Stems.	Leaves.	Stems.
SiO_2	1.42	1.40	1.78	2.31
SO_3	1.70	1.60	1.70	2.60
P_2O_5	6.57	7.80	8.20	9.75
	23.92	31.60	26.50	24.46
	39.67	24.76	36.87	23.26
MgO	12.05	9.14	15.55	11.74

The above mentioned results of 10 analyses will be sufficient to show the difference in chemical composition of the healthy and diseased plants. The following tables show the average of the above 10 analyses.

Average of the above 10 analyses.

	Healthy.	Diseased	Ratio.
Average length of 1 stem	95.8 cm.	49.5	100 : 51.7
Average fresh weight of 1 stem	57.07	11.10	100 : 19.4
Average dry weight of 1 stem	9.64	2.98	100 : 31.0
Moisture in stems	80.16	78.68	100 : 98.2
Dry matter in stems	19.84	21.32	100 : 107.3
Number of leaves on 1 stem	75.	57.	100 : 76.0
Dry matter in leaves of 1 stem	9.17	3.35	100 : 36.6
Average fresh weight of 1 leaf	0.954	0.168	100 : 17.6
Average dry weight of 1 leaf	0.275	0.052	100 : 18.8
Moisture in fresh leaves	74.87	70.88	100 : 94.7
Dry matter in fresh leaves	25.13	29.12	100 : 116.0

Average composition of leaves.

	Healthy.	Diseased.	Ratio.
Moisture	74.87	70.88	100 : 94.7
Dry matter	25.13	29.12	100 : 116.0

In 100 parts of dry matter,

Crude proteids	31.47	25.76	100 : 81.8
Crude fats	4.42	3.80	100 : 86.0
Crude fibres	10.00	8.14	100 : 81.4
Nitrogen-free extracts	47.97	57.60	100 : 120.0
Ash (free from carbon and sand)	8.52	7.75	100 : 91.0
Total nitrogen	5.02	4.12	100 : 81.8
Albuminoid nitrogen	3.85	3.34	100 : 86.6
Non-albuminoid nitrogen	1.17	0.78	100 : 66.6

In 100 parts of ash (free from carbon and sand),

SiO_2	2.68	3.03	100 : 113.1
SO_3	3.18	3.09	100 : 97.2
P_2O_5	12.48	12.69	100 : 101.6
K_2O	27.15	25.05	100 : 92.3
CaO	26.63	28.09	100 : 105.5
MgO	10.15	12.25	100 : 120.6

Average composition of stems.

	Healthy.	Diseased.	Ratio.
Moisture	80.14	78.68	100 : 98.2
Dry matter	19.86	21.32	100 : 107.3

In 100 parts of dry matter,

Crude proteids	13.51	15.43	100 : 114.2
Crude fats	1.50	2.26	100 : 151.0
Crude fibres	49.16	43.64	100 : 88.8

	Healthy.	Diseased.	Ratio.
Nitrogen-free extracts	32.08	33.55	100 : 104.6
Ash (free from carbon and sand)	6.92	7.19	100 : 103.9
Total nitrogen	2.16	2.47	100 : 114.2
Albuminoid nitrogen	1.02	1.19	100 : 117.0
Non-albuminoid nitrogen	1.14	1.28	100 : 112.3

In 100 parts of ash (free from carbon and sand),

SiO_2	1.14	2.12	100 : 186.0
SO_3	3.40	4.48	100 : 132.0
P_2O_5	9.86	12.07	100 : 123.0
K_2O	34.85	32.59	100 : 93.5
CaO	21.23	23.30	100 : 110.0
MgO	11.60	13.90	100 : 126.0

We see from the above tables that the average length of the diseased stems is nearly half that of the healthy ones, and the fresh weight nearly $\frac{1}{5}$. Both the diseased stems and leaves contain a little less moisture, but the difference is not so remarkable, being only 2% in the stems and 5% in the leaves. The total dry weight of the leaves of one diseased stem is about $\frac{1}{3}$ of those of a healthy one, while the average dry weight of one diseased leaf is far less than that of a healthy one, being only $\frac{1}{6}$ of the latter.

Comparing the chemical constituents of the leaves, we find that nitrogen compounds, fibres, and fats are remarkably deficient in the diseased ones, being nearly $\frac{1}{5}$ of the healthy, while, on the contrary, nitrogen-free extracts are more abundant. The reason why the leaves remain so small and shrivel may be due to the deficiency of nitrogen compounds in the living cells, in consequence of which the chemical activity of the living protoplasm must be much retarded, and also to the bad development of fibres. The assimilation products in the leaves remain unchanged, and the conversion by the living protoplasm, of soluble sugars into cellulose seems to be especially retarded.

It is a most remarkable fact that the diseased leaves contain only $\frac{2}{3}$ of the non-albuminoid nitrogen compounds, compared with the healthy ones. The decrease of non-albuminoid nitrogen

in the leaves is a clear evidence of the retardation of vital activity.

The deficiency of nitrogen compounds in the diseased leaves is not caused by an insufficient supply of nitrogen manures, since the disease is always rampant on fertile soils and on soils treated with soluble manures. The principal cause of the retardation of vital activity must be either the cutting of the stems in the growing season, or the exhaustion of reserve materials in the roots and stems by excessive drain of the leaves. This question will be discussed fully in the next chapter. As regards the ash contents, we find on the average less in the diseased leaves, but sometimes there are exceptions, so that we can not draw any definite conclusion on this point. In the ash of the diseased leaves we generally find less silica, sulphuric acid and phosphoric acid and more lime and magnesia, but the difference is not very regular. At any rate, we may conclude that the disease is not caused by the deficiency of some special mineral nutriments in the soil, and the difference in the ash ingredients may be simply an effect of the disease.

The diseased stems contain also less fibres, but the percentage of nitrogen is apparently higher; this may be explained by the fact that in the healthy stems the development of fibres is enormous, in consequence of which the percentage of nitrogen seems apparently lowered. There must of course be remarkable difference in the absolute quantity of nitrogen contained in one stem. No regular difference is found in the ash ingredients of the diseased stems.

Chapter II. On the Reserve Materials of the Mulberry Tree and their Relation to the Disease.

In this chapter, the reserve materials of the mulberry tree, their migration, and their relation to cutting is fully discussed. The samples used for analysis were all taken from the mulberry farm of the Tokyo Sericultural Experiment Station at Nishigahara. Three varieties, Takasuke, Tsuruta, and Jūmonji, were selected for this purpose. They were all under the same treatment in the same farm, and eight years old (low cutting). Five plants of

each variety which were very similar in all respects, were analysed at different seasons of the year, and the quantity of the reserve materials, the rapidity of their migration, etc. were compared. As the cutting in the growing season has a special relation to the disease, it was my chief aim to find out the relation of the reserve materials to the period of cutting, and finally the conclusion was arrived at that the cutting in the growing season is the principal cause of the disease.

I. TAKASUKE.

(1). April 28, 1899.

		Fresh weight.	Dry weight.	Dry matter %	Moisture %
Roots	wood	2895 g.			
	bark	864	240.7	27.9	72.1
Stems	wood	812			
	bark	420	132.6	31.6	68.4
Leaves [1]		282	48.2	17.1	82.9

[1] As the time of gathering was a little too late, the leaves began to develop.

In 100 parts of dry matter,

	Root bark.	Stem bark.	Leaves.
Crude proteids	16.0	17.1	40.0
Crude fats	8.3	3.1	—
Crude fibres	27.3	35.9	—
Total carbohydrates (as starch)	18.4	—	—
Crude ash	8.2	6.7	10.9
Nitrogen-free extracts	40.2	37.2	—
Total nitrogen	2.56	2.74	6.40
Albuminoid nitrogen	1.38	1.60	4.10
Non-albuminoid nitrogen	1.18	1.14	2.30

(2) May 18, 1899.

		Fresh weight.	Dry weight.	Dry matter %.	Moisture %.
Roots	wood	3500.0			
	bark	1174.5	341.3	20.5	79.5
Stems	wood	1064.2			
	bark	456.5	146.2	32.0	68.0
New stems		305.2	43.0	14.1	85.9
Leaves		1220.7	266.8	21.9	

In 100 parts of dry matter,

	Root bark.	Stem bark.	New stem.	Leaves.
Crude proteids	10.8	11.9	19.38	31.25
Crude fats	9.1	3.5	—	—
Crude fibres	30.2	32.7	—	—
Total carbohydrates	14.0	—	—	—
Crude ash	7.3	6.2	8.3	8.8
Nitrogen-free extracts	42.6	45.7	—	—
Total nitrogen	1.73	1.91	3.10	5.00
Albuminoid nitrogen	1.12	1.30	—	3.40
Non-albuminoid nitrogen	0.61	0.61	—	1.60

(3). July 11, 1899.

Number of new shoots 32; average length of 1 shoot 50 cm.

		Fresh weight.	Dry weight.	Dry matter %.	Moisture %.
Roots	wood	2000.0			
	bark	717.2	186.5	26.0	74.0
New shoots		116.3	19.6	16.8	83.2
Leaves		214.1	55.4	25.9	74.1

In 100 parts of dry matter,

	Root bark.	New shoots.	Leaves.
Crude proteid	8.0	10.77	33.0
Crude fats	10.5	—	—
Crude fibres	33.1	—	—
Total carbohydrates	12.5	—	—
Crude ash	7.7	10.7	11.0
Nitrogen-free extracts	40.7	—	—
Total nitrogen	1.28	1.72	5.28
Albuminoid nitrogen	0.95	—	—
Non-albuminoid nitrogen	0.33	—	—

(4). November 28, 1899.

Number of stems 21.

		Fresh weight.	Dry weight.	Dry matter %.	Moisture. %.
Roots	wood	2207.5			
	bark	660.8	269.2	40.7	59.3
Stems	wood	357.5			
	bark	172.1	72.7	42.2	57.8

In 100 parts of dry matter,

	Root bark.	Stem bark.
Crude proteids	9.3	16.4
Crude fats	6.6	5.3
Crude fibres	25.1	33.4
Total carbohydrates	28.2	18.6
Crude ash	7.1	6.7
Nitrogen-free extracts	51.9	38.2
Total nitrogen	1.49	2.62
Albuminoid nitrogen	0.92	1.79
Non-albuminoid nitrogen	0.57	0.83

(5). December 2, 1899. (no-cutting)

Number of stems 24.

		Fresh weight.	Dry weight.	Dry matter %.	moister %.
Roots	wood	4962.4			
	bark	1791.3	591.1	33.0	67.0
Stems	wood	5229.4			
	bark	1715.0	919.3	53.7	46.3

In 100 parts of dry matter,

	Root bark.	Stem bark.
Crude proteids	12.3	14.3
Crude fats	6.8	5.6
Crude fibres	27.9	36.3
Total carbohydrates	21.6	12.9
Crude ash	7.8	5.6
Nitrogen-free extracts	45.2	38.2
Total nitrogen	1.97	2.28
Albuminoid nitrogen	1.16	1.51
Non-albuminoid nitrogen	0.81	0.77

II. TSURUTA.

(1). April 28, 1899.

		Fresh weight.	Dry weight.	Dry matter %.	Moisture %.
Roots	wood	3120.0			
	bark	1000.0	338.3	33.8	66.2
Stems	wood	610.0			
	bark	285.0	97.5	34.2	65.8
Leaves		231.0	40.8	17.7	82.3

In 100 parts of dry matter,

	Root bark.	Stem bark	Leaves.
Crude proteids	13.4	12.5	42.5
Crude fats	10.5	4.4	—
Crude fibres	25.8	34.2	—

Total carbohydrates	20.0	—	—
Crude ash	7.7	7.0	10.0
Nitrogen-free extracts	42.6	41.9	—
Total nitrogen	2.15	2.00	6.80
Albuminoid nitrogen	1.20	1.40	—
Non-albuminoid nitrogen	0.95	0.60	—

(2). May 28, 1899.

		Fresh weight.	Dry weight.	Dry matter %.	Moisture %.
Roots	wood	2520.0			
	bark	866.2	293.4	66.1	33.9
Stems	wood	1271.7			
	bark	370.9	125.6	66.0	34.0
New stems		415.2	80.5	80.6	19.4
Leaves		1473.7	377.1	74.4	25.6

In 100 parts of dry matter,

	Root bark.	Stem bark.	New bark.	Leaves.
Crude proteids	8.6	9.3	13.75	26.9
Crude fats	11.5	4.5	—	—
Crude fibres	30.0	31.8	—	—
Total carbohydrates	14.4	—	—	—
Crude ash	8.7	6.9	7.0	8.6
Nitrogen-free extracts	41.2	47.5	—	—
Total nitrogen	1.37	1.48	2.2	4.3
Albuminoid nitrogen	0.95	1.00	1.1	—
Non-albuminoid nitrogen	0.42	0.48	1.1	—

(3). July 11, 1899.

Number of new shoots 27; average length of one shoot 60 cm.

		Fresh weight.	Dry weight.	Dry matter %.	Moisture %.
Roots	wood	3380.0			
	bark	945.8	268.8	28.4	71.6
New shoots		311.5	50.4	16.2	83.8
Leaves		433.6	92.1	21.2	78.8

In 100 parts of dry matter,

	Root bark.	New shoots.	Leaves.
Crude proteids	8.9	10.4	32.7
Crude fats	12.6	—	—
Crude fibres	34.0	—	—
Total carbohydrates	10.9	—	—
Crude ash	10.1	7.2	10.2
Nitrogen-free extracts	34.6	—	—
Total nitrogen	1.42	1.67	5 23
Albuminoid nitrogen	0.92	—	—
Non-albuminoid nitrogen	0.50	—	—

(4). December 2, 1899.

Number of stems 34.

		Fresh weight.	Dry weight.	Dry matter %.	Moisture %.
Roots	wood	4558.5			
	bark	1493.1	602.6	41.7	58.3
Stems	wood	742.4			
	bark	313.4	172.3	54.9	45.1

In 100 parts of dry matter,

	Root bark.	Stem bark
Crude proteids	10.7	12.9
Crude fats	9.1	5.9
Crude fibres	26.5	30.7
Total carbohydrates	24.0	16.4
Crude ash	7.4	6.3
Nitrogen-free extract	46.3	44.2
Total nitrogen	1.71	2.07
Albuminoid nitrogen	1.02	1.48
Non-albuminoid nitrogen	0.69	0.59

(5). December 6, 1899. (*no cutting.*)

Number of stems 10.

		Fresh weight.	Dry weight.	Dry matter %.	Moisture %.
Roots	wood	4135.1			
	bark	1495.7	534.0	35.7	64.3
Stems	wood	2368.4			
	bark	806.9	381.0	47.2	52.8

In 100 parts of dry matter,

	Root bark.	Stem bark.
Crude proteids	13.3	13.4
Crude fats	11.2	5.9
Crude fibres	27.8	37.1
Total carbohydrates	17.4	14.7
Crude ash	6.6	6.4
Nitrogen-free extracts	41.1	37.2
Total nitrogen	2.13	2.14
Albuminoid nitrogen	0.99	1.36
Non-albuminoid nitrogen	1.14	0.78

III. JŪMONJI.

(1). April 28, 1899.

		Fresh weight.	Dry weight.	Dry matter %.	Moisture %
Roots	wood	3158.0			
	bark	778.0	311.1	40.0	60.0
Stems	wood	660.0			
	bark	295.0	90.2	30.6	69.4
Leaves		130.0	25.5	19.6	80.4

In 100 parts of dry matter,

	Root bark.	Stem bark.	Leaves.
Crude proteids	11.3	15.1	36.0
Crude fats	10.4	5.5	—
Crude fibres	25.4	36.3	—
Total carbohydrates	23.6	—	—

Crude ash	7.7	7.5	10.7
Nitrogen-free extracts	45.2	35.6	—
Total nitrogen	1.80	2.41	5.76
Albuminoid nitrogen	1.10	1.82	4.00
Non-albuminoid nitrogen	0.70	0.59	1.76

(2). May 29, 1899.

		Fresh weight.	Dry weight.	Dry matter %	Moisture %
Roots	wood	1180.0			
	bark	1018.5	382.3	37.5	62.5
Stems	wood	1176.1			
	bark	435.1	149.6	34.4	65.6
New stems		315.1	50.3	16.0	84.0
Leaves		1200.5	316.4	26.4	73.6

In 100 parts of dry matter,

	Root bark.	Stem bark.	New stems.	Leaves.
Crude proteids	8.1	10.2	14.4	25.0
Crude fats	10.6	5.3	—	—
Crude fibres	30.8	28.6	—	—
Total carbohydrates	16.9	—	—	—
Crude ash	7.4	6.3	8.0	10.7
Nitrogen-free extracts	43.1	49.6	—	—
Total nitrogen	1.29	1.63	2.30	4.00
Albuminoid nitrogen	0.95	1.10	1.30	3.30
Non-albuminoid nitrogen	0.34	0.53	1.00	0.70

(3). July 11, 1899.

Number of new shoots 27; average length of one shoot 55 cm.

		Fresh weight.	Dry weight.	Dry matter %.	Moisture %.
Roots	wood	5650.0			
	bark	1307.8	412.2	31.5	68.5
New shoots		198.5	28.4	14.3	85.7
Leaves		255.3	49.9	19.5	80.5

In 100 parts of dry matter,

	Root bark.	New shoots.	Leaves.
Crude proteids	8.0	12.88	32.75
Crude fats	11.5	—	—
Crude fibres	34.9	—	—
Total carbohydrates	14.6	—	—
Crude ash	9.2	9.10	11.9
Nitrogen-free extracts	36.4	—	—
Total nitrogen	1.28	2.06	5.24
Albuminoid nitrogen	0.79	1.03	—
Non-albuminoid nitrogen	0.49	1.03	—

(4). December 2, 1899.

Number of stems 21.

		Fresh weight.	Dry weight.	Dry matter %.	Moisture %.
Roots	wood	6233.0			
	bark	1378.2	605.3	43.9	56.1
Stems	wood	793.6			
	bark	383.7	183.0	47.7	52.3

In 100 parts of dry matter,

	Root bark.	Stem bark.
Crude proteids	9.7	12.9
Crude fats	10.0	6.2
Crude fibres	28,4	29.1
Total carbohydrates	25.5	17.9
Crude ash	7.0	6.0
Nitrogen-free extracts	44.9	45.8
Total nitrogen	1.55	2.07
Albuminoid nitrogen	0.92	1.48
Non-albuminoid nitrogen	0.63	0.59

From the above mentioned data, we have prepared the following tables, from which one can clearly see that the reserve materials in the barks of roots and stems rapidly decrease with the development of the leaves, and that at the time of cutting, i.e. May 18-28, they reach the minimum. Compare also Plates XIX–XXX.

I. TAKASUKE.

A) Root bark.

	April. 28	May 18.	July 11.	November 28.	December 2. (no cutting)
Moisture	72.1	79.5	74.0	59.3	67.0
Dry matter	27.9	20.5	26.0	40.7	33.0

In 100 parts of dry matter,

Crude proteids	21.1	10.8	8.0	9.3	12.3
Crude fats	8.3	9.1	10.5	6.6	6.8
Crude fibres	27.3	30.2	33.1	25.1	27.9
Total carbohydrates	18.4	14.0	12.5	28.2	21.6
Crude ash	8.2	7.3	7.7	7.1	7.8
Nitrogen-free extracts	40.2	42.6	40.7	51.9	45.2
Total nitrogen	3.38	1.73	1.28	1.49	1.97
Albuminoid nitrogen	1.82	1.12	0.95	0.92	1.16
Non-albuminoid nitrogen	1.56	0.61	0.33	0.57	0.81

Total nitrogen.	100.0	51.2	37.9	44.1	58.3
Albuminoid nitrogen	53.9	33.1	28.1	27.2	34.3
Non-albuminoid nitrogen	46.1	18.1	9.8	16.9	24.0

Total nitrogen	100.0	51.2	37.9	44.1	58.3
Albuminoid nitrogen	100.0	61.5	52.2	50.6	63.7
Non-albuminoid nitrogen	100.0	39.1	21.2	36.5	51.9

B) Stem bark.

	April 28.	May 18.	November 28.	December 2. (no cutting)
Moisture	68.4	68.0	57.8	46.3
Dry matter	31.6	32.0	42.2	53.7

In 100 parts of dry matter,

Crude proteids	22.3	11.9	16.4	14.3
Crude fats	3.1	3.5	5.3	5.6
Crude fibres	35.9	32.7	33.4	36.3
Total carbohydrates	—	—	18.6	12.9
Crude ash	6.7	6.2	6.7	5.6
Nitrogen-free extracts	37.2	45.7	38.2	38.2
Total nitrogen	**3.56**	**1.91**	**2.62**	**2.28**
Albuminoid nitrogen	**2.08**	**1.30**	**1.79**	**1.51**
Non-albuminoid nitrogen	**1.48**	**0.60**	**0.83**	**0.77**

Total nitrogen.	**100.0**	**53.7**	**73.6**	**64.0**
Albuminoid nitrogen	**58.4**	**36.5**	**50.3**	**42.4**
Non-albuminoid nitrogen	**41.6**	**17.2**	**23.3**	**21.6**

Total nitrogen.	**100.0**	**53.7**	**73.6**	**64.0**
Albuminoid nitrogen	**100.0**	**62.5**	**86.6**	**72.6**
Non-albuminoid nitrogen	**100.0**	**41.3**	**56.1**	**52.0**

II. TSURUTA.

A) Root bark.

	April 28.	May 29.	July 11.	December 2.	December 6. (no cutting)
Moisture	66.2	66.1	71.6	58.3	64.3
Dry matter	33.8	33.9	28.4	41.7	35.7

In 100 parts of dry matter,

Crude proteids	17.4	8.6	8.9	10.7	13.3
Crude fats	10.5	11.5	12.6	9.1	11.2
Crude fibres	25.8	30.0	34.0	26.3	27.8
Total carbohydrates	20.0	14.4	10.9	24.0	17.4
Crude ash	7.7	8.7	10.1	7.4	6.6
Nitrogen-free extracts	42.6	41.2	34.6	46.3	41.1
Total nitrogen	**2.79**	**1.37**	**1.42**	**1.71**	**2.13**
Albuminoid nitrogen	**1.56**	**0.95**	**0.92**	**1.02**	**0.99**
Non-albuminoid nitrogen	**1.28**	**0.42**	**0.50**	**0.69**	**1.14**

Total nitrogen	**100.0**	**49.1**	**50.9**	**61.3**	**76.3**
Albuminoid nitrogen	**55.8**	**34.1**	**33.0**	**36.6**	**35.5**
Non-albuminoid nitrogen	**44.2**	**15.0**	**17.9**	**24.7**	**40.8**

Total nitrogen	**100.0**	**49.1**	**50.9**	**61.3**	**76.3**
Albuminoid nitrogen	**100.0**	**60.9**	**59.0**	**65.4**	**63.5**
Non-albuminoid nitrogen	**100.0**	**34.1**	**40.7**	**56.1**	**92.7**

B) Stem bark.

	April 28.	May 29.	December 2.	December 6. (no cutting)
Moisture	65.8	66.0	45.1	52.8
Dry matter	34.2	34.0	54.9	47.2

In 100 parts of dry matter,

Crude proteids	16.5	9.3	12.9	13.4
Crude fats	4.4	4.5	5.9	5.9
Crude fibres	34.2	31.8	30.7	37.1
Total carbohydrates	—	—	16.4	14.7
Crude ash	7.0	6.9	6.3	6.4
Nitrogen-free extracts	41.9	47.5	44.2	37.2
Total nitrogen	2.64	1.48	2.07	2.14
Albuminoid nitrogen	1.85	1.00	1.48	1.36
Non-albuminoid nitrogen	0.79	0.48	0.59	0.78

Total nitrogen	100.0	56.6	78.4	81.1
Albuminoid nitrogen	70.0	37.9	56.6	51.5
Non-albuminoid nitrogen	30.0	18.7	21.8	29.6

Total nitrogen	100.0	56.6	78.4	81.1
Albuminoid nitrogen	100.0	54.0	80.0	73.5
Non-albuminoid nitrogen	100.0	60.8	74.7	98.7

III Jūmonji.

A) Root bark.

	April 28.	May 29.	July 11.	December 3.
Moisture	60.0	62.5	68.5	56.1
Dry matter	40.0	37.5	31.5	43.9

In 100 parts of dry matter,

Crude proteids	13.5	8.1	8.0	9.7
Crude fats	10.4	10.6	11.5	10.0
Crude fibres	25.4	30.8	34.9	28.4
Total carbohydrates	23.6	16.9	14.6	25 5
Crude ash	7.7	7.4	9 2	7.0
Nitrogen-free extracts	45.2	43.1	36.4	44.9
Total nitrogen	2.16	1.29	1.28	1.55
Albuminoid nitrogen	1.32	0 95	0.79	0.92
Non-albuminoid nitrogen	0 84	0.34	0 49	0.63

Total nitrogen	100.0	59.7	59.1	71.8
Albuminoid nitrogen	61.1	44.0	36.6	42.6
Non-albuminoid nitrogen	38.9	15.7	22.5	29.2

Total nitrogen	100.0	59.7	59.1	71.8
Albuminoid nitrogen	100.0	72.0	60.0	70.0
Non-albuminoid nitrogen	100.0	40.5	58.3	75.0

B) Stem bark.

	April 28.	May 29.	December 3.
Moisture	69.4	65.6	52.3
Dry matter	30.6	34.4	47.7

In 100 parts of dry matter,

Crude proteids	18.3	10.2	12.9
Crude fats	5.5	5.3	6.2
Crude fibres	36.3	28.6	29.1
Total carbohydrates	—	—	17.9
Crude ash	7.5	6.3	6.0
Nitrogen-free extracts	35.6	49.6	45.8
Total nitrogen	2.77	1.63	2.07
Albuminoid nitrogen	2.09	1.10	1.48
Non-albuminoid nitrogen	0.68	0.53	0.59

Total nitrogen	100.0	58.8	74.7
Albuminoid nitrogen	75.5	40.0	53.4
Non-albuminoid nitrogen	24.5	18.8	21.3

Total nitrogen	100.0	58.8	74.7
Albuminoid nitrogen	100.0	52.6	70.8
Non-albuminoid nitrogen	100.0	78.0	86.9

The above tables clearly show that the reserve materials in the roots and stems undergo a very remarkable change during the development of leaves in spring. The greater part is transported to the growing parts, especially to the leaves, and there used partly for the formation of new cells and partly for the respiration process. Thus we see that in summer, when the leaves are in full growth, the roots and stems contain only very little reserve materials, but in autumn, the assimilation products formed in the leaves, again come down to the stems and roots and are there stored up to provide for future growth. Such a process is quite natural and every plant follows the same course; still there obtains a great difference in different plants, as to the quantity of the reserve materials and the rapidity of their migration. Some plants store up large quantities, while others contain very little. These differences must naturally exist not only among plants of different species, but also among different plants of the same species.

The reserve materials in plants are generally fats, carbohydrates and nitrogen compounds, but in the case of the mulberry, fats seem to play no important rôle.

It is a remarkable fact that non-albuminous nitrogen compounds (most probably amido-compounds) are contained as reserve materials in large quantities, corresponding, as they do, to nearly one-half of the total nitrogen.

As amido-compounds are soluble in water and easily transportable they may be conveniently used during the energetic development of the plant. Here we must not forget that, not the whole of the nitrogen compounds contained in the stems and roots, serves as reserve materials, but only some portions of them are capable of transformation; since there is a not inconsiderable quantity of nitrogen in the form of insoluble compounds

The greater part of the reserve carbohydrates in the mulberry consists of starch, the presence of which may be easily demonstrated under the microscope. Further, we can easily prove the migration of starch during development without the aid of quantitative analysis. Thus, if we examine the bark of stems or roots under the microscope, we shall soon discover that in winter and in early spring, the bark is remarkably rich in starch grains, which, on the application of iodine, cover the entire field with violet spots. But in summer, when the leaves are fully

developed, we can hardly find a single starch grain under the same treatment.

The importance of reserve materials in the first stages of development is self-evident, and hardly needs an explanation, since the youngest buds can only develop by the aid of reserve materials, and even the green leaves, in their youngest stages, have very little assimilative faculty and must chiefly rely upon reserve materials until they become tolerably large. No plants can develop their leaves without the aid of reserve materials But the precise extent to which the mulberry stands in need of reserve materials for proper development, is still open to question and must be discussed fully. There is no doubt that different plants needs different quantities of reserve materials, and if a plant contains a large quantity of reserve materials, it shows that the plant wants so much until it becomes able to assimilate the neccssary nutriments from outside. Otherwise the plant would never keep in store an unnecessarily large amount of reserve materials.

As already shown in the preceding tables, a considerable amount of reserve materials is stored up in the stems and roots of the mulberry, the greater part of which is consumed during the development of the leaves; hence there is no doubt that this plant has need of a large amount of reserve materials, and it may naturally be expected that when the reserve materials are insufficient, the plant can not attain normal development. It is an evident fact that an enormous quantity of reserve materials moves toward the growing points of the buds and roots to build up new cells, and a still larger quantity of fats and carbohydrates must be consumed there by the energetic respiration which commences long before the new leaves are unfolded. The necessity of reserve materials will be the greater the quicker the development of young leaves, and the nutriments absorbed by the roots must be insufficient to meet the demand, as the absorptive power of the roots depends greatly upon the intensity of transpiration; and it may naturally be expected that in the first stages of development, when the leaves are very small the absorption of the nutriments must be very slow and thus makes the necessity of reserve materials still more urgent.

According to some recent investigations, proteids can be formed from amido-compounds or other inorganic nitrogen com-

pounds (ammonium salts or nitrates) in the absence of light, although much more slowly than in full day-light; the amido-compounds seeming, when the light is insufficient, to be far more conveniently used than the nitrates for the synthesis of proteids.

It is therefore very probable that the amido-compounds, which are present in the stems and roots of the mulberry, are far more efficiently and quickly used for the formation of new proteids in the growing roots and in the buds where light has no access, and can never be replaced by the inorganic nitrogen compounds absorbed from the soil.

As can be seen from the tables, the root-bark of Takasuke and that of Tsuruta and Jūmonji contained on May 18 and 29 respectively (the dates of cutting) only very little reserve materials. The new shoots are therefore compelled to commence their development with a very scanty source of reserve materials, so that it may sometimes happen that the reserve materials become exhausted before the new leaves can duly perform their function. The natural result in such a case would be an imperfect development of the leaves, as a consequence of which the assimilation process would be retarded and the nourishment of the new roots become insufficient and their development be more and more retarded until at last they would be compelled to die off, and the consequent deficient absorption of nutriments from the soil would react injuriously on the leaves. This may be compared to babies deprived of milk and nourished only with solid food, before the digestive organs have developed their powers. Only those plants which have effected a certain degree of development before the exhaution of reserve materials can attain normal growth.

We can now clearly understand why the variety Jūmonji, which requires the least amount of reserve materials as compared with the other varieties, is so rarely subject to the disease.

The following calculations will bring out this point more clearly;—

A) Takasuke, on April 28, had already developed new leaves, the fresh weight of which was 282 grams and the dry weight 48.2 grams for one stock, the nitrogen amounting to about 3.072 grams. This nitrogen must have come almost entirely from reserve materials in the stems and roots and not from the soil. Notwithstanding so much con-

sumption of reserve nitrogen for the development of new leaves, not an inconsiderable amount of nitrogen was, still afterwards, transported from the stems and roots to the leaves. That is to say, on April 28, the bark of the roots still contained 2.56% and that of the stems 2.74% of nitrogen; this, however, gradually decreased, and on May 18, we found only 1.73% in the bark of the roots and 1.91% in that of the stems. As the dry matter of the bark of the roots was nearly 250 grams and that of the stems 140 grams, we can calculate the total amount of the nitrogen transported after April 28; thus :—

$250 \times (2.56 - 1.73) = 250 \times 0.83\% = 2.075$ grams..... transported from the roots

$140 \times (2.74 - 1.91) = 140 \times 0.83\% = 1.162$ grams..... transported from the stems.

Sum total = **3.237** grams.

If now we add the nitrogen consumed before April 28, then we have :—

$3.237 + 3.072 = \mathbf{6.31}$ grams nitrogen, transported during the development of the leaves, of which nearly **4.15** grams was in the roots.

B) Tsuruta.

On April 28, new leaves contained already	2.79 gram.
After the 28th. transported from the roots	2.34
,, ,, ,, from the stems	0.52
Sum total.....	**5.65** grams nitrogen

Nearly 4.26 grams therefore was in the roots.

C) Jūmonji.

On April 28, the new leaves contained..	1.45 grams.
After the 28th. transported from the roots	1.50
,, ,, ,, from the stems	1.00
Sum total.....	**3.95** grams nitrogen.

Of this nearly 2.60 grams was in the roots.

The following table shows the decreased percentage of nitrogen in the dry matter.

		Takasuke.	Tsuruta.	Jumonji.
Total nitrogen	in the bark of roots	1.65%	1.42	0.87
	„ stems	1.65	1.16	1.14
Albuminoid nitrogen	„ roots	0.70	0.61	0.37
	„ stems	0.78	0.85	0.99
Non-albuminoid nitrogen	„ roots	0.05	0.81	0.50
	„ stems	0.84	0.31	0.15

Let us now try to see how much nitrogen was contained in the leaves and the new stems on May 18 and 28 respectively, viz. at the time of the full development of leaves.

	Takasuke (May 18.)	Turuta (May 28.)	Jumonji (May 28.)
Nitrogen in the new stems	1.330 g	1.782 g	1.150 g
„ „ „ leaves	13.340 „	16.210 „	12.640 „
Sum total	**14.670** „	**17.992** „	**13.790** „

The quantity of nitrogen transported from the roots and stems to the leaves is :—

Takasuke.	Tsuruta.	Jumonji.
6.31 g.	**5.65** g.	**3.95** g.

That is to say, Takasuke consumed 6.31 grams of reserve nitrogen to develop the new stems and leaves containing **14.670** grams nitrogen.

Therefore $\frac{6.310}{14.670}=43\%$ of the nitrogen in the new stems and leaves came from the reserve materials.

In Tsuruta $\frac{5.65}{17.992}=31\%$

In Jūmonji. $\frac{3.950}{13.790}=29\%$

It is surprising to find that the mulberry consumes a far greater quantity of reserve nitrogen than we supposed. We observe at the same time that Takasuke consumes a far greater quantity of reserve nitrogen than Tsuruta or Jumonji. This is especially interesting, since Takasuke is more liable to suffer from the disease than the other two varieties.

Of course, the above mentioned figures represent only approximate values, because there are individual differences among the same varieties, and absolutely exact comparisons are impossible. Further, the reserve nitrogen does not only migrate into the leaves, but a portion of it must, of course, be consumed in the roots. Nevertheless, the above is sufficient to show how important a part the reserve materials play during the development of new leaves in the mulberry.

We shall now turn our attention to the question, whether the new shoots that come out from the stump after cutting can be sufficiently nourished with the reserve materials remaining in the bark of the roots.

At the period of cutting, the bark of the roots contained the following quantities of nitrogen :—

	Takasuke (May 18.)	Tsuruta (May 28.)	Jūmonji. (May 28.)
Total nitrogen.....	1.73%	1.37	1.29
Albuminoid nitrogen	1.12	0.95	0.95
Non-albuminoid nitrogen	0.61	0.42	0.34

Absolute quantities in the root-bark of one stock.

	Takasuke. (May 18.)	Tsuruta. (May 28.)	Jumonji. (May 28.)
Dry matter in the bark of roots...	341 grams	203	38.2
Total nitrogen.....	5.900	4.020	4.930
Albuminoid nitrogen	3.820	2.780	3.630
Non-albuminoid nitrogen	**2.080**	**1.240**	**1.300**

Although the above are only approximate values yet there will be no great error in assuming that nearly 4–6 grams nitrogen is still contained in the roots after cutting; but this quantity of nitrogen is not entirely available for the young shoots, because a portion of it is in the form of insoluble compounds, and only a portion is capable of being transported to the growing parts. If we assume the quantity of non-albuminoid nitrogen as representing the quantity of available nitrogen, there will be no great error; and in that case 1.3–2.0 grams or at most 3 grams can be utilized by the new developing shoots. Let us now see whether this quantity is sufficient for the energetic development of the new shoots.

On July 11, when the new shoots had reached the height of 40–60 cm., they were analyzed, and the following results were obtained:

	Total dry matter.	Nitrogen in same.	Sum total of nitrogen.
1) Takasuke.			
New stems.....	**19.6**	0.337	**3.262** g.
Leaves.	**55.4**	2.925	
2) Tsuruta.			
New stems.....	**50.4**	0.842	**5.66** g.
Leaves	**92.1**	4.817	
3) Jūmonji.			
New stems.	**28.4**	0.585	**3.20** g.
Leaves.....	**50.0**	2.620	

We see now that when the young shoots had reached the height of about 50 cm and the dry matter of the leaves amounted to about 50 grams, there were more than 3 grams of nitrogen

in the latter. The reserve nitrogen is therefore hardly sufficient for the normal development of the shoots, and some plants may suffer in consequence and become diseased.

One may ask now, how can we explain the fact that some varieties, like Takasuke, become diseased more easily than others, like Jūmonji. This I shall explain fully in the following pages.

As we see from the results of the analyses, the decrease of nitrogen in the bark of the roots of Jūmonji during the growing period, is smaller than in Takasuke and Tsuruta, and the total quantity of reserve nitrogen is also far smaller in Jūmonji than in the other two. This means that Jūmonji needs a far smaller quantity of reserve nitrogen to build up the same quantity of leaves and new shoots. The roots of Jūmonji seem to be especially fitted for the absorption of soil nutriments, and to make good the deficiency of the reserve materials already present. If now we compare the nitrogen contents of the leaves in the three varieties we find :

	Takasuke.	Tsuruta.	Jūmonji.
April 28,	6.40	6.80	5.76
May 18,	5.00	4.30	4.00

From this it is clear that Jūmonji can build up the same quantity of leaves with a far smaller quantity of nitrogen, that is, the necessity for nitrogen must be far smaller than in the other two varieties. Further, we have calculated that Takasuke consumes 6.31 grams reserve nitrogen to produce 1220 grams fresh leaves (=266.8 grams dry matter, containing 13.34 grams nitrogen), while in Jūmonji only 3.95 grams reserve nitrogen is spent to produce the same amount of fresh leaves (=316 grams dry matter, containing 12.64 grams nitrogen). Thus we see that Jūmonji needs only 62% or nearly $\frac{3}{5}$ of the reserve nitrogen required by the other two varieties to produce the same quantity of leaves. This evidently shows that Jūmonji has a stronger absorptive power for the nitrogen in the soil and manures, and does not rely upon reserve nitrogen so much as the other two. It is therefore not to be wondered at that, Jūmonji is less liable to attack by the disease, since the comparatively small quantity of reserve materials in the roots is soon made good by the newly absorbed nutriments.

But we must here remember that the power of resistance to the disease is not absolutely confined to certain fixed varieties, but may be gradually changed by climatic conditions, soil, and treatment. Thus we always observe that, when certain varieties are induced to rapid growth by the addition of soluble manures in excess, and then cut, then even the favored varieties may become diseased, while on the contrary, such varieties, as Takasuke, will never become diseased if they are cultivated in infertile soil with poor manuring. Thus, one and the same variety may undergo a very remarkable change in its character, especially as regards its conduct towards the disease. Overgrowth accelerates the migration of reserve materials. If the development of new shoots after cutting is very energetic, then the want for reserve materials must be correspondingly great, and the absorbed nutriments must be insufficient for the demand, the result being an emaciated condition of the shoots. Over manuring will never directly increase the quantity of reserve materials in the roots and stems in the growing periods, the nutriments supplied as manures being all transported to the growing parts. So if we cut such over-grown plants, the new shoots will develop more energetically and the reserve materials will soon be exhausted, just the natural condition for the disease. Thus we can understand why the power of resistance to the disease is not absolutely confined to certain fixed varieties. Young plants are rarely attacked; this may be explained by the fact that the cells of young plants are more active, and after cutting can develop small rootlets more easily, and thus gain a stronger absorptive power, than old plants, so that they can easily recover from the deficiency of reserve materials.

Old plants are less active, and after cutting, the development of new rootlets takes place with more difficulty, and accordingly, their absorptive power must remain comparatively stationary and the recovery will consequently be slower.

For the same reason, the disease appears oftener after cutting in late summer or autumn, while it is less frequent when the plants are cut earlier. The development of new rootlets and the consequent recovery after cutting, must be much more difficult in autumn.

The young rootlets must once lose their activity after cutting, and the entire root-system will become gradually ineffici-

ent, unless the development of the new rootlets is exceptionally good; and after successive cuttings, the small roots must entirely die off. So it is quite natural that we always find decayed roots when the disease is much advanced. But in the earlier stages of the disease, we find only small rootlets dead. The roots and leaves are closely correlated to each other, a bad condition in the one accelerating the degeneration of the other; and the primary cause of the disease must be sought in the practice of cutting in the growing period.

We see [Plates XIX–XXVI] that the quantity of reserve nitrogen in the roots of Takasuke, Tsuruta etc. is not restored to its norm even in December. This evidently shows a gradual wearing of the plants, and after two or three years, they must become diseased unless they are kept from cutting.

In the foregoing pages, we have considered only the nitrogen compounds; but we must remember that the carbohydrates, especially starch, have also the same physiological importance during the first stages of development That the cutting in the growing period, when the starch contents of the roots have reached the minimum, has a specially injurious effect upon the new shoots hardly requires any further explanation.

The view above developed finds an additional support from the following facts:—

(1). The disease does not affect those plants which are not cut. This is true throughout the whole country and there is no exception to it. We have observed this in such provinces as Fukushima, Yamagata, Akita, Hyogo, etc., where the cutting method is not practiced. The plants in these provinces attain their maximum height and are 30–40 years, or sometimes even more than 100 years old. Further, we have observed a very instructive case in these provinces, viz. some of the farmers adopted the method of cutting, and all suffered from the disease, while in the neighbouring farms where the method was not adopted the plants were perfectly healthy. A more striking fact was observed in Tanba. A farmer cut the same plants 3 times in one ummer, in consequence of which, all the plants in a farm without exception became diseased, while in the neighbouring farm in which the plants were cut only once, the disease did not appear. That the cutting is the principal cause of the disease may also be shown by the fact that, by keeping the diseased plants for

several years from cutting, most of them can be made to recuperate.

Many experiments have been made to prove this fact. Thus we see :—

				Result.			
Variety.	Date of cutting.	Number of cutting.	Number of plants used for experiments.	Became partly diseased.	Diseased.	Remained healthy.	Death.
Nezumi-gayeshi	July 5.	1	10	4	1	5	—
Jumonji	,,	1	5	1	1	3	—
Obata	,,	1	5	1	2	2	—
Hikojiro	,,	1	5	1	1	3	—
Tsuruta	,,	1	5	1	3	1	—
Takowase	,,	1	5	—	4	1	—
Hosoye	August 19.	1	50	6	9	23	12
,,	Late autumn (once cut before budding in spring.)	2	25	5	10	10	—
,,	,,	2	15	5	4	5	1
,,	August 26.	1	30	3	13	6	8
Yotsume	,, 28.	1	30	4	6	16	4
Hosoye	,, 26.	1	15	4	6	3	2
,,	,, 23.	1	15	—	12	3	—
Total			**215**	**35**	**72**	**81**	**27**
Ratio			**100**	**16**	**34**	**38**	**12**

The disease did not appear in the control (not cut) plants. A similar result has been reported by Mr. Ichikawa. Though he does not mention the age of the experimented plants, yet I do not doubt that they were moderately old ; young plants do not show, in my opinion, such a remarkable effect.

We have also made a similar experiment in 1898 with the variety Nezumigayeshi, eight years old :—

Date of cutting.	Number of cutting.	Number of plants.	Healthy.	Partly diseased.	Diseased.
May 16.	1	28	26	—	2
„	1	11	11	—	—
„	1	11	11	—	—
June 1	1	20	20	—	—
„	1	11	11	—	—
„	1	11	11	—	—
„ 20	1	20	14	—	6
„	1	11	9	—	2

The same experiment was repeated in 1899 with the same variety in the same farm :—

Date of cutting.	Number of cuttings.	Number of plants.	Healthy.	Diseased.
June 1	1	20	16	4
June 20	1	20	13	7
„	1	11	3	8
Total		**51**	**32**	**19**
Percentage		**100**	**63**	**37**

The control plants all remained healthy.

Hence it is quite evident that the cutting is the principal cause of the disease.

(II). If my assumption be true, then the disease must be induced in healthy plants by simply picking off the leaves after their full expansion, as by this means the reserve materials in the roots and stems must as well become exhausted. Following is the result of such an experiment.

Plot.	Variety.	Number of plants.	Date of picking off the leaves.	Result.
All the leaves taken.	Komaki.	5	3 times. (May 7-Sept 9)	Healthy 3 Diseased 2
A portion of leaves taken.	Komaki	5	3 times. (Apr. 28-Sept. 9)	Healthy 5 Diseased 0
New stems, together with leaves taken.	Rosō, Tsuruta, Takasuke.	980	2 times. (May 18-July 15)	Healthy 995 Partly diseased 96 Diseased 198
,,	Takasuke.	6	1 times. (May 28)	Healthy 5 Diseased 1
Stem cut off.	Takasuke, Hosoye, Akagi.	122	1 times. (May 21)	Healthy 75 Diseased 34 Dead 13
Total.		**1118**		Healthy **783** Diseased **322**

This table shows that by frequent picking off of the leaves, nearly $\frac{1}{3}$ of the plants may become diseased.

Second Experiment.

Variety.		Date of picking.	Number of plants.	Healthy.	Diseased.	Partly diseased.	Dead.
Takasuke,	All the leaves taken.	August 14	92	81	2	9	—
,,	A portion of leaves taken.	,, 17	82	68	2	12	—
,,	All the leaves taken.	July 14 ,, 30 August 15 ,, 30 Sept. 13	84	69	1	14	—
Rosō.	A portion of leaves taken.	August 20 ,, 30 Sept. 8 ,, 15 ,, 23	561	530	6	20	2

Third experiment.

Plot.	Variety.	Date of picking.	Number of plants.	Healthy.	Diseased.
A portion of the leaves on new shoots taken.	Nezumi-gayeshi.	August 16	35	32	3
All the leaves on new shoots taken	,,	June 1.	40	28	12
A portion of the leaves taken 3 times.	,,	June 1. June 10. Sept. 20.	40	37	3
All the leaves taken 3 times	,,	June 1. July 10. Sept. 20.	40	31	9

Fourth experiment.

Plot.	Variety.	Date of picking.	Number of plants.	Healthy.	Diseased.	Dead.
A portion of leaves on new shoots taken.	Nezumi-gayeshi.		35	28	5	2
A portion of new shoots picked.	,,		40	25	15	—
New shoots wholly cut.	,,		40	16	24	—
A portion of leaves picked 3 times.	,,	May 28. July 10. Sept. 20.	40	35	5	—
All the leaves, picked 3 times.	,,	May 28. July 10. Sept. 20.	40	20	20	—

No case of the disease was found in the control plants, which were not deprived of their leaves. See Plates XXXV–XXXVI.

The age of the plants, varietal peculiarities and other conditions have a great influence upon the disease.

It is a well known fact that in those provinces where silkworm culture was recently introduced, the disease is especially prevalent, because in these provinces, the farmers try to get the largest possible crop of the leaves from rather small farms, and consequently, over-manuring and frequent picking of the leaves, sometimes followed by cutting, is commonly practiced. Further, those varieties such as Takasuke, Hoɔoye, etc., which grow rather rapidly, are mostly preferred. Moreover, silkworm culture in late summer or autumn is especially injurious for the mulberry, since the leaves are taken off in autumn, and the assimilation products in them to be utilized by the stems and roots for the next year's growth, are neccessarily lost ; and thus the principal cause of the wearing of the plants is brought about.

One might suppose that by giving an excess of soluble manures, the injurious effect of the frequent picking of the leaves or of cutting, can be counterbalanced ; such a view is erroneous. In the growing period, manuring does not directly nourish the stems or roots ; the nutriments being all transported to the growing parts, especially to the leaves ; and in late autumn, the assimilation products in the leaves come down again to the roots and stems and are stored there until the next spring. In the growing period, the roots and stems merely serve as a passage for the nutriments. Therefore if we cut off these over-grown plants in the growing season, the new shoots will grow energetically and the reserve materials must be rapidly exhausted, the disease being the result.

Here I shall cite one more example showing that the disease is nothing but the result of the deficiency of the reserve materials. Healthy and diseased plants in the same farm were cut on August 30, and the new shoots coming from the diseased stems showed distinct signs of the disease even in the earliest stage. On October 15, the plants were cut down and analyzed, with the following result :—

	Leaves.			Stems.		
	Healthy	Diseased.	Ratio.	Healthy.	Diseased	Ratio.
Moisture.	78.90	73.64	100 : 93.0	87.35	85.05	100 : 97.3
Dry matter.	21.10	26.36	100 : 125.0	12.65	14.95	100 : 118.0

In 100 parts of dry matter,

Ash.	11.76	7.82	100 : 66.5	12.5	8.90	100 : 71.2
Total nitrogen.	5.28	3.70	100 : 70.0	3.16	3.29	100 : 104.1
Albuminoid nitrogen.	3.80	2.70	100 : 71.0	1.40	1.30	100 : 92.9
Non-albuminoid nitrogen.	1.48	1.00	100 : 67.9	1.76	1.99	100 : 113.0

We see from this that the new shoots coming from the diseased stocks are considerably poorer in nitrogen and ash, which evidently shows that their roots contained very little reserve materials, which were insufficient to meet the demand of the new shoots even in the first stages of development.

(III). By grafting diseased shoots on healthy roots or stems, many of them can be made to recover and grow normally. We may therefore infer that the disease is exclusively due to deficiency of nutriments, and may be prevented by replacing worn-out roots with vigorous ones. The following experiments prove this point.

First experiment.

Plot.	Variety.	Number of experiments.	Positive results.	Negative result.	Positive but diseased.
Diseased roots of Takowase to Healthy stems of Nezumigayeshi		20	0	18	2
Diseased stems of Hosoye on Healthy roots of Rosō		20	6	13	1

Second experiment.

Plot.	Variety.	Number of experiment.	Positive result.
Diseased stems of ..... to healthy stems of	Takowase Nezumigayeshi	30	8
,, ...	,,	,,	21
Diseased stems ..... to healthy root.....	,,	,,	8
		,,	12
Diseased stems ..... to healthy *Misho*.....	,,	,,	10
	,,	,,	15
Diseased stems ..... on diseased roots.....	,,	,,	0
Healthy stems ..... Diseased roots or stems	,,	,,	0

In the control experiments (grafting of healthy shoots on healthy roots) nearly 60% were successful.

(IV). By layering i,e. burying a portion of the diseased shoots bent into the earth, as shown in Pl. XXXVII–XXXIII. The new rootlets are produced from the covered part, and absorb nutriments from the soil, and the shoots recover from the disease. Numerous experiments have shown this fact, and even the severet cases of the disease may sometimes be suecessfully treated in this way. See Plates XXXVIII–XXXVIII.

(V). By keeping diseased plants from cutting for two or three years and well manuring, many can be made to recover, as has been proved by many experiments. But if these plants are again cut in the growing season they will become again diseased. Further, if we cut down the diseased shoots in early spring before the leaves are developed, the next new shoots will grow normally. This may be explained by considering that the roots of diseased plants may still contain a moderately large quantity of reserve materials during the winter, if they were kept from cutting; so that the new shoots may develop normally without suffering from the deficiency of reserve materials. In the experiment made in 1898, in which 22 diseased plants were kept from cutting, 11 plants recovered and 3 more showed signs of imperfect recovery. In another experiment made in the same year 15 recovered out of 22, while those that were subjected to cutting in the growing season, did not only not recover, but became even more severely diseased. The result obtained in 1899 is equally interesting, 13 plants having recovered out of 22 diseased plants; while in the case of plants subjected to cutting none recovered.

(VI). After cutting in the growing season, a considerable amount of the sap flows out from the cut stems for several days, amounting sometimes to several hundred cubic centimenters for one plant. And as this sap has been believed by many to have an intimate relation to the exhaustion of the reserve materials, I have directed my attention to its chemical analysis. For collecting the flowing sap India rubber tubes were connected at one end with the cut stems and at the other with tightly corked test tubes.

a). Sap of Obata. Collected on May 20.

Colour.—Slightly turbid; after standing for several hours, a little precipitate was formed at the bottom.

Reaction.—Faintly acid.

Specific gravity.—1.001.

Qualitative tests :—

1. Nitrates.—Diphenylamine reaction, dark blue colouration :—

   Nitrates present in tolerable quantity.

2. Ammonia.—Nessler's reagent produced brown precipitate. The solution boiled with a little caustic potash, ammonia gas evolved, which turned red litmus paper distinctly blue and yellow turmeric paper brown. Doubtful whether the ammonia was present as such in the original fresh solution or was derived from the decomposition of proteids or amido-compounds. Normal sap commonly contains no ammonia. Further, a brown precipitate may also be produced by Nessler's reagent in the presence of sugars.

3. Lime salts.—A little present, white precipitate by ammonium oxalate.

4. Sulphate.—Doubtful trace.

5. Chlorides.—Tolerably much, white precipitate by silver nitrate.

6. Iron salts.—Absent.

7. Proteids.—Biuret reaction, only faintly violet.

   No precipitate by nitric acid.

   Almost no precipitate by potassium ferrocyanide and acetic acid.

   From these reactions, the presence of proteids is doubtful, or it must have been decomposed during the collecting of the sap.

8. Sugars.—Slightly reduces Fehling's solution.

100 c.c. of the sap yielded 0.0172 grams nitrogen.

b). Sap of Jūmonji. June 2.

Colour.—Same as in Obata.

Specific graviiy.—1.0007.

100 c.c. contained.—0.007 grams nitrogen.

0.185 grams dry matter.

0.080 grams ash (76% of which dissolves in hydrochloric acid.)

0.095 grams organic matter.

The sap of Jūmonji contained far less nitrogen than that of Obata. The composition of the sap may therefore be very different according to varieties, time of gathering and other conditions. The nitrogen contained in the sap may come partly from the reserve materials in the roots and partly from the freshly absorbed nutriments. I have not determined the exact quantity of the sap that flows out from one plant, as it is rather difficult. But it is plain enough that Takasuke and Tsuruta furnish a far greater quantity of it than Jūmonji, which fact may have some significance for the resisting capacity of these varieties towards the disease, though I do not believe it has so much influence upon the disease as many suppose.

The numerous facts above mentioned are in full accord with my view.

I must here add a few words on a case that I have observed very often, viz. that the cut ends of the stems decay and the putrefaction preceeds between the wood and the bark towards the inner part, and interrupts the connection between the vascular bundles of the old stems and those of the new shoots, and thus prevents a free circulation of the nutriments into the growing shoots. For a time I thought that this putrefaction was the

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Sometimes the disease appears on plants which have never been cut, or on young plants which has not yet attained the age of cutting; but these cases are very rare and may have some special cause, either attack of fungi or insects, etc, or some physiological abnormality. I have analyzed a young plant which has never been cut, but some leaves of which showed signs of the disease, and obtained the following results :—

	Leaves.		Stems.	
	Healthy.	Diseased.	Healthy.	Diseased.
Total nitrogen	3.42	3.04	2.15	2.00
Albuminoid nitrogen	2.94	2.37	1.50	1.53
Non-albuminoid nitrogen	0.48	0.67	0.65	0.47
Ash	10.7	8.6	6.0	6.1

	Leaves.		Stems.	
	Healthy.	Diseased.	Healthy.	Diseased.
Total nitrogen	100.0	88.8	100.0	93.2
Albuminoid nitrogen	„	80.6	„	102.2
Non-albuminoid nitrogen	„	140.4	„	72.3
Ash	„	80.4	„	101.0

Thus we see that the diseased leaves and stems are considerably poorer in nitrogen. So we may conclude that the disease appears when the nitrogen supply is reduced beyond a certain limit, whatever the efficient cause may be.

cause of the disease. But as my observation became more extensive, I found that in many diseased plants there was no putrefaction, and also that it was not generally observed in the first stage of the disease. So it is very probable that this putrefaction is only a secondary phenomenon accopanying the disease and not its primary cause. Nevertheless the putrefaction unavoidably accelerates the disease and hastens the death of the whole plant. Further, the sap flowing out from the cut stems may be a good nourishment for small orgamsms. As the sap is very abundant in Takasuke, Tsuruta etc, it is natural that these varieties are especially prone to putrefaction.

Again many believe that the principal cause of the disease lies in the decay of the roots. It is true that the roots of the diseased plants are generally in a very bad condition, the small roots having almost entirely decayed; but at the beginning of the disease, the roots are generally quite normal. Moreover, if the decay of the roots be the cause of the disease we could not understand why those plants which are not subjected to cutting are entirely free from the disease.

It is very interesting to see whether other plants are also liable to the same disease when subjected to repeated cutting or when frequently robbed of their leaves in the growing season. Japanese Salix is cultivated in Tanba and Tango just in the same manner as the mulberry, but the stems are cut in late autumn when the leaves have fallen down. This is a very rational mode of precedure and entirely in harmony with the teachings of plant physiology, and we have never observed the disease in this plants.

Japanese tea plant is generally subjected to frequent picking of the leaves, sometimes even 3 or 4 times in a year, and we observe very often that in such a case the leaves become smaller and can not develop well, and that sometimes even the whole plant dies; but the disease is soon healed by good manuring.

Summary and Conclusions.

1). The diseased leaves are remarkably poor in nitrogen and the development of fibres is considerably retarded; no other peculiarity is regularly found. The shrinking of the leaves and the retardation of growth may be due to the deficiency of nitrogen and the bad development of fibres.

The deficiency of nitrogen in the diseased plants is not caused by an insufficient supply of nitrogen manures in the soil, but must be due to a diminution of the absorptive power of the roots, and also of chemical activity in the plant cells; since the disease is always observed in those places where soluble manure is given in excess and over-growth is induced. Further, the diseased plants can never recover on simple application of nitrogen manures.

The diminution of chemical activty in the living cells, owing to the deficiency of nitrogen, may also be the cause of the bad development of fibres, since the formation of cellulose from soluble carbohydrates is performed by the living protoplasm.

2). During winter a considerable amount of reserve materials (especially nitrogen compounds and starch) is stored up in the bark of stems and roots, and in spring, when the development of new leaves commences, the greater part is transported to the growing parts, and the assimilation products in the leaves again come down in late autumn, when the leaves begin to fall. Therefore the stems and roots are considerably poorer in reserve materials during the growing season. Now it will be at once evident that the cutting of the plants in the growing season must have a very bad effect on the new developing shoots, since they must be nourished with scanty reserve materials, and moreover, it is not impossible that the reserve materials may be entirely exhausted before the new shoots have attained a certain height and become able to assimilate independently enough nutriments from outside. In such a case the shoots will not grow normally and the disease must be the result. Many facts support this view :—

a). The first appearance of the disease is always in the new shoots, after cutting in the growing season.

b). The disease is not observed where the plants are not cut, and even plants already diseased may recover when kept from cutting for some years.

c). Many experiments show that cutting in the growing season is followed by the disease, while it is not observed when the plants are left without cutting.

d). The disease is not induced when the stems are cut during winter or in early spring before the leaves are unfolded.

3. Not only by cutting in the growing season, but also by frequent picking of the leaves, the reserve materials in the roots and stems, may be exhausted, and the new leaves developed subsequently become diseased, as proved by many experiments.

4. Some varieties, such as Takasuke, store up a large quantity of reserve materials, while others, like Jūmonji, contain comparatively little. The former need a far greater amount of reserve materials during the devlopment of the leaves, while the latter require only a comparatively small amount. This indicates that Takasuke has a weaker absorptive power for soil nutriments in the first stages of development. So it must be very difficult for it to counterbalance the deficiency of reserve materials with the nutriments absorbed from the soil. The consequence is that Takasuke is more liable to the disease than Jūmonji. But such a propensity is not absolutely confined to certain varieties ; on the contrary, it must have a wide range of variation even among the same variety and is subject to modicification by various conditions. Generally speaking, a plant becomes more liable to the disease by accelerating its growth with abundant soluble manures, and by some other treatment. Therefore no variety would be absolutely free from the disease if they were cut in the growing season, or if the leaves were picked frequently.

5. Young plants become very rarely diseased. This may be due to energetic development of the roots and a large capacity for the absorption of nutriments in the young plants ; while old plants have less power of developing new roots, and consequ-

ently the deficiency of reserve materials can not be made good by the nutriments absorbed from outside. For the same reason, cutting in late autumn produces more cases of the disease than cutting in early summer. Since the activity of plant cells is already diminished in late autumn the development of new roots must be more difficult.

6. Micro-organisms are not the cause, because they are not always present in the diseased plants. Further, the decay of the roots of the diseased plants may be a secondary phenomenon, since we can not understand why the disease is not observed when the plants are left without cutting, or why the diseased plants may even recover by being kept from cutting, for some years. Shoots of the diseased plants may develop normally when they are grafted on healthy roots or stems, or when they are covered with earth and propagated by cutting. But there is no doubt that the mirco-organisms have some accelerating influence upon the disease and at last cause the death of the plants.

7. As regards the methods for the prevention and cure of the disease, we must leave them to future investigations, since we have not succeeded in finding any that promises success.

I. Comparison of healthy and diseased plants.

	Tsuruta (I) May 6.		Tsuruta (II) Oct. 15.		Hosoye (I) May 6.		Hosoye (II) Oct. 15.		Yotsume May 22.		Takasuke (I) May 22.		Takasuke (II) July 9.		Takasuke (III) Sept. 9.		Jumouji (I) July 9.		Jumonji (II) Sept. 9.		Average.		Ratio.	
	Healthy	Diseased.	Healthy	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased	Healthy	Diseased	Healthy.	Diseased.	Healthy	Diseased.	Healthy.	Diseased	Healthy	Diseased	Healthy	Diseased
Average length of shoots	96.1	45.0	—	—	195.0	82.5	39.0	33.0	66.0	45.0	90.00	45.00	30.0	18.0	180.0	90.0	16.50	12.00	150.0	75.0	95.80	49.5	100.0	51.7
Fresh weight of 1 shoot	59.7	15.1	—	—	205.0	18.85	2.22	0.904	29.3	6.00	50.0	78.70	5.45	1.04	101.0	36.3	2.50	1.02	58.5	12.00	57.07	11.10	100.0	19.4
Dry weight of 1 shoot	—	—	—	—	—	—	0.28	0.135	—	—	—	—	0.87	0 24	29.56	11.06	0.30	0.115	17 19	3.33	9.64	2.98	100.0	31.0
Misture in shoots	—	—	—	—	—	—	87.35	85.05	—	—	—	—	84.04	78.00	70.66	69 49	88.04	88.60	70 60	72.27	80.14	78.68	100.0	98.2
Dry matter in shoots	—	—	—	—	—	—	12.65	14.95	—	—	—	—	15.96	22.00	29.34	30.51	11.96	11.40	29.40	72.73	19.86	21.32	100.0	107.3
Number of leaves on 1 shoot	57.0	31.0	—	—	224.0	5.0	10.0	1.30	140.0	40.0	136.0	45.0	18.00	19.00	36.0	26 6	7.00	11.00	45.00	37.00	75.00	57.00	100.0	76.0
Dry matter in leaves of 1 shoot	2.36	0.42	—	—	5.37	0.52	0.695	0.422	7.15	0.77	15 09	2.02	1.622	0.647	31.02	20.34	0.82	0.28	18.42	4.75	9.17	3.35	100.0	36.6
Fresh weight of 1 leaf	0.229	0.065	3.22	0.36	0.111	0.049	0.304	0.126	0 181	0.072	0.376	0.141	0.39	0.11	2.57	0.16	0 982	0.240	1.18	0 35	0.054	0.168	100.0	17.6
Dry weight of 1 leaf	0.041	0.014	0.964	0.135	0.024	0.010	0.066	0.033	0.0513	0.0195	0.111	0.045	0.10	0.032	0.87	0.076	0.113	0 024	0.41	0.127	2.275	0.052	100.0	18.8
Moisture in leaves	81.20	78.14	70.00	62.60	79.84	78.81	78.90	73.64	71.63	72.60	70.50	68.00	76.70	69.15	66.23	52.70	88.50	90 0	65.24	63.12	74 87	70 88	100.0	94.7
Dry matter in leaves	18 80	21.86	30.00	37.40	20.16	21.19	21.10	26.36	28.37	27.40	29 50	32.00	23.30	60.85	33.77	47 30	11.50	10.0	34 76	36 88	25 13	29 12	100.0	116.0

II. Comparison of healthy and diseased plants (Leaves).

	Tsuruta (I) May 6.		Tsuruta (II) Oct. 15.		Hosoye (I) May 6.		Hosoye (II) Oct. 15.		Yotsume May 22.		Takasuke (I) May 22.		Takasuke (II) July 9.		Takasuke (III) Sept. 9		Jumonji (I) July 9.		Jumonji (II) Sept. 9.		Average.		Ratio.	
	Healthy	Diseased.	Healthy.	Diseased	Healthy.	Diseased.	Healthy	Diseased.	Healthy.	Diseased.	Healthy	Diseased	Healthy	Diseased	Healthy.	Diseased.	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased.	Healthy	Diseased.
Moisture	81.2	7.14	70.00	62.60	79.84	78.81	78.90	73.64	71.63	7.60	70.50	68.00	76.70	60.15	66.23	52.70	88.50	90.00	65.24	63.12	74.87	70.88	100.0	94.7
Dry matter	18.80	21.86	70.00	27.4	20.16	21.19	21.10	26.36	28.37	27.40	29.50	32.00	23.30	30.85	33.77	47.30	11.50	10.00	34.76	36.88	25.13	29.12	100.0	116.0
In 100 part of dry matter,																								
Crude proteids	47.04	34.56	26.88	23.13	41.00	38.00	33.00	23.13	28.75	27.94	23.75	18.44	30.50	23.13	22.88	20.25	30.63	32.50	22.38	16.50	31.47	25.76	100.0	81.8
Crude fats	1.92	3.10	5.80	4.40	2.26	1.51	5.19	4.96	3.83	3.45	3.46	3.34	2.84	3.05	7.34	4.34	4.20	3.08	7.29	6.72	4.42	3.80	100.0	86.0
Crude fibres	41.27	54.20	8.74	6.70	40.18	52.33	10.53	7.26	59.66	60.07	0.61	8.60	11.14	8.05	9.59	7.11	11.04	0.56	0.32	0.73	10.00	8.14	100.0	81.4
Nitrogen-free extracts			51.23	57.71			30.52	56.83			56.84	63.40	47.26	58.00	52.42	60.88	37.67	45.04	50.88	60.50	47.97	57.60	100.0	120.0
Ash	7.87	8.14	7.35	8.06	7.50	8.16	11.76	7.82	7.76	8.54	6.34	6.13	8.20	6.89	7.77	7.42	10.40	9.82	10.13	6.55	8.52	7.75	100.0	91.0
Total nitrogen	7.67	5.53	4.30	3.70	6.56	6.08	5.28	3.70	4.60	4.47	3.80	2.95	4.88	3.70	3.66	3.24	5.86	5.20	3.58	2.64	5.02	4.12	100.0	81.3
Albuminoid nitrogen	5.15	4.07	3.60	3.00	4.08	3.94	3.80	2.70	4.34	4.15	3.05	2.70	3.85	3.35	3.37	2.08	4.18	4.10	3.10	2.33	3.85	3.34	100.0	86.6
Non-albuminoid nitrogen	2.52	1.46	0.70	0.70	2.48	2.14	1.48	1.00	0.26	0.32	0.75	0.16	1.03	0.35	0.29	0.16	1.68	1.1	0.48	0.31	1.17	0.78	100.0	66.6
In 100 parts of pure ash																								
SiO_2	3.71	3.37	1.68	1.85	1.07	2.50	4.87	4.00	3.44	4.60	2.64	3.77	4.03	4.85	1.35	1.54	1.00	2.10	1.42	1.58	2.68	3.03	100.0	113.1
SO_3	6.23	3.86	2.32	2.04	5.15	4.27	3.25	3.66	3.26	3.34	3.64	4.00	2.00	2.81	1.22	1.00	2.43	2.70	1.70	1.70	3.18	3.00	100.0	97.2
P_2O_5	11.34	13.55	9.44	7.52	12.08	14.00	8.56	11.80	13.86	13.14	17.00	10.02	14.00	11.00	6.41	6.10	20.40	20.00	6.57	8.20	12.48	12.60	100.0	101.6
K_2O	35.20	30.53	27.40	14.10	30.30	32.00	24.35	23.20	31.81	28.30	28.51	29.43	21.15	18.70	19.2	18.10	33.3	24.66	23.1	20.50	27.15	25.05	100.0	92.3
CaO	15.20	18.9	22.10	38.60	17.23	15.75	27.80	30.00	25.07	23.08	23.64	23.02	27.21	32.43	40.28	35.54	28.16	26.77	30.07	30.87	24.13	28.00	100.0	105.5
MgO	8.22	10.46	9.60	12.20	8.45	7.26	8.50	7.00	11.48	11.60	10.44	12.82	10.13	14.12	13.78	18.65	8.85	12.56	12.05	15.55	10.15	12.23	100.0	120.6

II. Comparison of healthy and diseased plants (Leaves).

	Tsuruta (I) May 6.		Tsuruta (II) Oct. 15.		Hosoye (I) May 6.		Hosoye (II) Oct. 15.		Yotsume May 22.		Takasuke (I) May 22.		Takasuke (II) July 9.		Takasuke (III) Sept. 9		Jumonji (I) July 9.		Jumonji (II) Sept. 9.		Average.		Ratio.	
	Healthy.	Diseased	Healthy.	Diseased	Healthy.	Diseased.	Healthy	Diseased.	Healthy.	Diseased.	Healthy	Diseased	Healthy	Diseased	Healthy.	Diseased	Healthy.	Diseased	Healthy	Diseased	Healthy	Diseased.	Healthy	Diseased
Moisture	81.20	7[illegible].14	70.00	62.60	79.84	78.81	78.00	73.64	71.0	7[illegible].0	70.50	68.00	70.70	69.15	66.23	52.70	88.50	90.60	65.24	[illegible].12	74.87	70.88	100.0	94.7
Dry matter	18.80	21.86	70.00	27.40	[illegible].16	21.1	21.10	26.36	28.37	27.40	29.50	32.00	23.30	30.85	33.77	47.30	11.50	10.00	34.76	[illegible].88	25.13	29.12	100.0	116.0
In 100 part of dry matter,																								
Crude proteids	47.4	34.56	26.88	23.13	41.0	38.00	[illegible].00	23.13	28.75	27.14	23.75	18.44	[illegible].53	23.13	22.88	20.25	[illegible]	25.[illegible]	32.38	19.[illegible]	31.47	25.70	100.0	81.8
Crude fats	1.62	3.30	5.8	4.40	2.[illegible]	1.51	[illegible].30	4.06	3.[illegible]3	3.15	3.40	3.34	[illegible].84	3.05	7.34	4.34	1.[illegible]	3.[illegible]8	7.20	[illegible].27	4.12	3.80	100.0	86.0
Crude fibres	41.27	34.20	8.74	6.70	4.18	[illegible].33	10.[illegible]3	7.20	5.[illegible]0	[illegible].07	[illegible].61	8.00	11.14	8.[illegible]	[illegible].51	7.11	11.64	[illegible].30	[illegible].[illegible]	7.[illegible]	10.[illegible]	8.14	100.0	81.4
Nitrogen-free extracts			51.23	57.71			30.52	50.[illegible]3			[illegible].[illegible]4	[illegible].[illegible]0	47.26	58.[illegible]	52.42	66.88	57.[illegible]7	45.[illegible]4	5.88	10.[illegible]	47.07	57.[illegible]0	100.0	120.0
Ash	7.57	8.14	7.35	8.[illegible]0	7.50	8.16	11.76	7.82	7.76	8.54	6.[illegible]3	[illegible].13	8.[illegible]0	[illegible].80	7.77	7.42	[illegible].[illegible]0	6.82	10.13	[illegible].[illegible]	8.32	7.75	100.0	91.0
Total nitrogen	7.97	5.33	4.30	3.70	[illegible].[illegible]	6.08	5.28	3.70	4.[illegible]0	4.47	[illegible].[illegible]8	2.[illegible]5	4.88	3.7[illegible]	3.[illegible]0	3.24	5.80	5.20	3.58	2.[illegible]4	5.02	4.12	100.0	81.3
Albuminoid nitrogen	5.15	4.[illegible]7	3.60	3.[illegible]0	4.[illegible]8	3.94	3.[illegible]8	2.70	4.34	4.15	3.[illegible]5	2.70	3.[illegible]2	2.35	3.37	[illegible].[illegible]8	4.18	4.[illegible]0	3.[illegible]6	2.[illegible]3	3.[illegible]8	3.34	100.0	86.6
Non-albuminoid nitrogen	2.82	1.[illegible]0	0.7	0.7[illegible]	2.48	2.14	1.48	1.[illegible]0	0.20	0.32	[illegible].77	0.[illegible]0	1.[illegible]3	0.[illegible]5	[illegible].2		1.[illegible]8	1.1	[illegible].48	.[illegible]	1.17	0.7[illegible]	100.0	66.6
In 100 parts of pure ash																								
SiO_2	3.71	3.[illegible]7	1.08	1.85	1.67	2.[illegible]5	4.87	4.[illegible]0	3.44	4.[illegible]0	2.[illegible]4	3.77	4.[illegible]0	4.[illegible]5	1.[illegible]5	1.[illegible]1	1.[illegible]	2.[illegible]0	1.42	1.[illegible]8	2.[illegible]8	3.[illegible]3	100.0	113.1
SO_3	[illegible].23	3.[illegible]6	2.32	2.[illegible]4	5.15	4.27	3.25	3.[illegible]0	3.20	3.34	3.[illegible]4	4.[illegible]0	2.[illegible]0	2.[illegible]1	1.22	1.[illegible]	2.43	2.70	1.70	1.[illegible]0	3.18	3.[illegible]0	100.0	97.2
P_2O_5	14.34	13.55	0.44	7.52	12.[illegible]8	14.[illegible]0	8.[illegible]0	11.80	13.[illegible]0	13.14	17.90	10.02	14.[illegible]0	11.[illegible]0	[illegible].41	[illegible].[illegible]0	20.40	20.[illegible]0	[illegible].57	8.2[illegible]	12.[illegible]8	12.[illegible]4	100.0	101.6
K_2O	35.20	33.53	27.40	14.10	30.30	32.[illegible]0	24.35	23.20	31.81	28.30	28.51	20.43	21.[illegible]3	18.70	1.[illegible]2	18.1	27.[illegible]3	24.[illegible]0	2.[illegible]	20.[illegible]5	27.15	25.[illegible]5	00.0	92.3
CaO	15.20	18.0	22.10	38.60	17.23	15.75	27.80	30.00	25.07	23.08	23.64	23.02	27.[illegible]1	32.13	[illegible].[illegible]8	35.51	27.[illegible]0	[illegible].77	30.[illegible]7	3.[illegible]7	29.03	2.[illegible]0	100.0	105.5
MgO	8.22	10.46	0.60	12.20	8.45	7.26	8.50	7.[illegible]0	11.48	11.60	10.44	12.82	10.[illegible]3	14.[illegible]2	13.[illegible]5	18.[illegible]5	8.85	12.56	12.[illegible]5	15.55	10.[illegible]5	12.25	100.0	120.6

III. Comprison of healthy and diseased plonts (Stems.)

	Takasuke (I) Oct. 15.		Takasuke (II) July 9.		Takasuke (III). Sept. 9.		Jūmonji (I) July 9.		Jūmonji (II). Sept. 9.		Average.		Ratio.	
	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased.	Healthy.	Diseased	Healthy.	Diseased.	Healthy.	Diseased.
Moisture	87.35	85.05	84 04	78.00	70.66	69.49	88.04	88.60	70.60	72.27	80.14	78.68	100.0	98 2
Dry matter	12.65	14.95	15 96	22.00	29.34	30.51	11.96	11 40	29.40	27 73	19.86	21.32	100 0	107.3
In 100 parts of dry matter,														
Crude proteids	19 75	20.56	15.38	14 44	5 38	10 06	21.75	26 63	5.31	5.44	13 51	15 43	100.0	114.2
Crude fats	2 35	3 70	0.83	2.20	1.10	1.57	2.20	2.54	1.00	1 30	1.50	2 26	100 0	151.0
Crude fibres	65 40	66 84	46 30	40.10	54 60	50.54	36.72	27 67	59 00	56.20	49.16	43 64	**100.0**	**88.8**
Nitrogen-free extracts			31 54	36 93	34 58	32 56	31.85	31 52	30.36	33.19	32 08	33 55	100.0	104.6
Crude ash	12 50	8 90	5.95	6 29	4.34	5.27	7.48	11.64	4 33	3 87	6 92	7 19	100 0	103.9
Total nitrogen	3 16	3.29	2.46	2.31	0 86	1.61	3 48	4 26	0.85	0.87	2.16	2.47	100.0	114 2
Albuminoid nitrogen	1.40	1.30	1.10	1.20	0 54	0 98	1.47	1.79	0.57	0 66	1.02	1.19	100.0	117.0
Non-albuminoid nitrogen	1.76	1.99	1.36	1.11	0 32	0.63	2.01	2.47	0.28	0.21	1.14	1.28	100.0	112.3
In 100 parts of pure ash,														
SiO_2	1.02	1.90	1.19	2.90	0.94	1.36	—	—	1.40	2.31	1.14	2.12	100.0	186 0
SO_3	6 00	7.10	4.20	4.20	2.00	4.00	—	—	1.60	2.60	3.40	4.48	100.0	132.0
P_2O_5	8.10	10.90	14.64	18.87	8 90	8.72	—	—	7 80	9.75	9.86	12.07	100.0	123.0
K_2O	29.7	29.5	38.5	31.3	39.6	34.9	—	—	31.6	34 66	34 85	32.59	100.0	93 5
CaG	19 10	24.00	22.26	28.90	18.80	16.62	—	—	24 76	23.26	21.23	23 30	100.0	110.0
MgO	14.10	15.00	13.70	16.43	7.04	12.46	—	—	9.14	11.74	11.00	13 90	100.0	126 0

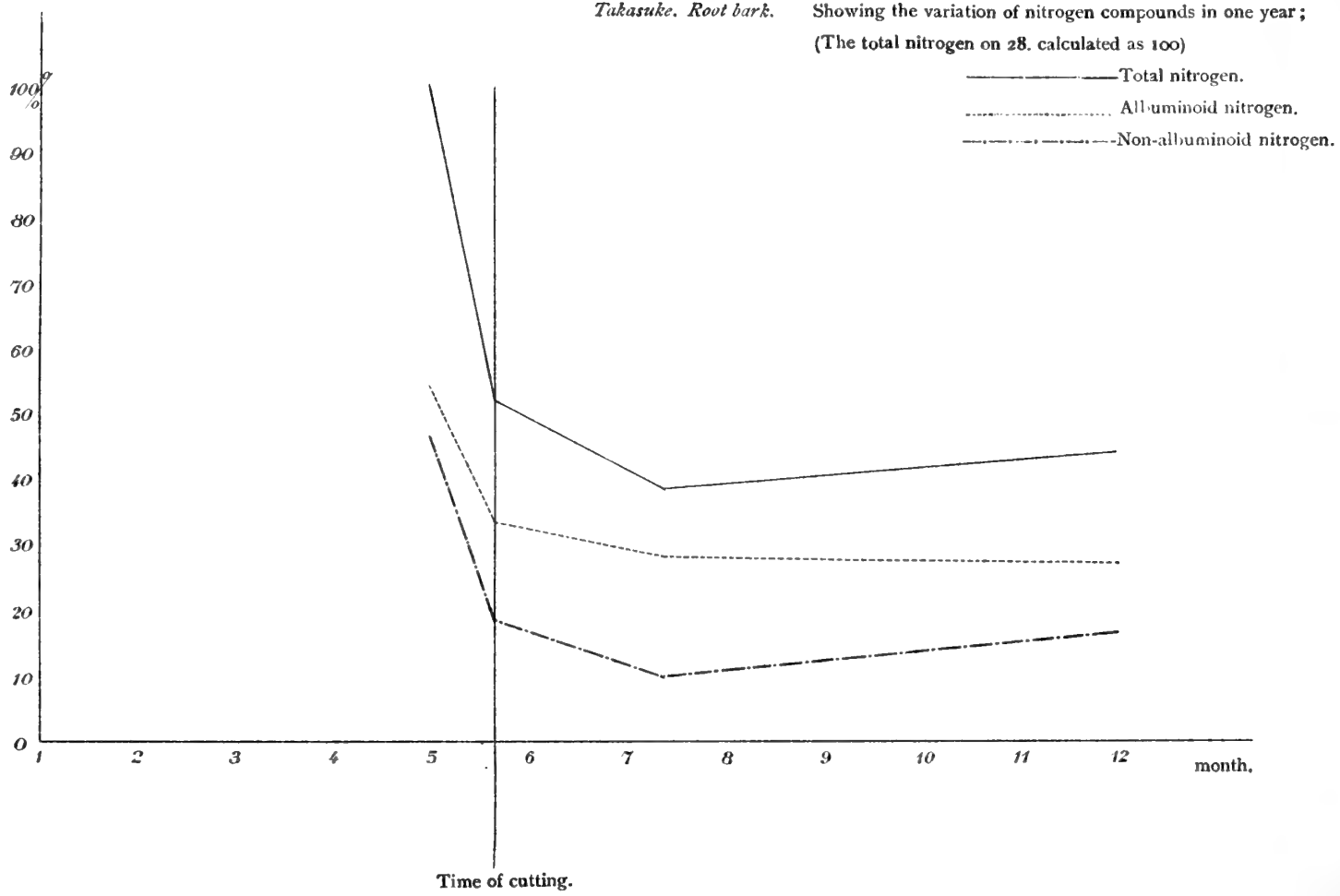
IV. Comprison of the chemical composition of different varieties (Healthy plants.)

	Leaves (May 6).		Leaves (May 22.)		Leaves (July 9.)		Stems (July 9.)		Leaves (Sept. 9.)		Stems (Sept. 9).	
	Tsurata	Hosoye	Yotsume	Takasuke	Takasuke	Jûmonji	Takasuke	Jûmonji	Takasuke	Jûmonji	Takasuke	Jûmonji.
Average length of shoots							30.00	16.5			180.0	150.0
Fresh weight of 1 shoot							5.45	2.50			101.0	58.5
Dry weight of 1 shoot							0.87	0.30			29.56	17.19
Fresh weight of 1 leaf	0.229	0.111	0.181	0.376	0.39	0.982			2.57	1.18		
Dry weight of 1 leaf	0.041	0.024	0.051	0.121	0.10	0.113			0.87	0.41		
Moisture	81.20	79.84	71.63	70.50	76.70	88.50	84.04	88.04	66.23	65.24	70.66	70.60
Dry matter	18.80	20.16	28.37	29.50	23.30	11.50	15.96	11.96	33.77	34.76	29.34	29.34
In 100 parts of dry matter,												
Crude proteids	48.44	41.00	28.75	23.75	30.50	36.63	15.38	21.75	22.88	22.38	5.38	5.31
Crude fats	1.62	2.26	3.83	3.46	2.84	4.26	0.83	2.20	7.34	7.29	1.10	1.00
Crude fibres	41.27	49.18	59.66	9.61	11.14	11.04	46.30	36.72	9.59	9.32	54.60	59.00
Nitrogen-free extracts				56.84	47.20	37.67	31.54	31.85	52.42	50.88	34.58	30.36
Ash	7.87	7.56	7.76	6.34	8.26	10.40	5.95	7.48	7.77	10.13	4.34	4.38
Total nitrogen	7.77	6.56	4.60	3.80	4.88	5.86	2.46	3.48	3.66	3.58	0.86	0.85
Albuminoid nitrogen	5.15	4.08	4.34	3.05	3.85	4.18	1.10	1.47	3.37	3.10	0.54	0.57
Non-albuminoid nitrogen	2.52	2.48	0.26	0.75	1.03	1.68	1.36	2.01	0.29	0.48	0.32	0.28
In 100 parts of pure ash,												
SiO_2	3.71	1.07	3.44	2.64	4.68	1.90	1.19		1.35	1.42	0.94	1.40
SO_3	6.23	5.15	3.26	3.64	2.60	2.43	4.00		1.22	1.70	2.00	1.00
P_2O_5	14.34	12.68	13.86	17.90	14.60	20.40	14.64		6.41	6.56	8.90	7.80
K_2O	35.20	36.30	31.81	28.51	21.15	23.63	38.50		19.20	23.92	30.60	31.60
CaO	15.20	17.23	25.07	23.64	27.21	28.10	22.26		40.28	39.67	18.80	24.76
MgO	8.22	8.45	11.48	10.44	10.13	8.85	13.70		13.78	12.05	7.04	9.14

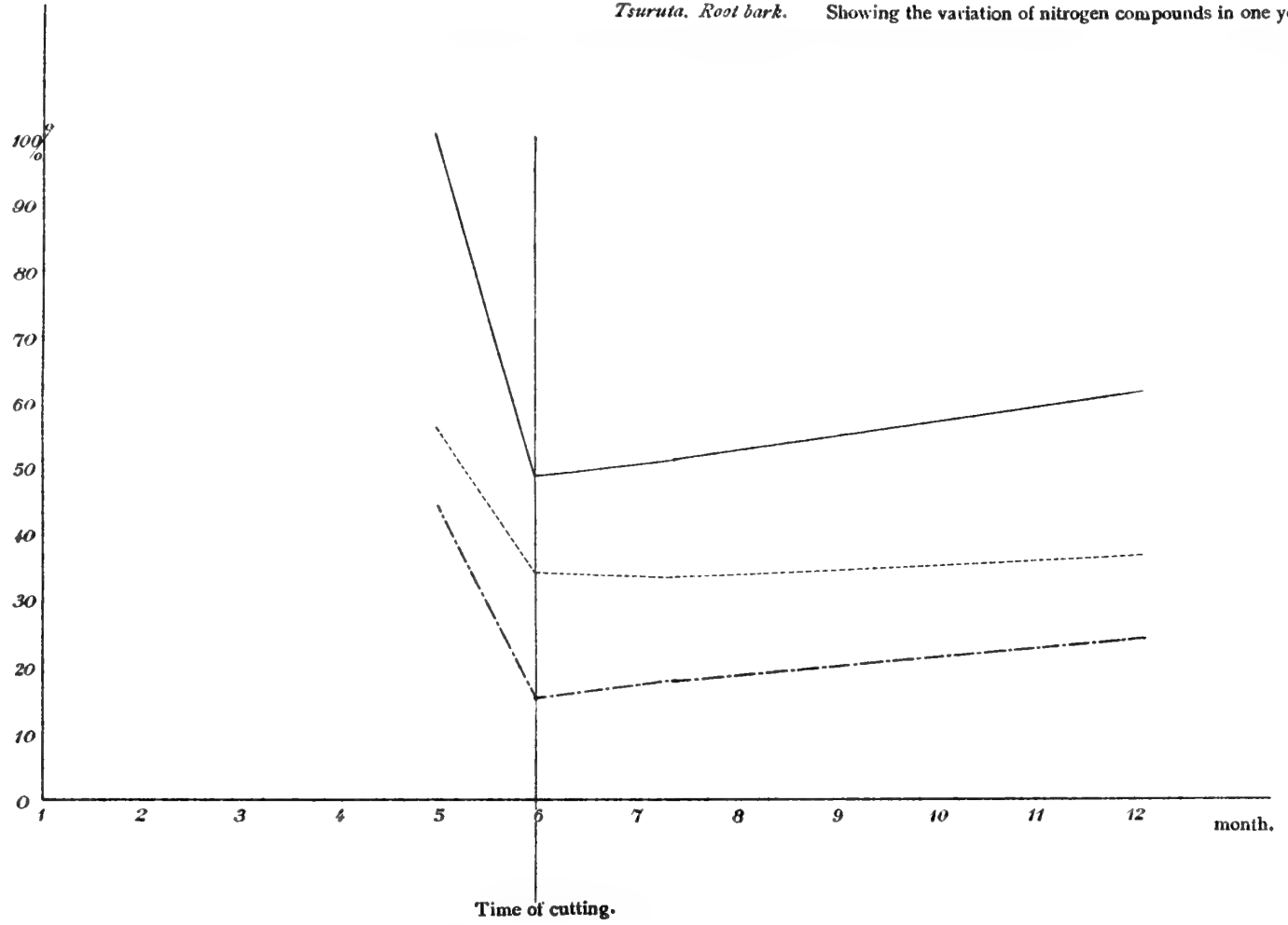
V. Showing the variation of Chemical composition according to seasons.

	Tsuruta		Hosoye		Takasuke					Jûmonji			
	May 6.	Oct. 15.	May 6.	Oct. 15.	May 22.	July 9.	Sept. 9.	July 9.	Sept. 9.	July 9.	Sept. 9.	July 9.	Sept. 9.
	Before cutting	After cutting	Before cutting	After cutting at autumn	Before cutting	After cutting	After cutttng	After cutting	After cutting	After cutting	After cutting	After cutting	After cutti
	Leaves	Leaves	Leaves	Leaves	Leaves	Leaves	Leaves	Stems	Stems	Leaves	Leaves	Stems	Stems
Average length of shoots								30.	180.			16.5	150.
Fresh weight of 1 shoot								5.45	101.			2.50	58.5
Dry weight 1 shoot								0.87	29.50			0.30	17 19
Fresh weight of 1 leaf	0.229	3.22	0.111	0.304	0.376	0.39	2.57			0 982	1.18		
Dry weight of 1 leaf	0.041	0.964	0.024	0.060	0.110	0.10	0.87			0.113	0.41		
Moisture	81.20	70.00	79.84	78.90	70.50	76.70	66.23	84.04	70.66	88.50	65.24	88 04	70.60
Dry matter	18.80	30.00	20.16	21.10	29.50	23.30	33.77	15.96	29.34	11.50	34.76	11.96	29 40
In 100 parts of dry matter,													
Crude proteids	48.94	26.88	41.00	33.00	23.75	30.50	22.88	15.38	5.38	36.63	22.38	21.75	5.31
Crude fats	1.92	5.80	2.26	5.19	3.46	2.84	7.34	0.83	1.10	4 26	7.29	2.20	1.00
Crude fibres	41.27	8.74	49.18	10.53	9.61	11.14	9.59	46.30	54.60	11 04	9.32	36.72	59.00
Nitrogen-free extracts		51.23		39.52	56.84	47.26	52.42	31.54	34.58	37.67	50.88	31.85	30.36
Ash	7.87	7.35	7.56	11.76	6.34	8.26	7.77	5.95	4.34	10.40	10.13	7.48	4.83
Total nitrogen	7.67	4.30	6.56	5.28	3.80	4.88	3.66	2.46	0.86	5.86	3 58	3.48	0.85
Albuminoid nitrogen	5.15	3.60	4.08	3.80	3.05	3.85	3.37	1.10	0.54	4.18	3.10	1.47	0 57
Non-albuminoid nitrogen	2.52	0.70	2.48	1.48	0·75	1.03	0.29	1.36	0.32	1.68	0.48	2.01	0.28

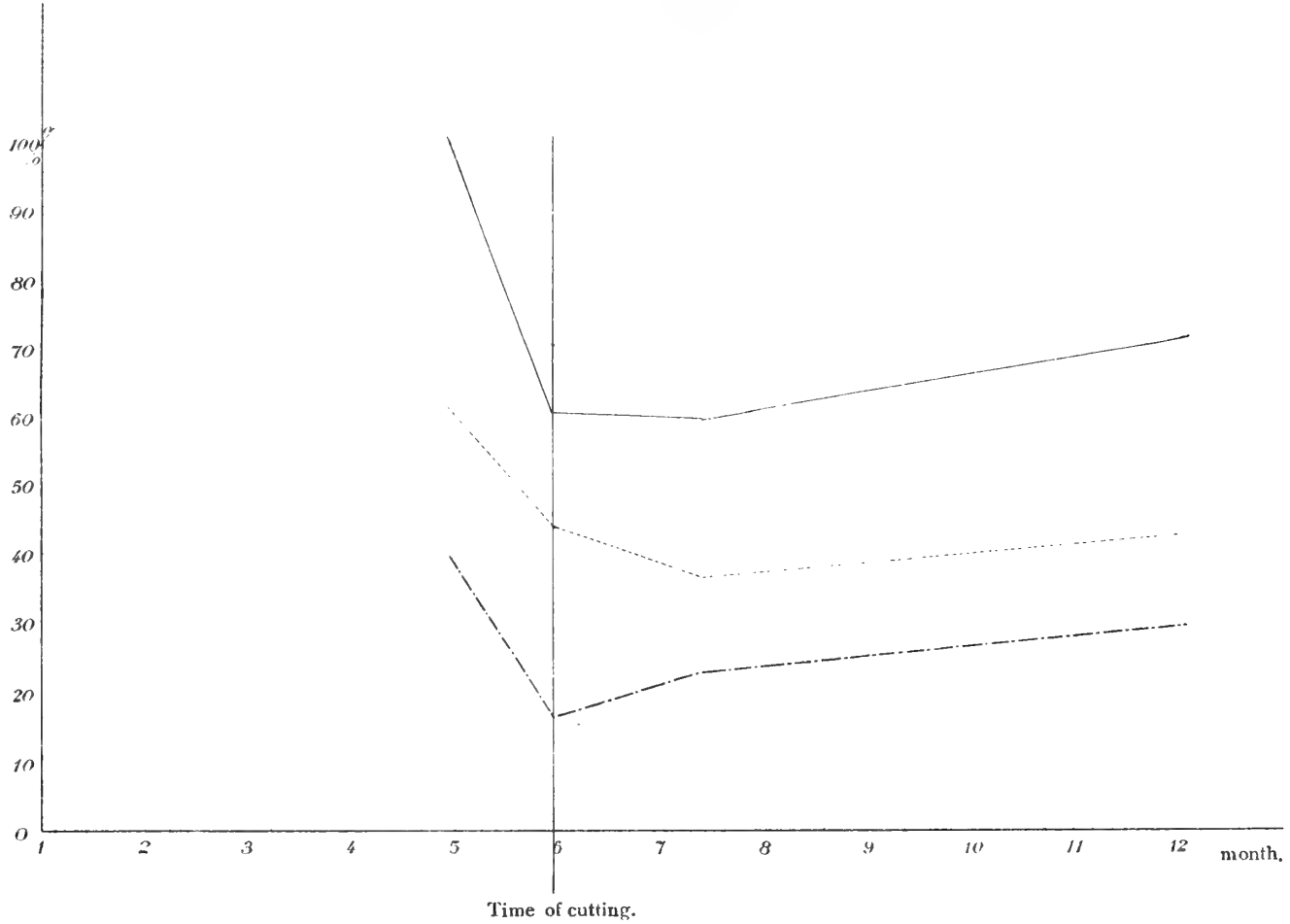
Takasuke. Root bark. Showing the variation of nitrogen compounds in one year;
(The total nitrogen on 28. calculated as 100)



Tsuruta. Root bark. Showing the variation of nitrogen compounds in one year.

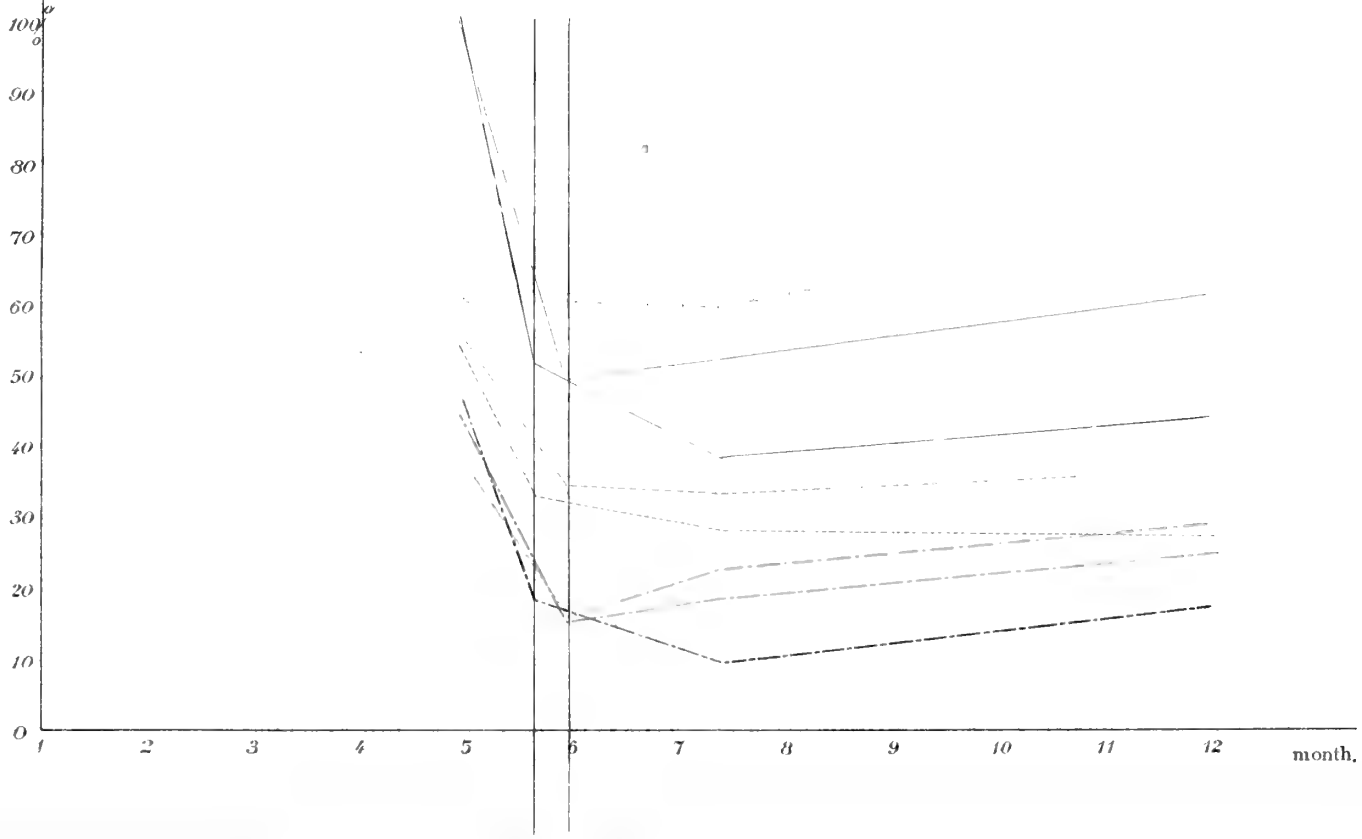


Fumonji. Root bark. Showing the variation of nitrogen compounds in one year.



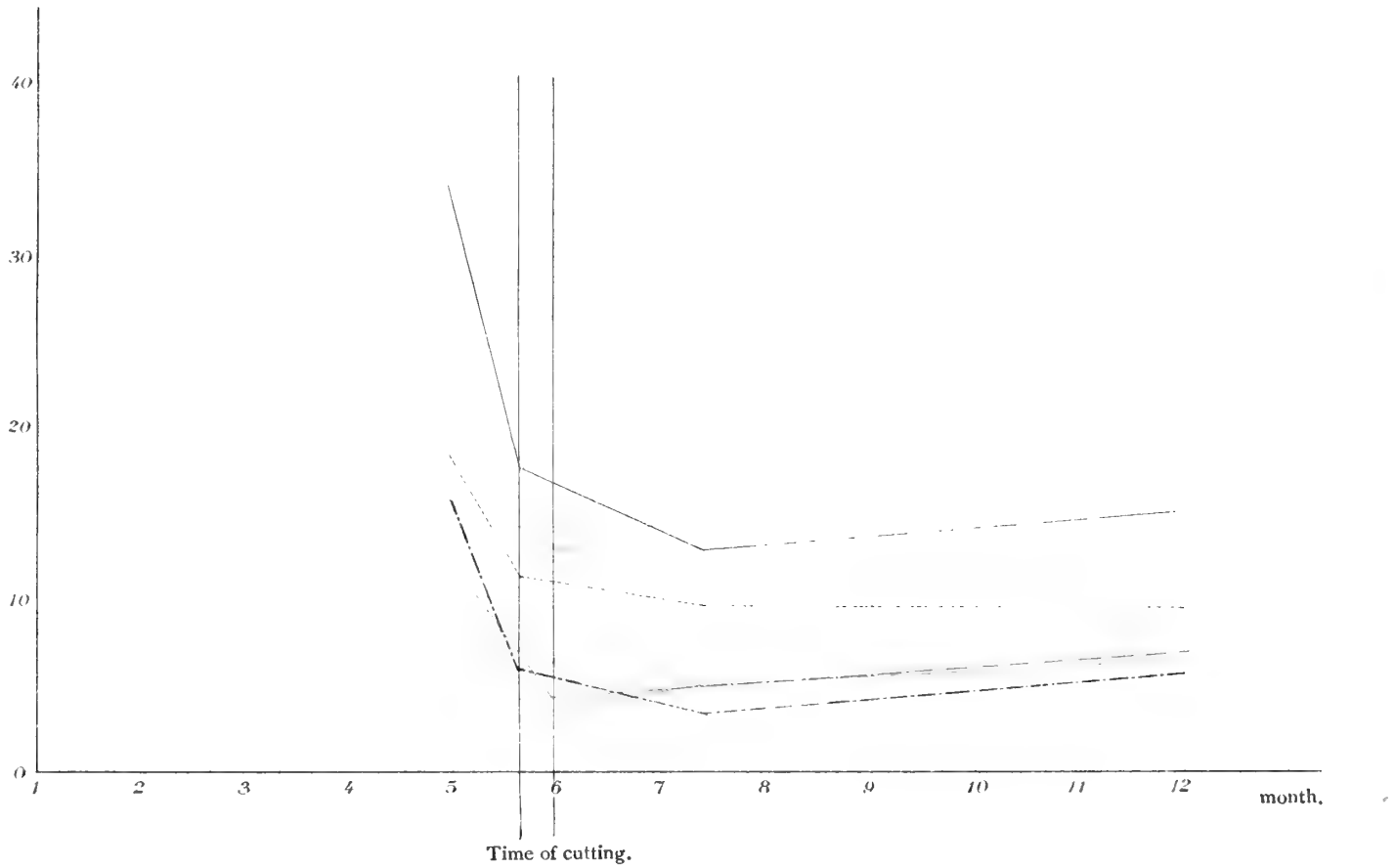
Showing the relative rapidity (intensity) of the variation of nitrogen compounds in one year.
(Total nitrogen on April 28. calculated as 100)

Black lines.....Takasuke
Red lines.....Tsuruta } Root bark.
Yellow lines.....Jūmonji

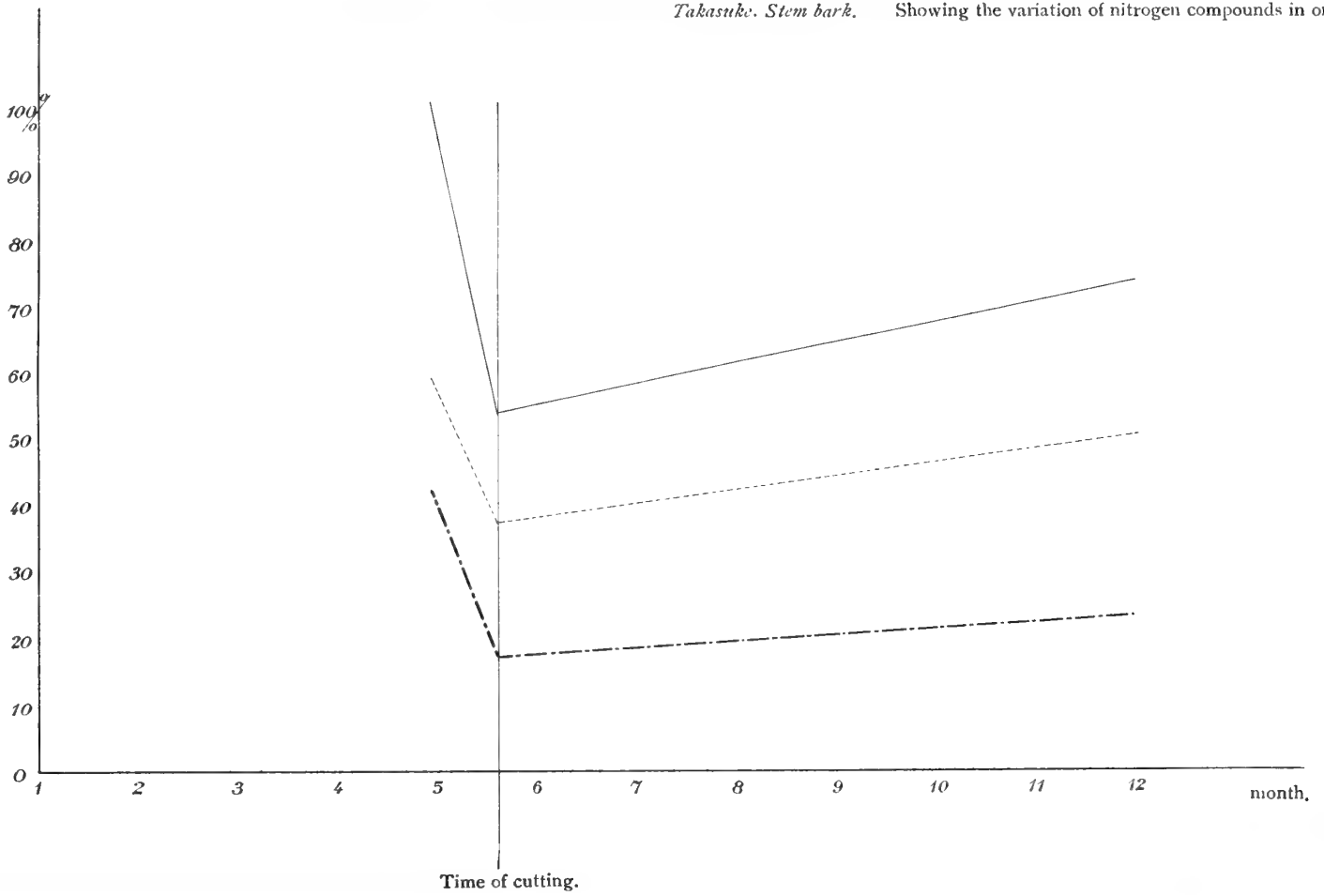


Showing the relative intensity of the migration (variation) of nitrogen compounds in one year.
(Percentage in the dry matter of root bark)

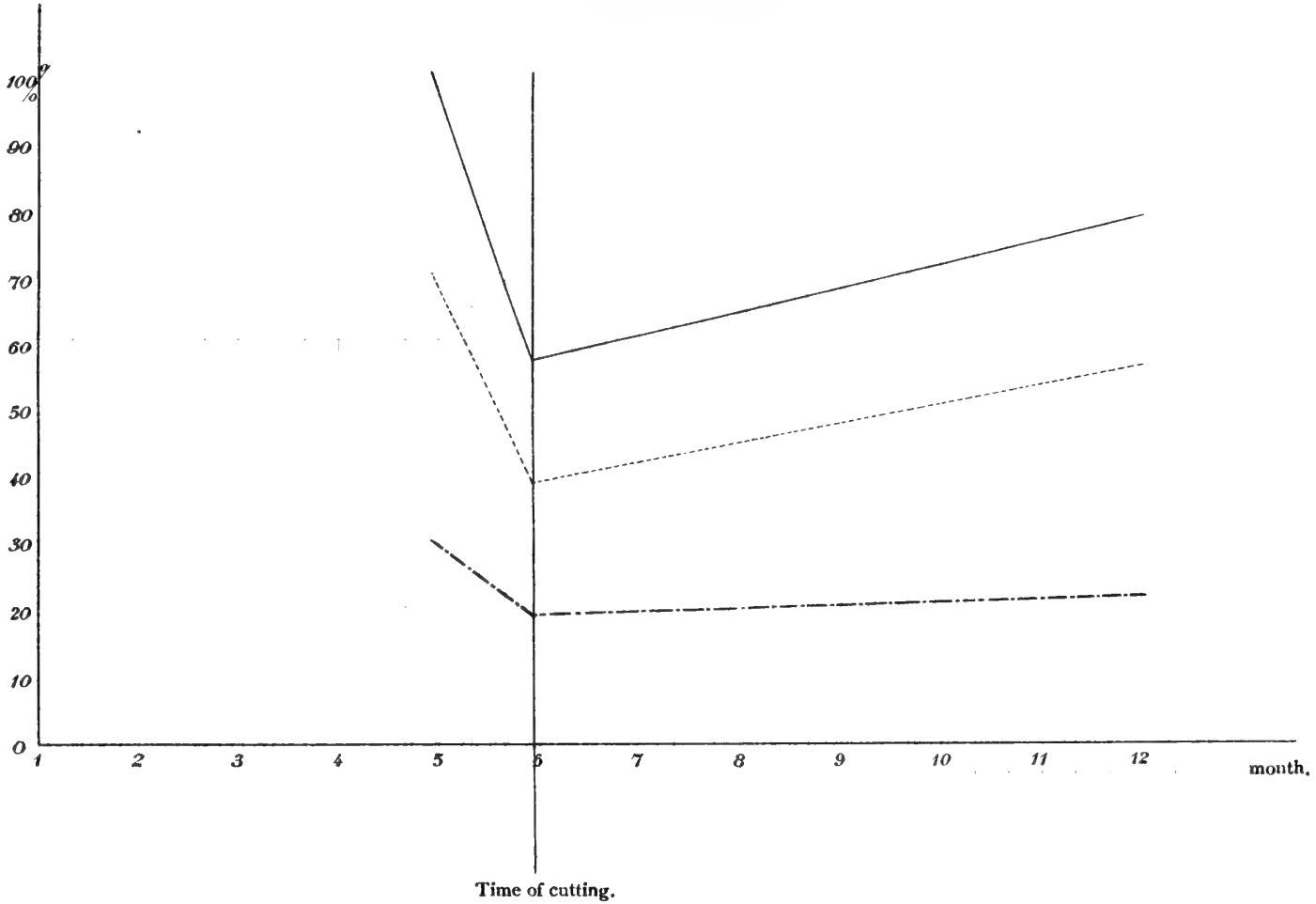
Black lines.....Tôkasuke
Red lines.....Tsuruta } Root bark.
Yellow lines.....Jumonji



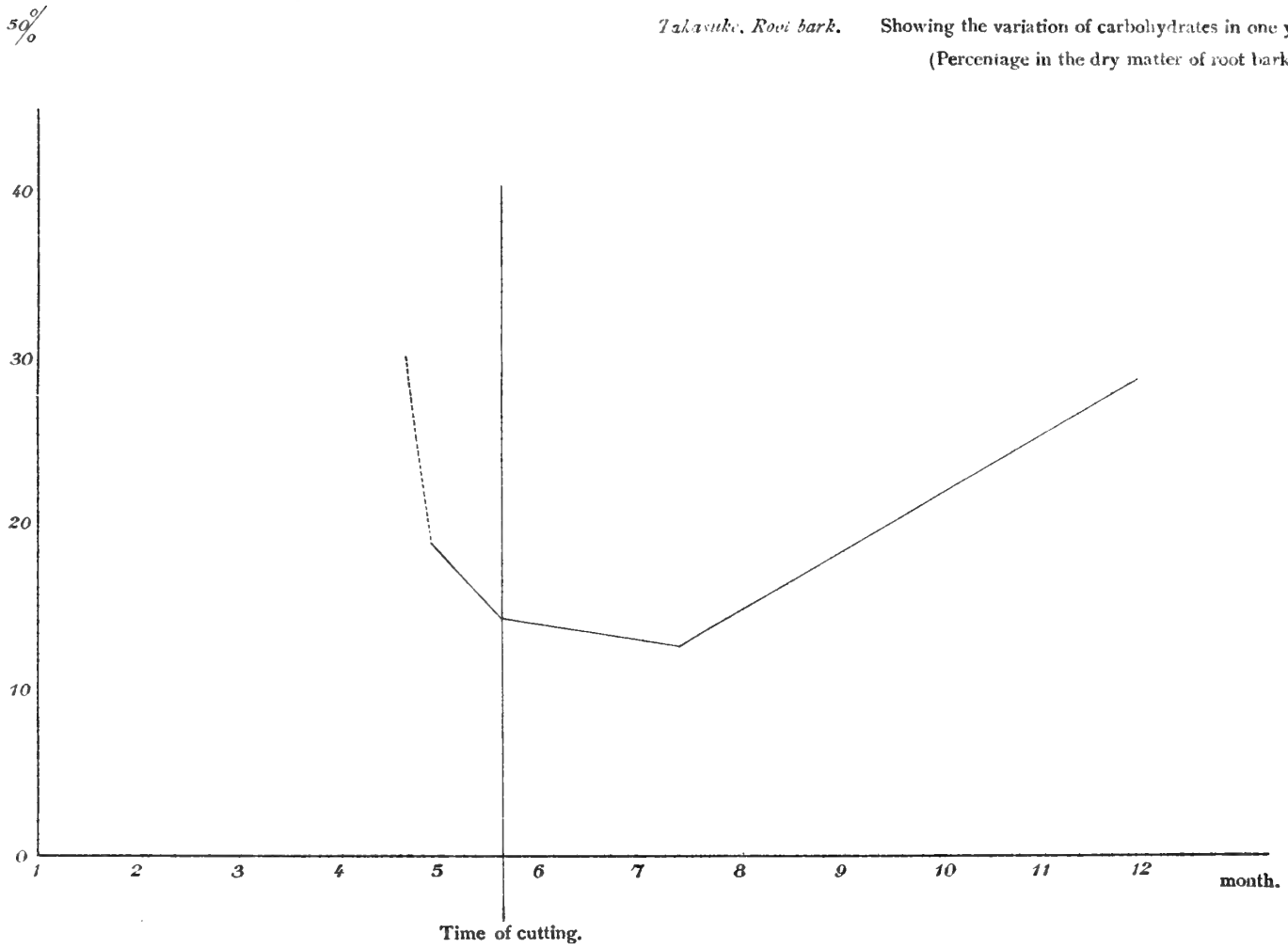
Takasuke. Stem bark. Showing the variation of nitrogen compounds in one year



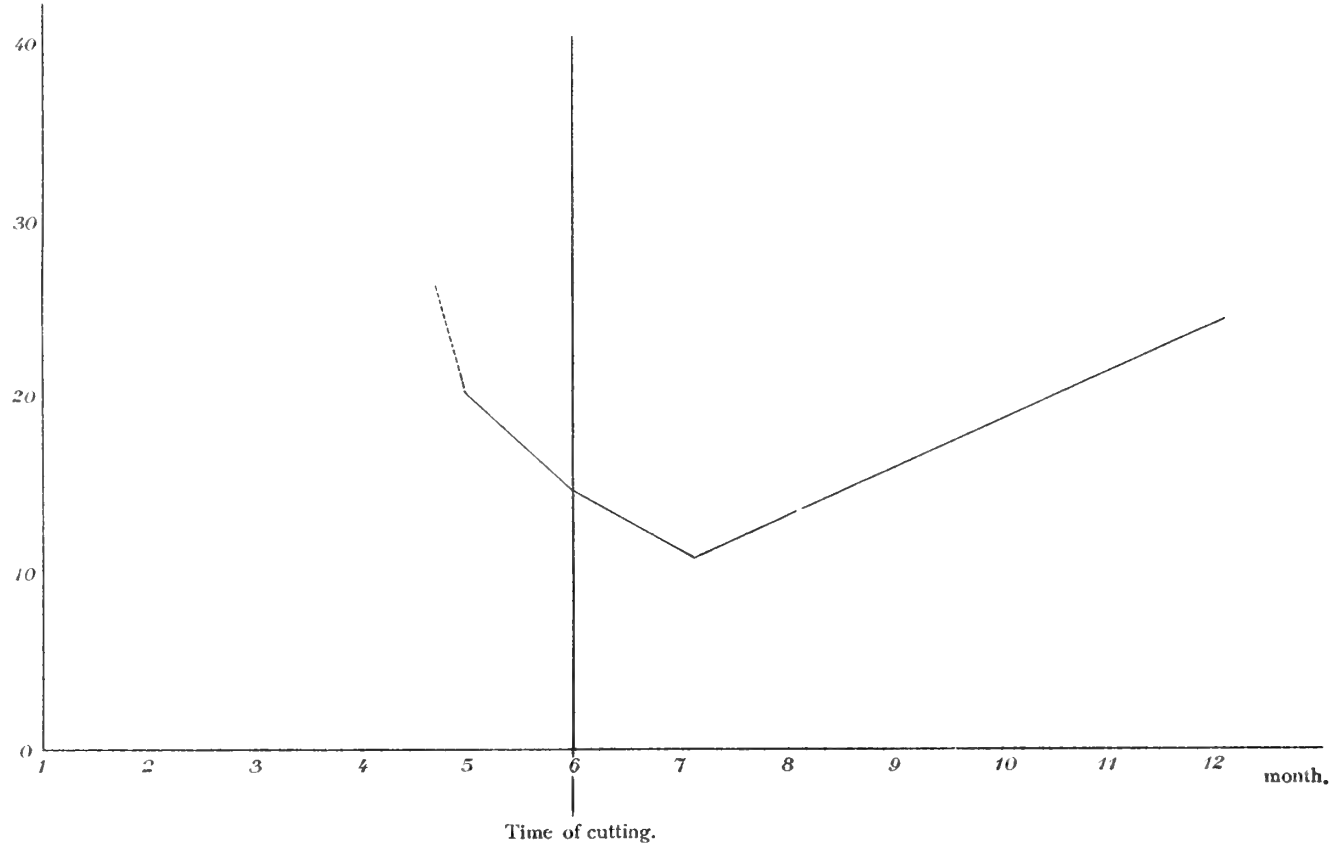
Tsuruta. Stem bark. Showing the variation of nitrogen compounds in one year.



Takayuke. Root bark. Showing the variation of carbohydrates in one year.
(Perceniage in the dry matter of root bark)

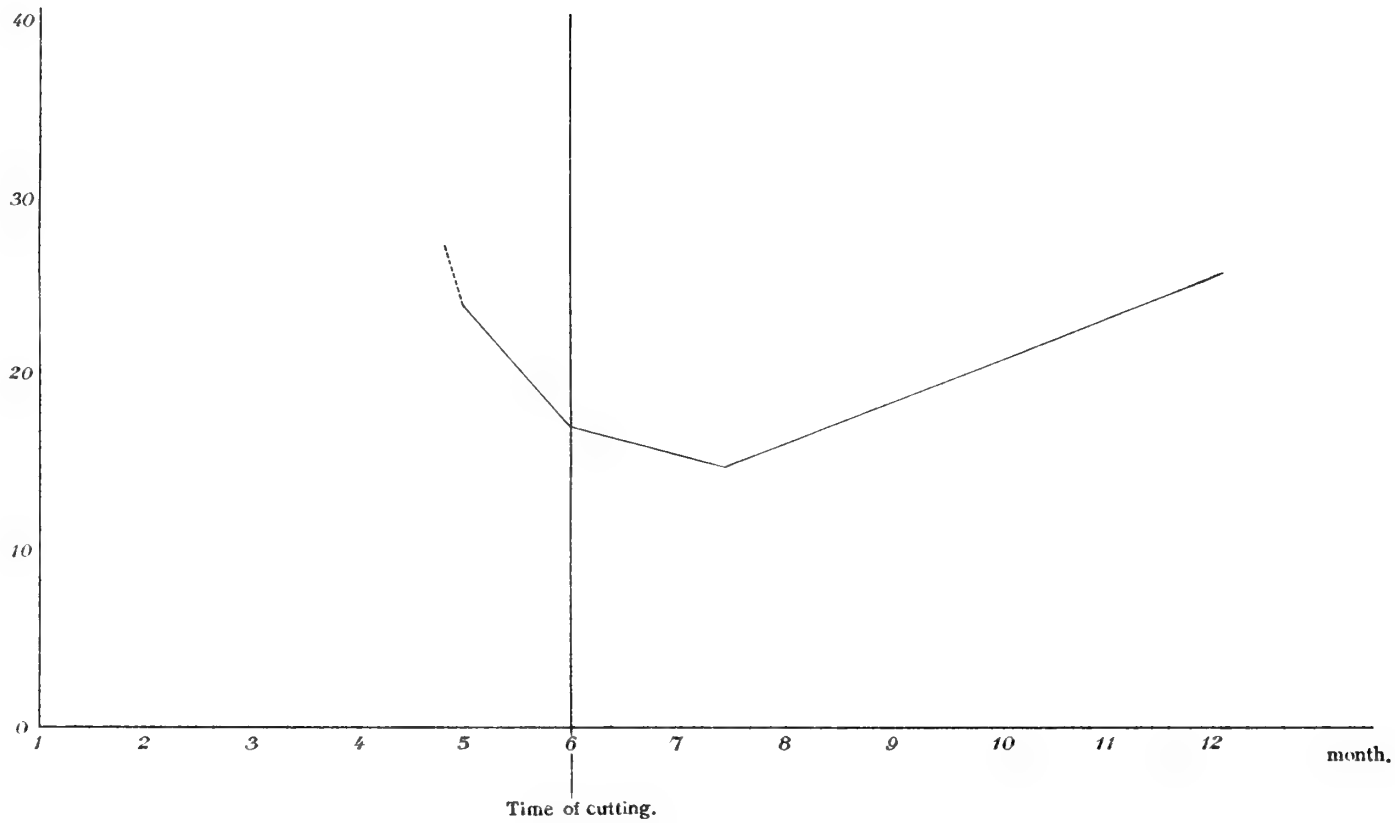


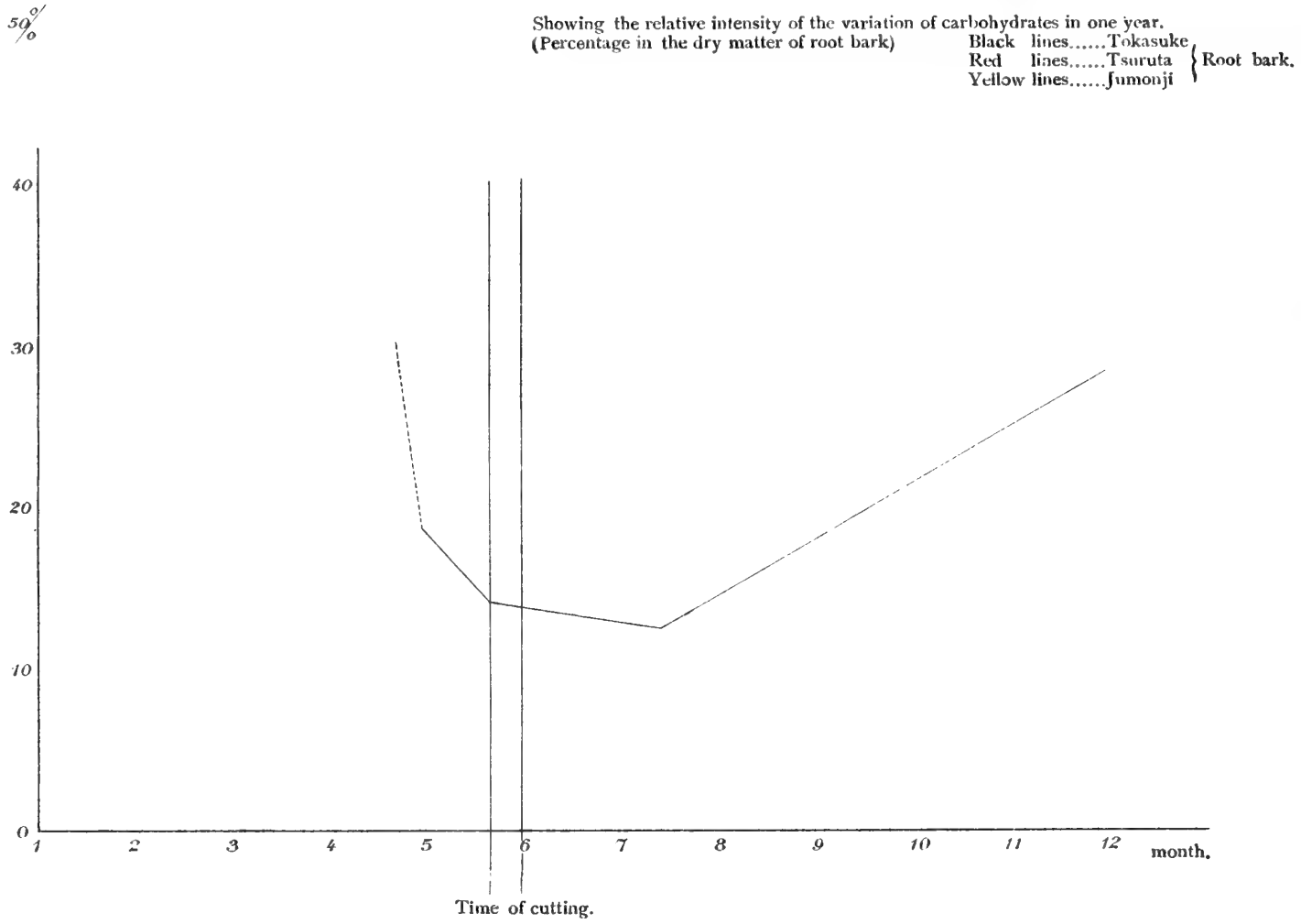
Tsurutra. Root bark. Showing the variation of carbohydrates in one year.



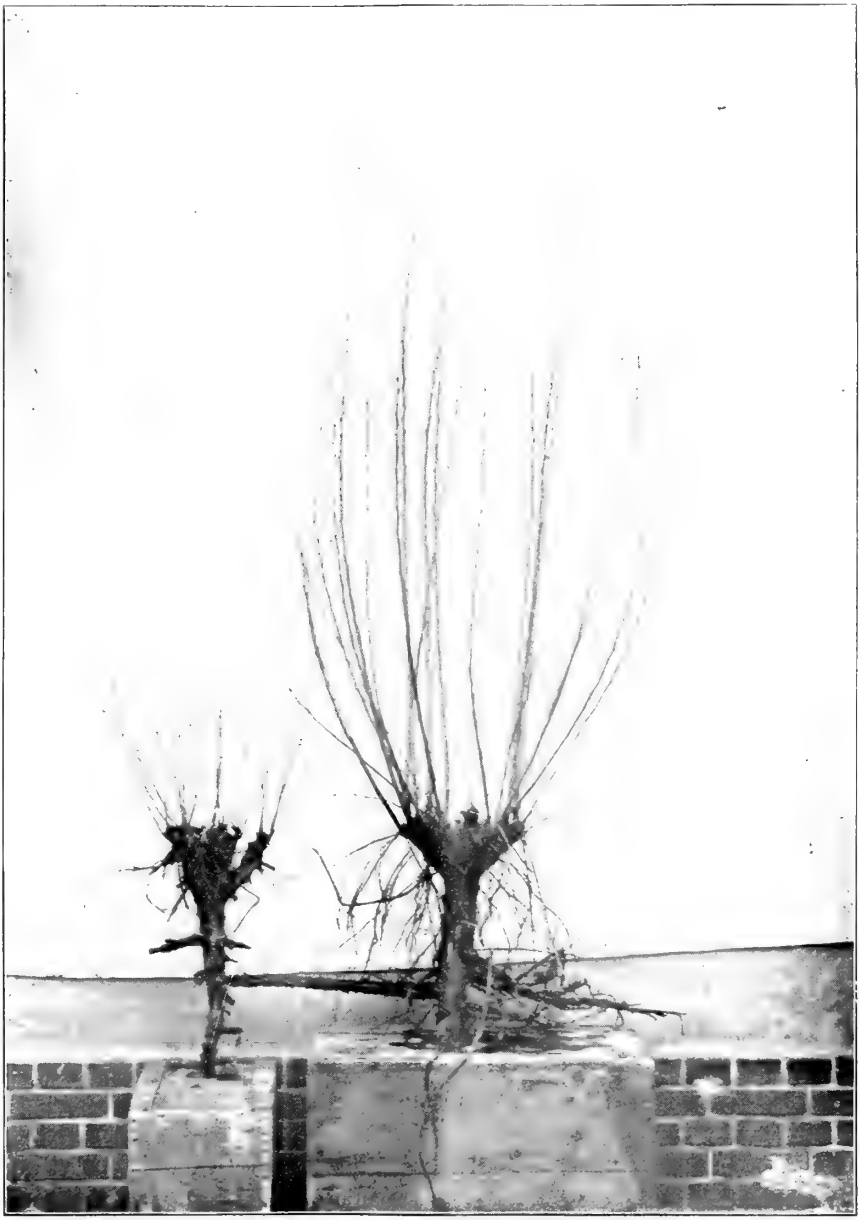
50%

Fūmonji. Root bark. Showing the variation of carbohydrates in one year.




50%
Showing the relative intensity of the variation of carbohydrates in one year.
(Percentage in the dry matter of root bark)
Black lines.....Tokasuke
Red lines.....Tsuruta
Yellow lines.....Jumonji
Root bark.
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
month.
Time of cutting.

A healthy plant (low cutting).



A healthy and a diseased plant compared.

Diseased plants in winter.

Normal growth without cutting.

A portion of stems diseased by frequent plucking off of the leaves.

Plants diseased by plucking off of all the leaves.

Two stems of a diseased plant recovered by layering.

Diseased plants recovered by layering.

Diseased plants.

Plants diseased by cutting.

A healthy and a diseased plant compared.

明治三十三年七月廿七日印刷
明治三十三年七月三十日發行

編輯兼發行者　農科大學

印刷者　佐久間衡治
東京市牛込區市ヶ谷加賀町一丁目十二番地

印刷所　株式會社秀英舍第一工場
東京市牛込區市ヶ谷加賀町一丁目十二番地

Zur Physiologie des Bacillus pyocyaneus.

VON

O. Loew und Y. Kozai.

Da der Bacillus pyocyaneus ein beträchtliches Interesse darbietet, haben wir eine Anzahl von Versuchen unternommen, welche über Abhängigkeit der Farbstoff- und besonders der Enzymproduction von den jeweiligen Nährsubstanzen weitere Anhaltspunkte liefern sollten. Ueber seine Entwicklung in verschiedenen Nährlösungen liegen zwar bereits Mittheilungen vor, besonders von *F. Hüppe* und von *Thum*,[1] doch hatten diese nicht speciell die Enzymbildung, sondern hauptsächlich die Farbstoffbildung verfolgt. Ausser dem blauen Pyocyanin ($C_{14}H_{14}N_2O$, nach *Leidderhose*) wurden bekanntlich noch zwei weitere Farbstoffe in Culturen des Pyocyaneus beobachtet, das gelbbraune Pyoxanthin (*Fordas*) und ein weiterer, dessen ammoniakalische Lösung eine grüne Fluorescenz zeigt und in durchfallendem Lichte gelb erscheint (*Kunz*). Doch scheinen gelegentlich auch davon verschiedene Farbstoffe aufzutreten, wie durch *Babes* wahrscheinlich geworden ist. Jedenfalls hat die Varietät des Pyocyaneus einen beträchtlichen Einfluss in dieser Richtung.[2]

Erhöhtes Interesse kann aber die Production eines Enzyms beanspruchen, welches in genügender Concentration nicht nur den Bacillus selbst wieder auflöst, sondern in verschiedenem Energiegrade auch Cholera-, Typhus-, Anthrax-, und Diphtherie-

[1] Arbeiten aus dem bacteriologischen Institut der technischen Hochschule in Karlsruhe, Bd. I, p. 337 (1897). *Thum* verwendete Asparagin, Ammonsalze organischer Säuren, sowie Gemische von Harnstoff mit Glucose und mit Mannit.

[2] Es ist von wesentlichem Interesse, dass auch die Anwesenheit von Magnesium als Sulfat notwendig ist, die Farbstoffe zu bilden (*Noesske*), was mit analogen Beobachtungen am Bac. fluorescens (*Thum*) und am Bac. prodigiosus (*Kuntze*) übereinstimmt.

bacillen.[1] Reaction und Concentration des Nährmediums, sowie die chemische Structur der organischen Nährsubstanzen üben einen beträchtlichen Einfluss in dieser Richtung aus. Neutrale oder nur sehr schwach alkalische Reaction sind am günstigsten. Ferner ist der Luftzutritt in Betracht zu ziehen. *Krause*[2] beobachtete üppige Entwickelung in einer Wasserstoffatmosphäre, aber keine Farbstoffbildung. Diese erfolgt erst, wenn man Luft zutreten lässt. In einer Kohlensäureatmosphäre gedeiht nach ihm dieser Bacillus nicht, ja soll er nach 24 Stunden absterben. Dass auch die Toxinbildung bei verschiedenen Baeterienarten durch manche Umstände bedeutend beeinflusst wird, ist bekannt.

Dasselbe gilt aber auch für die Bildung von „Schleim," welche beim Bac. pyocyaneus auftreten oder ausbleiben kann. Die Schleimbildung ist bekanntlich nach *Nägeli* Folge einer weit getriebenen Aufschwellung der Zellmembranen. Man kann also folgern, dass bei ausbleibender Schleimbildung weit weniger Membransubstanz gebildet wird, oder dass in der betreffenden Nährlösung Enzyme gebildet werden, welche die Membranen völlig wieder auflösen. *Marpmann*[2] schreibt in Bezug auf die Membranbildung (Centr. Blatt. f. Bakt. II Abt. 6, 674): „Es steht somit fest, dass die Substanz der Hülle, resp. Kapsel, nicht bei allen Spaltpilzen aus denselben Stoffen gebildet ist und dass die Stoffe nach Art der chemischen Zusammensetzung des Nährbodens sehr variiren und endlich, dass diese Hüllen bei besonderen Verhältnissen von Temperatur und Nährboden überhaupt nicht gebildet werden."

Was die Enzymbildung betrifft, so hat *Auerbach*[3] mitgetheilt, dass bei *Bacterium vulgare* die Bildung des Gelatine verflüssigenden Enzyms durch Gegenwart von Zucker verhindert wird.[4] Aehnliches haben wir beim B. pyocyaneus beobachtet betreffs

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[1] *Emmerich* und *Loew*, Zeitschrift für Hygiene Bd. 31 (1899). Von der erhaltenen Pyocyanase-Lösung konnte 1 cc. in 2 Stunden über 4 Millionen Milzbrandbacillen, in 6 Stunden 27 Millionen Pestbacillen, in 24 Stunden 23 Millionen Staphylococcen, in 3 Tagen 29 Millionen Typhusbacillen und in 4 Tagen 20 Millionen Diphtheriebacillen auflösen. In neuester Zeit wurde ein noch weit stärkeres Enzym erhalten.

[2] Centralbl. für Bakt. Bd. 27, p. 769 (1900).

[3] Archiv f. Hygiene 1897.

[4] Auch Anaërobiose scheint manchmal Einfluss auf die Enzymbidung zu äussern. So verliert nach *San Felice* der *B. pyocyaneus* bei anaërober Kultur seine Fähigkeit zu peptonisiren.

der Bildung des bacteriolytischen Enzyms. Die Bildung lässt sich leicht schon daran erkennen, dass nach anfänglich reichlicher Entwickelung bald ein Zeitpunkt eintritt, wo nicht nur die weitere Entwickelung sistirt wird, sondern die vorhandenen Bacterienmassen Agglutination zeigen, alle Bacterien absterben und bis auf minimale Granula und Kryställchen das ganze Sediment gelöst wird.

In manchen Lösungen tritt dieser Zeitpunkt der Wiederauflösung bald, in andern sehr spät, in manchen vielleicht gar nicht ein. Während in bloser Peptonlösung mit mineralischen Nährsalzen dieses Enzym reichlich gebildet wird, entsteht weit weniger, wenn ausserdem (1–2%) Glycerin zugezetzt wird, ja in einer Mischung von 1% Pepton mit 2% Rohrzucker blieb das Bakteriensediment selbst nach neun Wochen noch ungelöst.

In unserer ersten Versuchsreihe wandten wir auf eine relativ grosse Menge stickstofffreien Materials absichtlich nur sehr geringe Mengen stickstoffhaltigen Materials (mit Ausnahme von No. VI.) an. Nach der üblichen dreimaligen Behandlung im Dampftopf wurden die Lösungen mit einer pathogenen Art des B. pyocyaneus, der in Berlin aus Eiter gezüchtet worden war, inficirt. Jede Lösung, 100 cc, befand sich in einem ca. 250 cc. fassenden, einen Watteverschluss tragenden *Erlenmeyer* Kolben. Die anorganischen Nährsalze bestanden, wo nicht speciell anders bemerkt wird, überall aus: 0,2% neutralem Kaliumphosphat, 0,2% Kochsalz, 0,1% Natriumsulfat und 0.01% Magnesiumsulfat. Letzteres Salz wurde in einer zweiprocentizen Lösung separat sterilisirt und bei der Infection zugesetzt, um die Ausfällung des meisten Magnesiums als Phosphat beim Sterilisiren zu vermeiden. Die organischen Nährsubstanzen bestanden aus:

No. I. Glycerin 1%; asparaginsaures Natron 0.1%.
No. II. Glycerin 1%; Pepton 0.1%.
No. III. Glucose 1%; Pepton 0.1%.
No. IV. Weinsaures Kali-Natron 1%; Pepton 0.1%.
No. V. Essigsaures Natron 1%; Pepton 0.1%.
No. VI. Glycerin 0.1%; Pepton 1%.[1]

Die im Brütschrank gehaltenen Proben zeigten folgende Erscheinungen:

[1] Das verwendete Pepton war nicht das an Albumosen reiche von Witte, sondern war albumosenfreies, von Gehe & Co. bezogenes.

No. I. Nach 3 Tagen sehr geringe Entwicklung, die auch nach acht weiteren Tagen nur sehr mässig blieb, Färbung schwach gelbgrünlich.

No. II. Nach 3 Tagen mässige Trübung und blaugrüne Färbung, welche bei weitererm Aufenthalt im Brutschrank verschwand, um beim mässigen Schwenken des Gefässes immer wieder zu erscheinen. Reaction allmälig spurenweise sauer; offenbar ermöglichte das Glycerin anaërobes Leben, wobei der Farbstoff zu einer Leukoverbindung reducirt wurde, die unter Sauerstoffaufnahme wieder in den Farbstoff überging. Eine Wiederauflösung der gebildeten Bacterienmassen war nach 2 Wochen nicht zu bemerken.[1]

No. III. Hier trat gar keine blaugrüne Färbung, sondern nur weisse Trübung auf. Selbst nach 10 Tagen war die Vegetation nur gering und trat selbst beim Umschwenken des Glases keine Färbung auf; es war also auch keine Leukoverbindung vorhanden. Offenbar kann der Bacillus Glucose nur gut verwerthen, wenn eine grössere Menge stickstoffhaltigen Materials vorhanden ist. Die Reaction war kaum merklich sauer.

No. IV. Hier waren die Erscheinungen wesentlich dieselben wie in III, nur war die Vegetation noch geringer. Auch Zusatz geringer Mengen kohlensauren Natrons brachte in III und IV keine wesentliche Aenderung zustande. Weinsäure kann daher kaum von diesem Bacillus verwerthet werden, so lange die gleichzeitig gebotene Menge stickstoffhaltiger Nährsubstanz sehr gering ist. Ueber ihre Verwendbarkeit zur Farbstoffproduction gehen die Meinungen noch auseinander; *Thum* folgerte, dass die Weinsäure hierzu nicht geeignet sei, während *Hüppe* die entgegengesetzte Beobachtung machte. Wahrscheinlich verhalten sich verschiedene Varietäten des B. pyocyaneus in dieser Hinsicht nicht gleich.

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[1] Auch nach Zusatz von geringen Mengen kohlensauren Kalis nicht; doch trat dann allmälich Agglutination ein.

No. V. Hier war nach fünf Tagen eine schön dunkelgrüne Färbung eingetreten, welche sich auch nach tagelangem Stehen nicht verminderte. Hier war also das Nährmedium der Bildung eine Leukoverbindung nicht günstig; die Entfärbung fand nicht statt, weil hier keine anaërobe Existenz in den unteren Schichten der Flüssigkeit ermöglicht war, wie in No. II. Jeden Tag war eine dünne Haut an der Oberfläche gebildet, welche beim Umschütteln zu Boden fiel. Die Vegetation hörte nach 10 Tagen auf, die Lösung wurde schleimig, die Bacterienmassen agglutinirten, doch schritt die Wiederauflösung nur sehr langsam fort. Die Schleimbildung erreichte jedoch nicht den starken Grad, wie er in Bouillonculturen beobachtet wird.

No. VI. Hier war die Vegetation am lebhaftesten und Farbe und Geruch des Pyocyaneusculturen am besten entwickelt. Nach 10 Tagen trat Agglutination und beginnende Wiederauflösung ein. Reaction schwach alkalisch, doch waren nur Spuren Ammoniak gebildet. Nach 15 Tagen liess eine in Bouillon abgeimpfte Probe erkennen, dass die Bacterien bereits wieder abgestorben waren, es trat keine Spur von Vegetation in dieser Probe auf. Schleimbildung war relativ gering, geringer als bei No. V.

Als wesentliche Resultate ergeben sich also:

1. Eine starke Vermehrung stickstoffreier Nährstoffe, resp. Verminderung stickstoffhaltiger, ist der Enzymbildung oder auch der Vegetation im Allgemeinen nicht günstig.
2. Glycerin ist unter dieser Bedingung ein besseres Nährmaterial als Glucose, aber ein schlechteres als essigsaures Natron.
3. Die Schleimbildung wurde durch essigsaures Natron mehr befördert als durch die anderen Nährsubstanzen, jedoch noch nicht in dem Grade wie durch Bouillon.
4. Am günstigsten für Farbstoff- und Enzymproduction erwies sich die 1% Peptonlösung, mit nur 0.1% Glycerin, auch der specifische Geruch war am besten entwickelt, die Schleimbildung war gering.

In der zweiten Versuchsreihe wurden sowohl verschiedene Stickstoffquellen, als auch einige stickstofffreie Substanzen betreffs ihres Nährwerthes mit einander verglichen. Die organischen Nährstoffe waren :

No. VII. Asparagin 0,5% ; Glycerin 1,0% ; Pepton 0,5%.
No. VIII. Asparagin 0,5% ; Glycerin 1,0%.
No. IX. Asparagin 0,5% ; Glycerin 0,2%.
No. X. Asparagin 0,5% ; Essigsaures Natron 0,6%.
No. XI. Asparagin 0,5% ; Methylalcohol 1%.[1]
No. XII. Tyrosin 0,2% ; Glycerin 1,0% ; Essigsaures Natron 0,6%.
No. XIII. Leucin 0,2%[2]; Glycerin 1,0% ; Essigsaures Natron 0,6%.
No. XIV. Harnstoff 1,0% ; Glycerin 1,0% ; Essigsaures Natron 0,6%.

Die Mengen der Nährlösungen waren die gleichen wie bei der ersten Reihe, doch war die Temperatur anfänglich niedriger (18—22° C., statt 30—35° C.) Nach drei Tagen ergab sich Folgendes :

No. VII. Viele Flocken aber nur geringe grüne Färbung.
No. VIII. Schöne Haut und etwas mehr Färbung wie bei VII.
No. IX. Mässige Vegetation, wenig Färbung.
No. X. Mehr Vegetation und Färbung als in IX.
No. XI. Nur spurenweise weissliche Trübung.
No. XII. Wenig entwickelt, nur gelbliche Färbung.
No. XIII. Geringe Entwickelung, aber blaugrüne Färbung.
No. XIV. Spur Trübung, farblos.

No. XII u. XIII wurden nun 2 Tage bei 36° C. gehalten, aber das änderte an dem Resultate nicht wesentlich, in beiden Fällen blieb die Vegetation sehr gering, und zwar war sie bei No. XII (Tyrosin) noch weit geringer als bei No. XIII. (Leucin). Bei No. XII stellte sich ferner nicht einmal eine grünliche

[1] Der Methylalcohol wurde nach dem Sterilisiren zugesetzt.

[2] Beim Vergleich der Stickstoffquellen sollten diese allerdings in gleichen Stickstoffmengen entsprechenden Quantitäten verwendet werden, doch scheitert dieses öfters an der Schwerlöslichkeit gewisser Verbindungen, wie Z. B. beim Tyrosin, andererseits wäre selbst bei Befolgung jenes Grundsatzes öfters schon desshalb eine strenge Beurtheilung ausgeschlossen, weil die Mengen des gleichzeitig mit den Stickstoffquellen eingeführten Kohlen- und Wasserstoffs zu grosse Differenzen aufweisen würden. Man vergleiche Z. B. Leucin mit Asparagin in dieser Hinsicht.

Färbung ein, es war lediglich eine schwache schmutzig gelbe Färbung vorhanden, welche auch beim Umschwenken des Gefässes und vermehrter Luftzufuhr sich nicht im Geringsten veränderte. Es konnte wohl keinem Zweifel bei Vergleich mit No. X unterliegen, dass Leucin und Tyrosin weit weniger günstige Stickstoffquellen für den Bac. pyocyaneus sind als Asparagin, denn die Unterschiede waren so auffallend, dass man jene geringe Vegetation nicht etwa nur der geringeren Menge Stickstoff hätte zuschreiben dürfen. Ferner wurde es evident, dass zur Farbstoffbildung Leucin und Asparagin geeignet sind, aber nicht Tyrosin. Auch weiterhin änderte sich an diesem Verhalten nichts und wenn auch beim Leucin etwas mehr Vegetation sich entwickelt hatte als beim Tyrosin, so war doch selbst nach 10 weiteren Tagen keine grössere Entwickelung zu beobachten. Die Ueberimpfung aus diesen Lösungen aber in sterilisirte Bouillon ergab das Vorhandensein von noch lebenden Bacillen.

Zehn Tage nach der Impfung zeigte sich bei den übrigen Proben Folgendes:

No. VII. Haut und starker Bodensatz, Färbung gelblich, beim Umschwenken grün.

No. VIII. Vegetation schwächer als bei VII, sonst gleiches Verhalten.

No. IX. Weniger Entwickelung als in VII. Farblos, beim Umschwenken sich allmälig grün färbend.

No. X. Dickere Haut als in IX., grüne Färbung.

No. XI. Flockige Massen am Boden. Schwach gelblichgrün, sich nicht ändernd bein Schütteln.

No. XIV. Dünne weisse Haut, Flüssigkeit farblos, nicht grün werdend beim Schütteln.

Als nun die Proben XI und XIV bei Brutwärme gehalten wurden, zeigten sie bald eine lebhafte grüne Färbung. Bei XIV trat allmälich eine lebhafte Vegetation ein und 30 Tage nach der Impfung war diese Probe ziemlich schleimig geworden, das Bacteriensediment zeigte Agglutination, war aber nicht merklich gelöst worden, auch enthielt es noch lebende Bacillen wie die Impfung in Bouillon bewies. Mässige Schleimbildung trat auch in den übrigen Proben ein. Eine völlige Wiederauf-

lösung der Bacterienmassen war nirgends zu bemerken.[1] Ammoniak war in mässiger Menge überall vorhanden, wo Asparagin als Nährstoff diente; ziemlich gering war die *Nessler*sche Reaction in XIV, Harnstoff wurde blos in geringem Grade gespalten.

Als wesentliche Resultate ergeben sich aus

Reihe II: *Leucin und Tyrosin sind schlechte Nährstoffe für die pathogene Varietät des Bacillus pyocyaneus; Asparagin ist ein besserer Nährstoff. Leucin ermöglicht die Farbstoffbildung während Tyrosin hiefür ungeeiget scheint. Glycerin ermöglicht die Bildung des Leukofarbstoffs und ist ein besseres Nährmittel als Methylalcohol.*

In der dritten Versuchsreihe wurden stickstoffhaltige Stoffe ohne Beimengung stickstofffreier im Bezug auf ihren Nährwerth für den B. pyocyaneus verglichen. Die Lösungen enthielten je ein Procent der folgenden Stoffe:

No. XV. Pepton.
No. XVI. Betain (das salzsaure Salz mit kohlensaurem Natron neutralisirt).
No. XVII. Asparagin.
No. XVIII. Glycocoll.
No. XIX. Hydantoin.
No. XX. Kreatin.

Nach 2 Tagen bei 36° ergab sich (Jan. 14) Folgendes:

Pepton: schmutzig gelbe, starke Trübung, weisse Haut; beim Umschwenken grünliche Farbe.
Betain: Starke Trübung und Haut, Färbung schwach grünlich, stärker grünblau werdend beim Schwenken.
Asparagin: Gelbgrüne Fluorescenz, beim Umschwenken blaugrüne Färbung. Starke Trübung und Haut.
Glycocoll: Äusserst schwache weisse Trübung.
Hydantoin: Mässige weisse Trübung, nicht verändert beim Schütteln.
Kreatin: Wie bei Hydantoin.

Nach zwei weiteren Tagen war nur bei der Asparagin-Nährlösung eine wesentliche Aenderung zu bemerken. Hier waren die Bacterienmassen zu einer dicken zähen Masse verei-

[1] In obigen Lösungen VIII u. IX ergab sich jedoch ein wesentlicher Unterschied; in VIII bedingte der grössere Glyceringehalt eine geringere Enzymbildung als in IX, wo eine partielle Wiederauflösung stattgefunden hatte und schliesslich nur sehr wenig Bacterienseliment vorhanden war.

nigt und keine neue Haut zu bemerken. Dieses auffallend frühe Aufhören der Vegetation nach der anfangs so überaus lebhaften Entwicklung ist allem Anschein nach nur der schädlichen Wirkung der entstandenen nicht unbeträchtlichen Menge kohlensauren Ammoniaks zuzuschreiben. Das Schleimigwerden der Flüssigkeit, sowie der flockigen Bacterienmassen erinnert ganz an das Verhalten desselben Bacillus in Bouillon.

Zehn Tage nach der Infection :

Pepton : Vegetation nahezu abgelaufen, Bodensatz sehr gering, Färbung schmutzig graugrün.

Betain : Neue Haut nach dem Umschütteln allmälig gebildet, viel flockiges Sediment. Färbung gelblich, nach dem Umschütteln grün.

Glycocoll, Hydantoin und Kreatin : Nur geringfügige weisse Trübung.

Dreizehn Tage nach der Infection war bei Pepton die Vegetation abgelaufen ; zwei Tage später auch bei Betain, wo nun auch die grüne Färbung nicht wieder in die gelbe überging. Ammoniakbildung hatte in beiden Fällen stattgefunden. Schleimbildung war in beiden ausgeblieben. Ein auffallender Unterschied bestand jedoch im Betreff des Sedimentes ; bei Pepton nur geringer krystallinischer Bodensatz, bei Betain nicht unbeträchtliche Mengen flockiger Massen, welche indessen nur zu kleinem Theile aus unveränderten Bacterien bestanden und ausser amorphen Zerfallsproducten noch lange dünne Krystallnadeln enthielten. Auffallend blieb der so geringe Nährwerth von Glycocoll, verglichen mit dem von Betain und Asparagin.

Fassen wir die wesentlichsten Punkte der drei Versuchsreihen zusammen so ergibt sich :

1. Asparagin ist ein besserer Nährstoff für den B. pyocyaneus als Leucin, Tyrosin, Glycocoll, Hydantoin und Kreatin.
2. Essigsaure Salze sind günstigere Nährstoffe als weinsaure.
3. Essigsaure Salze und Glycerin begünstigen die Schleimbildung, jedoch nicht in dem Grade wie Bouillon.
4. Pepton ist der günstigste Nährstoff für die Bildung des bacteriolytischen Enzyms und führt nicht zu einer wesentlichen Schleimbildung. Nachträgliche

Versuche ergaben, dass ein ganz geringer Glycerinzusatz zur Peptonlösung günstig auf die Entwickelung des Pyocyaneus wirkt. Eine Erhöhung der Menge der schwefelsauren Magnesia auf 1% brachte keinen Vortheil.

Ueber die Bestimmung von Humus in der Ackererde.

VON

Dr. K. Bieler und K. Asō.

Zur Bestimmung von Humus in der Ackererde sind mehrere Methoden vorgeschlagen und im Gebrauch, keine jedoch liefert ein wirklich richtiges Resultat. Je nach dem Grade der vorgeschrittenen Zersetzung besitzt ferner der Humus eine andere Zusammensetzung. Humus entspricht keinem einheitlichen Körper.

Als zuverlässigste Methode gilt allgemein die Elementaranalyse. Die gefundene Menge Kohlensäure wird mit dem Factor 0.471 multiplicirt, wobei angenommen ist, dass der in Betracht kommende Humus 58% Kohlenstoff enthält. Es ist klar, dass diese Methode oft zu hohe Resultate liefern wird, weil auch andere Kohlenstoffverbindungen vorhanden sein können. Auf der andern Seite ist es eine wohl bekannte Thatsache, dass die noch oft angewandte Knop'sche Methode zu niedrige Resultate giebt.

Eine andere Methode, welche vielfach in Gebrauch ist, ist die Extractionsmethode. Obgleich diese Methode modificiert und verbessert wurde, sind trotzdem die gefundenen Humuszahlen zu hoch. Die Methode basiert auf der Thatsache, dass die Humussubstanzen in verdünnten Alkalienlösungen löslich sind. Es gehen hierbei jedoch auch andere Bestandtheile des Bodens mit in Lösung.

Neuerdings wird von *Aschmann* und *Faber*[1] eine volumetrische Methode zur Humusbestimmung vorgeschlagen, und zwar wird durch diese Methode der Humus als Humussäure bestimmt.

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[1] Chem-Zeitg. 1899. Nr. 7. S. 61.

Keine der angeführten Methoden ist also unbedingt zuverlässig. Wir haben nach jeder dieser Methoden den Humusgehalt im Boden unserer Versuchstation bestimmt und geben im Weiteren die von uns gemachten Beobachtungen.

Die Probenahme des Bodens geschah im September 1898 in der üblichen Weise aus der oberen Culturschicht. Die gezogene Durchschnittsprobe wurde durch Zerreiben zwischen den Handflächen und mit den Fingern genügend zerkleinert, darauf an der Sonne getrocknet, im Wagezimmer gelassen bis er lufttrocken war und sodann durch das 1 m m. Sieb gebracht Der abgesiebte Boden wurde in eine Glasflasche aufbewahrt.

I. Elementaranalyse.

a. 1.8274 grm. des lufttrockenen Bodens ergaben 0,536 grm. $CO_2 = 29.33\%$.

b. 1.7829 grm. ergaben 0.522 grm. $CO_2 = 29.29\%$.

c. 3 grm. ergaben 0.883 grm $CO_2 = 29.43\%$.

d. 2 grm. ergaben 0.5975 grm. $CO_2 = 29.87\%$.

Im Mittel : 29.48% CO_2.

CO_2 in Form von Karbonaten im Boden wurde im Mittel von 2 Bestimmungen[1] gefunden : 0.104%.

$29.48 - 0.104 = 29.376$.

$29.376 \times 0.471 = 13.84$ Humus.

II. Knop's Methode.

a. 5 grm. des lufttrockenen Bodens ergaben :
0.939 grm. $CO_2 = 18.78\%$ Humus.

b. 5 grm. des lufttrockenen Bodens ergaben :
0.9394 grm. $CO_2 = 18.79\%$
Im Mittel : 18.785% CO_2
$18.785 \times 0.471 = 8.85\%$ Humus.

III. Extractionsmethode.[2]

10 grm. des lufttrockenen Bodens wurden nach vorhergegangener Behandlung mit verdünnter Salzsäure mit 3% tigem Ammoniakwasser extrahiert, und der Extract zu $\frac{1}{2}$ Liter auf-

[1] Mit verdünnter Salzsäure.

[2] *Harry Snyder :* Chem. Centralbl. Nr. 18. Bd. II. 1897.

gefüllt. 100 ccm. der alkalischen Lösung wurden sodann in einer gewogenen Platinschale verdampft und gewogen nachdem durch Trockenen bei 100° C. Gewichtsconstanz erreicht worden war. Darauf wurde verascht und das Gewicht der Asche ebenfalls festgestellt. Die Humusmenge wurde bestimmt durch Abzug der Aschenmenge von dem Gewicht der Gesammttrockensubstanz.

a.	Trockensubstanz	0.218
	Asche	0.026
	Humus	0.192

$0.192 \times 5 \times 10 = 9.60\%$ Humus.

b.	Trockensubstanz	0.2260
	Asche	0.0265
	Humus	0.1995

$0.1995 \times 5 \times 10 = 9.98\%$ Humus.

Im Mittel: 9.79% Humus.

IV. Volumetrische Methode.[1]

10 c.c. Oxalsäure entsprechend, 11.3 c.c. $KMnO_4$-Lösung, und 10 c.c. Oxalsäure + 5 c.c. Humussäurelösung entsprechend 20.6 c.c. $KMnO_4$-Lösung. (die erhaltenen Zahlen waren: 20.5, 20.8, 20.4, 20.5, 20.8; im Mittel 20.6 c.c.).

$20.6 - 11.3 = 9.3$ c.c. $KMnO_4$-Lösung entsprechened 5 c.c. Humussäurelösung. 930 c.c. $KMnO_4$-Lösung entsprechend 500 c.c. Humussäurelösung enthaltend 0.125 gr. reine Humussäure.

1. c.c. $KMnO_4$-Lösung = 0.0001344 grm. Humussäure.

25 gr. des lufttrockenen Bodens in vorgeschriebener Weise behandelt, und die alkaliche Lösung zu einem halben Liter aufgefüllt. Da diese Lösung sich als zu concentriert erwies im Vergleich mit der Humussäurelösung, so wurden 10 c.c. zu 150 c.c. verdünnt. 5 c.c. der so verdünnten Lösung wurden zur Titration verwandt. Die verbrauchten Volumina der $KMnO_4$-Lösung betrugen: 20. c.c., 19.7 c.c. 20.1 c.c. Im-Mittel: 19.92 c.c.

[1] Vergl. Chem-Zeitg. 1899. Nr. 7, S. 61.

$$19.92 - 11.3 = 8.62 \text{ c.c.}$$
$$8.62 \times 30 \times 50 = 12930 \text{ c.c.}$$
$$12930 \times 0.0001344 = 1.738 \text{ gr.}$$
$$1.738 \times 4 = 6.95\% \text{ Humus.}$$

Die hierzu benutzte Humussäure[1] wurde von uns analysiert, die Analyse ergab in 100 Theilen der lufttrockenen Substanz:

Hygroscopisches Wasser	10.76
Trockensubstanz	89.24

In 100 Theilen der Trockensubstanz:

C.....	52.62
H.	4.19
N.	4.22
O.	35.03
Asche.	3.94

Die Farbe der Asche war röthlich und bestand hauptsächlich aus Eisenoxyd und Thonerde, Schwefelsäure, Phosphorsäure etc.

Wenn wir unsere Resultate kurz zusammenfassen, so ist zu bemerken: Die durch die Elementaranalyse erhaltene Zahl war zu hoch. Die von uns benutzte Bodenprobe enthielt trotz der vorsichtigen Vorbehandlung immerhin einige durch das Sieb gegangene Pflanzenreste, mehr oder weniger in Zersetzung begriffen, deren Kohlenstoffgehalt ebenfalls mit zur Berechnung der Humusmenge herangezogen wurde. Die durch die Extractionsmethode erhaltene Zahl betrug nur 71% der mit Hülfe der Elementaranalyse berechneten Humusmenge.

Die Humusmenge, welche durch die volumetrische Methode gefunden wurde, war die kleinste. Es wurden in 100 Theilen lufttrockenen Bodens gefunden bei:

Elementaranalyse.....	13.84	Theile	Humussubstanz.
Knop'sche Methode.....	8.85	;,	,,
Extractionsmethode	9.79	,,	,,
Volumetrische Methode ..	6.95	,,	,,

Wir sind der Ansicht, dass ähnliche Arbeiten wie die unserige mit den verschiedenartigsten Bodenarten ausgeführt werden müssen, um weitere Beiträge zu diesem wichtigen Gegenstande zu liefern.

[1] bezogen von Dr. Koenig, chemische Fabrik, Leipzig.

Ueber die Aufnahme von Stickstoff und Phosphorsäure durch verschiedene Kulturpflanzen (3 Cerealien und 2 Cruciferen) in drei Vegetationsperioden.

VON

Dr. K. Bieler und K. Asō.

Die hier beschriebenen Versuche hatten den Zweck, die Aufnahme von Stickstoff und Phosphorsäure durch einige Culturgewächse—Winterfrüchte in Japan—in 3 verschiedenen Vegetationsstadien zu vergleichen. Obgleich natürlich nur Durchschnittszahlen, gewonnen durch öfters wiederholte Versuche, diesen Vergleich zu illustrieren im Stande wären, so glauben wir doch, dass die Resultate des im Winter 1899 von uns ausgeführten Versuches als Beitrag zu diesem Gegenstande gut verwendbar sind.

Die Pflanzen wurden in Töpfen im Glashause unter genau denselben Vegetationsbedingungen gezogen und empfingen vollständige Düngung. Es wurden allen 5 verschiedenen Pflanzen dieselben Quantitäten verabreicht; nämlich 50 Kgr. P_2O_5 als Doppelsuperphosphat, 50 Kgr. K_2O als Kaliumcarbonat und 50 Kgr. N. in Gestalt von Ammoniumsulfat per Hektar. Ausserdem wurde Calcium carbonat angewandt und zwar für je 1 Kgr Boden, 1 gr. $CaCO_3$. Die von uns benutzten Toepfe waren aus Porzellan, im Übrigen jedoch ganz wie die gebräuchlichen Vegetations gefässe beschaffen. Der Durchmesser der Gefässe betrug 25 cm. und die Fläche, welche für die Pflanzen in Betracht kam, betrug circa $\frac{1 \text{ Hectar}}{20000}$. Jeder Versuch wurde dreifach angesetzt, so dass für jedes der fünf Culturgewächse im Ganzen 9 Gefässe bestellt wurden. Die für die Oberfläche des Bodens

in den Gefässen berechneten Düngerquantitäten betrugen pro Topf:

0.54 gr. Doppelsuperphosphat enthaltend 0.25 gr. P_2O_5.
0.39 gr. Kaliumcarbonat „ „ 0.25 gr. K_2O.
75 c.c. einer Ammoniumsulfatlösung „ „ 0.25 gr. N.

Das Beschicken der Töpfe, welche mit grobem Kies in der üblichen Weise auf dasselbe Gewicht gebracht wurden, fand am 9. November 1899 statt. Jedes Gefäss wurde mit 6.4 Kgr. gesiebten Bodens unserer Versuchsfelder in Komaba gefüllt, welcher vorher mit der bestimmten Menge kohlensauren Kalkes innig gemischt worden war. Am nächsten Tage wurde die Kalidüngung gegeben und zwar in Form einer wässerigen Lösung. Am 13. November wurde die Phosphorsäuredüngung und zwar in fester Form als Doppelsuperphosphat am Vormittage in die Toepfe gebracht und am Nachmittage desselben Tages die Stickstoffdüngung in flüssiger Form verabreicht. Die Samen wurden am Tage darauf, am 14. November, in der für Vegetationsversuche üblichen Weise in die Toepfe gelegt und zwar 20 in jedes einzelne Gefäss.

Die von uns angewandten Pflanzen waren:

1) Weizen (Sōshu Shirakawa).
2) Gerste (Golden Melon).
3) Hafer (Golden).
4) Raps (Tōkyo Wase).
5) Senf (Gewöhnliche Art).

Der Aufgang der Pflanzen war nicht gleichmässig, zuerst kamen die Senf- darauf die Rapspflanzen zum Vorschein. In den ersten Tagen des Decembers jedoch waren in allen Toepfen die Pflanzen aufgegangen; es wurden nun alle übrigen Pflanzen herausgezogen, so dass in jedem Gefäss nur 10 verblieben. Was die Wasserzufuhr anbelangt, so wurden alle Gefässe während des ganzen Versuches in einem für die betreffenden Pflanzen günstigsten Feuchtigkeitszustande erhalten.

In der Mitte des Monates Januar 1900 war der Stand der Senfpflanzen am üppigsten, jedoch allmälig gewannen die Rapspflanzen die Oberhand. Die drei Cerealien befanden sich zu derselben Zeit ebenfalls im üppigen Wachsthum; jedoch gegen Ende des Januars hatten die Gerstenpflanzen einige gelbe Blätter, verursacht durch die Winterkälte, welche sich auch in gewissem Grade im Glashause geltend machte. Bei

den Haferpflanzen war dieses in ganz bedeutend schwächerem Massstabe zu constatieren und beim Weizen nur vereinzelt. In den um Komaba gelegenen Farmen konnten wir allerdings zu derselben Zeit auch ein Gelbwerden von Blättern der Gerste- und auch Weizenpflanzen[1] und zwar in bedeutend stärkerem Masse als bei unsern Versuchen beobachten.

Die I Vegetationsperiode endete am 2. Februar, also ca $2\frac{1}{2}$ Monate nach der Samenlegung. Die Pflanzen aus je drei Töpfen wurden zusammen mit ihren Wurzelgebilden mit grösster Vorsicht und Sorgfalt, um Substanzverluste so viel es überhaupt möglich, zu verhüten. Zu diesem Zwecke wurden die Vegetationsgefässe 2 Tage vor dem Abernten aus dem Glashause in einen für derartige Zwecke vorgesehenen Raum gebracht, um dort etwas abzutrocken. Die Durchschnittszahl der Blätter betrug bei den einzelnen Pflanzen:

	Durchschnittszahl.
Weizen	5
Gerste	6
Hafer	5
Raps	8
Senf	7

Trockensubstanz der verschiedenen je 30 Pflanzen (3 Töpfe) betrug:

Weizen	2.966. grm.
Gerste	4.777. ,,
Hafer	2.884. ,,
Raps	8.416. ,,
Senf	2.898. ,,

ASCHE (Rohasche).[2]

	Prozente der Trocken-substanz.	Absolute Menge.
Weizen	9.38	0.278 grm.
Gerste	7.58	0.362 ,,
Hafer	9.40	0.271 ,,
Raps	18.04	1.518 ,,
Senf	15.85	0.459 ,,

[1] Hafer wird in dieser Gegend Japans nicht angebaut.

[2] Die verschiedenen Aschen zeigten verschiedene Farben: beim Weizen: röthlich braun, Gerste: gelblich, Hafer: gräulich braun, Raps: grau und Senf: dunkelgrau. Die Asche der jungen Senfpflanzen liess bei Behandlung mit Salpetersäure deutlich eine Entwickelung von Schwefelwasserstoff erkennen.

STICKSTOFF.

	Prozente der Trocken-substanz.	Absolute Menge.
Weizen	2.65	0.079 grm.
Gerste	3.55	0.170 ,,
Hafer	3.12	0.090 ,,
Raps	5.04	0.424 ,,
Senf	5.60	0.162 ,,

PHOSPHORSÄURE.

	Prozente der Trocken-substanz.	Absolute Menge.
Weizen	1.18	0.035 grm.
Gerste	0.95	0.045 ,,
Hafer	0.89	0.026 ,,
Raps	0.95	0.080 ,,
Senf	0.78	0.023 ,,

Das Verhältniss zwischen den N- und Phosphorsäuremengen (N=1) war

in den Weizenpflanzen	1 : 0.443
,, ,, Gerste ,,	1 : 0.265
,, ,, Hafer ,,	1 : 0.289
,, ,, Raps ,,	1 : 0.189
,, ,, Senf ,,	1 : 0.142

ENDE DER II PERIODE.

Am 19. März 1900, also ungefähr 4 Monate nach der Samenlegung wurden von je drei Töpfen zuerst die über der Erdoberfläche befindlichen Pflanzentheile abgeschnitten und gesammelt. Die Wurzeln wurden nachher mit grosser Sorgfalt den Töpfen unter Vermeidung von Substanzverlusten entnommen. Um die anhängenden erdigen Theile zu entfernen, wurden die aus je drei Töpfen stammenden Wurzeln 1—2 Tage getrocknet, darauf mit grosser Vorsicht gewaschen und in den Trockenschrank gebracht.

Die Länge der Pflanzen betrug :

	bis zu
beim Weizen	19.5 cm.
,, Gerste	20.5 ,,
,, Hafer	17.5 ,,
,, Raps	33.0 ,,
,, Senf	17.0 ,,

Die Wurzeln hatten in allen Fällen den Boden der Toepfe erreicht.

Trockensubstanz von je 3 Toepfen (30 Pflanzen).

	Obere Pflanzentheile.	Wurzeln.	Im Ganzen.
Weizen	3.211 grm.	5.098 grm.	8.309 grm.
Gerste	5.679 „	4.814 „	10.493 „
Hafer	3.405 „	4.379 „	7.784 „
Raps	24.605 „	8.621 „	33.226 „
Senf	8.240 „	4.404 „	12.644 „

ASCHE (Rohasche).

		Prozente der Trockensubstanz.	Absolute Menge.	Im Ganzen.
Weizen ..	Obere Theile	9.54	0.306 grm.	0.934 grm.
	Wurzeln	12.32	0.628 „	
Gerste ...	Obere Theile	9.49	0.539 „	1.023 „
	Wurzeln	10.06	0.484 „	
Hafer ...	Obere Theile	9.01	0.307 „	0.874 „
	Wurzeln	12.95	0.567 „	
Raps ...	Obere Theile	13.42	3.302 „	4.745 „
	Wurzeln	16.74	1.443 „	
Senf	Obere Theile	14.69	1.210 „	2.665 „
	Wurzeln	33.04	1.455 „	

STICKSTOFF.

Weizen ..	Obere Theile	3.30	0.106 grm.	0.191 grm.
	Wurzeln	1.67	0.085 „	
Gerste ...	Obere Theile	3.71	0.211 „	0.306 „
	Wurzeln	1.98	0.095 „	
Hafer ...	Obere Theile	3.48	0.119 „	0.185 „
	Wurzeln	1.52	0.067 „	
Raps ...	Obere Theile	2.11	0.519 „	0.644 „
	Wurzeln	1.45	0.125 „	
Senf	Obere Theile	4.55	0.375 „	0.466 „
	Wurzeln	2.07	0.091 „	

PHOSPHORSÄURE.

Weizen ..	Obere Theile	0.88	0.028 grm.	0.049 grm.
	Wurzeln	0.41	0.021 „	
Gerste ...	Obere Theile	0.40	0.023 „	0.043 „
	Wurzeln	0.41	0.020 „	
Hafer ...	Obere Theile	0.73	0.025 „	0.044 „
	Wurzeln	0.43	0.019 „	
Raps ...	Obere Theile	0.58	0.143 „	0.181 „
	Wurzeln	0.44	0.038 „	
Senf	Obere Theile	0.43	0.035 „	0.060 „
	Wurzeln	0.57	0.025 „	

Das Verhältniss zwischen den N- und Phosphorsäuremengen (N = 1) war

bei den	Weizenpflanzen	1 : 0.251
„ „	Gersten „	1 : 0.141
„ „	Hafer „	1 : 0.238
„ „	Raps „	1 : 0.281
„ „	Senf „	1 : 0.129

ENDE DER III PERIODE.

Die übriggebliebenen je 3 Toepfe wurden abgeerntet nach dem Abblühen der Pflanzen.

	Beginn der Blüthe.
Weizen	4. Mai.
Gerste	———
Hafer	12. Mai.
Raps	bereits am Ende der II Periode.
Senf	6. April.
	Ende des Blüthezustandes.
Weizen	24. Mai.
Gerste	———
Hafer	20. Mai.
Raps	1. April.
Senf	16. April.

Das Abernten der Töpfe geschah in derselben Weise wie am Ende der II Periode.

	Die Ernte fand statt:	Die ganze Zeit der Vegetation betrug:
beim Weizen	25. Mai	$6\frac{1}{3}$ Monate.
„ Gerste[1]	7. Juni	$6\frac{1}{3}$ „
„ Hafer[2]	25. Mai	$6\frac{1}{3}$ „
„ Raps	13. April	5 „
„ Serf[2]	27. April	$5\frac{2}{3}$ „

Die Höhe der Pflanzen betrug im Mittel:

Weizen	76 cm.
Gerste	46 „
Hafer	63 „
Raps	76 „
Senf	69 „

[1] Das Wachstum der Gerstenpflanzen machte in den letzten beiden Wochen keinerlei Fortschritte.

[2] Eine Pflanze war nicht zur völligen Entwickelung gelangt.

TROCKENSUBSTANZ.

	Obere Pflanzentheile.	Wurzeln.	im Ganzen.
Weizen	24.877 grm.	7.63 grm.	32.515 grm.
Gerste	14.915 „	10.267 „	25.182 „
Hafer	21.260 „	11.306 „	32.566 „
Raps	41.915 „	8.346 „	50.261 „
Senf	27.899 „	10.796 „	38.692 „

ASCHE (Rohasche).

		Prozente der Trockensubstanz.	Absolute Menge.	im Ganzen.
Weizen ..	Obere Theile	8.78	2 184 grm.	2.666 grm.
	Wurzeln	6.31	0.482 „	
Gerste ...	Obere Theile	10.07	1.502 „	3.475 „
	Wurzeln	19.22	1.973 „	
Hafer ...	Obere Theile	10.33	2.196 „	3 270 „
	Wurzeln	9.50	1.074 „	
Raps ...	Obere Theile	11.28	4.728 „	6.669 „
	Wurzeln	23.26	1.941 „	
Senf.....	Obere Theile	8.95	2.497 „	5.996 „
	Wurzeln	32.42	3.499 „	

STICKSTOFF.

Weizen ..	Obere Theile	2.06	0.512 grm.	0.627 grm.
	Wurzeln	1.50	0.115 „	
Gerste ...	Obere Theile	2 63	0.392 „	0 609 „
	Wurzeln	2.11	0.217 „	
Hafer ...	Obere Theile	2.36	0.501 „	0.629 „
	Wurzeln	1.13	0.128 „	
Raps ...	Obere Theile	1.22	0.509 „	0.609 „
	Wurzeln	1.20	0.100 „	
Senf.....	Obere Theile	2.05	0.572 „	0.705 „
	Wurzeln	1.23	0.133 „	

PHOSPHORSÄURE.

Weizen ..	Obere Theile	0.34	0.083 grm.	0.096 grm.
	Wurzeln	0.17	0.013 „	
Gerste ...	Obere Theile	0.30	0.044 „	0.067 „
	Wurzeln	0.22	0 023 „	
Hafer ...	Obere Theile	0.34	0.073 „	0.086 „
	Wurzeln	0.11	0.013 „	
Raps ...	Obere Theile	0.30	0.127 „	0.175 „
	Wurzeln	0.57	0.048 „	
Senf.....	Obere Theile	0.23	0.063 „	0.131 „
	Wurzeln	0.63	0.068 „	

Das Verhältniss zwischen den N- und Phosphorsäuremengen (N = 1) war

bei den	Weizen pflanzen	1 : 0.153
,, ,,	Gerste ,,	1 : 0.110
,, ,,	Hafer ,,	1 : 0.137
,, ,,	Raps ,,	1 : 0.288
,, ,,	Senf ,,	1 : 0.186

TABELLE I.

VERGLEICHSRESULTATE.

Periode der Vegetation.		Gesammt-trocken-substanz.	Gesammt-Asche.	Gesammt-N.	Gesammt-P_2O_5.
Weizen	I.	2.966 grm.	0.278 grm.	0.079 grm.	0.035 grm.
	II.	8.309 ,,	0.934 ,,	0.191 ,,	0.049 ,,
	III.	32.515 ,,	2.666 ,,	0.627 ,,	0.096 ,,
Gerste	I.	4.777 ,,	0.362 ,,	0.170 ,,	0.045 ,,
	II.	10.493 ,,	1.023 ,,	0.306 ,,	0.043 ,,
	III.	25.182 ,,	3.475 ,,	0.609 ,,	0.067 ,,
Hafer	I.	2.884 ,,	0.271 ,,	0.090 ,,	0.026 ,,
	II.	7.784 ,,	0.874 ,,	0.185 ,,	0.044 ,,
	III.	32.566 ,,	3.270 ,,	0.629 ,,	0.086 ,,
Raps	I.	8.416 ,,	1.518 ,,	0.424 ,,	0.080 ,,
	II.	33.226 ,,	4.745 ,,	0.644 ,,	0.181 ,,
	III.	50.261 ,,	6.669 ,,	0.609 ,,	0.175 ,,
Senf	I.	2.898 ,,	0.459 ,,	0.162 ,,	0.023 ,,
	II.	12.644 ,,	2.665 ,,	0.466 ,,	0.060 ,,
	III.	38.692 ,,	5.996 ,,	0.705 ,,	0.131 ,,

TABELLE II.

Vertheilung des N, der P_2O_5 und der Asche in den oberen Pflanzentheilen und Wurzeln.[1]

Periode der Vegetation	Obere Theile.			Wurzeln.		
	N.	P_2O_5.	Asche.	N.	P_2O_5.	Asche.
Weizen.						
II.	0.106 grm.	0.028 grm.	0.306 grm.	0.085 grm.	0.021 grm.	0.628 grm.
III.	0.512 „	0.083 „	2.184 „	0.115 „	0.013 „	0.482 „
Gerste.						
II.	0.211 grm.	0.023 grm.	0.539 grm.	0.095 grm.	0.020 grm.	0.484 grm.
III.	0.392 „	0.044 „	1.502 „	0.217 „	0.023 „	1.973 „
Hafer.						
II.	0.119 grm.	0.025 grm.	0.307 grm.	0.066 grm.	0.019 grm.	0.567 grm.
III.	0.501 „	0.073 „	2.196 „	0.128 „	0.013 „	1.074 „
Raps.						
II.	0.519 grm.	0.143 grm.	3.302 grm.	0.125 grm.	0.038 grm.	1.443 grm.
III.	0.509 „	0.127 „	4.728 „	0.100 „	0.048 „	1.941 „
Senf.						
II.	0.375 grm.	0.035 grm.	1.210 grm.	0.091 grm.	0.025 grm.	1.455 grm.
III.	0.572 „	0.063 „	2.497 „	0.133 „	0.068 „	3.499 „

[1] Die Pflanzen der I Periode wurden im Ganzen analysiert.

TABELLE III.

VERGLEICH DER PROZENT-ZAHLEN.

	Periode.	Theile.	N.	P_2O_5.	Asche.
Weizen.	I.	———	2.65	1.18	9.38
	II.	Obere.	3.30	0.88	9.54
		Wurzeln.	1.67	0.41	12.32
	III.	Obere.	2.06	0.34	8.78
		Wurzeln.	1.50	0.17	6.31
Gerste.	I.	———	3.55	0.95	7.58
	II.	Obere.	3.71	0.40	9.49
		Wurzeln.	1.98	0.41	10.06
	III.	Obere.	2.63	0.30	10.07
		Wurzeln.	2.11	0.22	19.22
Hafer.	I.	———	3.12	0.89	9.40
	II.	Obere.	3.48	0.73	9.01
		Wurzeln.	1.52	0.43	12.95
	III.	Obere.	2.36	0.34	10.33
		Wurzeln.	1.13	0.11	9.50
Raps.	I.	———	5.04	0.95	18.04
	II.	Obere.	2.11	0.58	13.42
		Wurzeln.	1.45	0.44	16.74
	III.	Obere.	1.22	0.30	11.28
		Wurzeln.	1.20	0.57	23.26
Senf.	I.	———	5.60	0.78	15.85
	II.	Obere.	4.55	0.43	14.69
		Wurzeln.	2.07	0.57	33.04
	III.	Obere.	2.05	0.23	8.95
		Wurzeln.	1.23	0.63	32.42

STICKSTOFF UND PHOSPHORSÄURE IN DEN SAMEN.

Zur Bestimmung von N benutzen wir je 30 Samen, zur Bestimmung der P_2O_5 jedoch 300 und zwar wurden in allen Fällen nur gute, auserlesene Samen verwandt.

30 Samen (den Pflanzen von 3 Töpfen entsprechend) enthielten:

	N.	P_2O_5.
beim Weizen	0.015 grm.	0.006 grm.
„ Gerste	0.023 „	0.012 „
„ Hafer	0.010 „	0.006 „
„ Raps	0.004 „	0.002 „
„ Senf	0.003 „	0.002 „

Die von je einer Pflanze aufgenommene Phosphorsäure- und N- Menge betrug am Ende des Versuches:

	N. Aufgenommen.	P_2O_5. Aufgenommen.
beim Weizen	0.021 grm.	0.003 grm.
„ Gerste	0.020 „	0.002 „
„ Hafer	0.021 „	0.003 „
„ Raps	0.020 „	0.006 „
„ Senf	0.023 „	0.004 „

Wenn wir zuerst die Resultate der Cerealienversuche ins Auge fassen, so constatieren wir, dass beim Weizen und Hafer eine Zunahme der Trockensubstanz von Periode zu Periode in ungefähr demselben Verhältnisse stattgefunden hatte, und von den Gesammtaschenmengen wäre dasselbe zu erwähnen. Die Gerstenpflanzen entwickelten sich in der ersten Periode viel üppiger als die andern Cerealien; und auch noch nach einer Vegetationszeit von 4 Monaten (Ende der Periode II) war das Gewicht der Trockensubstanz und ebenso das der Asche bei den 30 Gerstenpflanzen höher als die entsprechenden Zahlen beim Weizen sowohl wie bei dem Hafer. Nach dieser Zeit jedoch entwickelten die Weizen- und Haferpflanzen ein weiteres kräftiges Wachsthum, welches bis zum Schlusse der Versuches anhielt. Bei den Gerstenpflanzen war dieses aber nicht der Fall. Nach einer gewissen Zeit schien die Vegetation der Gerste stillzustehen und nicht eine einzige Pflanze erreichte das Blüthestadium. Die vollständig ausgebildeten Gerstenähren

blieben in der Blattscheide länger als 2 Wochen und in diesem Zustande wurde sodann am 7. Juni nach einer Vegetationsdauer von $6\frac{1}{3}$ Monaten abgeerntet. Das Gewicht der Gesammtasche betrug trotzdem bei den Gerstenpflanzen am Ende des Versuche mehr als das der völlig entwickelten Weizen- und Haferpflanzen.

Was die Stickstoffaufnahme anbetrifft, so stellen wir fest, dass am Ende des Versuches die in je 30 Pflanzen der 3 Cerealien enthaltene N-Menge ungefähr dieselbe war. Die Stickstoffmengen, welche am Ende der I und II Periode in den Gerstenpflanzen am höchsten waren, nahmen bei der Gerste vom Ende der II bis zum Schluss des Versuches im Verhältnisse von 1 : 2 zu, während dieses Verhältniss bei den Weizen- und Haferpflanzen 1 : 3 betrug.

In Hinsicht auf die Phosphorsäureaufnahme müssen wir zuerst erwähnen, dass die jungen Gerstenpflanzen mehr Phosphorsäure aufgenommen hatten als die andern Cerealien. Aber bei den Weizen- und Haferpflanzen nahm die Phosphorsäure von Periode zu Periode zu, was bei den Gerstenpflanzen nicht der Fall war.

Von dem Ende der I bis zum Ende der II Periode konnte bei den Gerstenpflanzen überhaupt keine Erhöhung der Phosphorsäureaufnahme constatiert werden. Eine Zunahme der Phosphorsäure vom Ende der II Periode bis zum Ende des Versuches konnte allerdings constatiert werden, war jedoch nur gering. Die aufgenommenen P_2O_5-Mengen in den Pflanzen der I Periode standen zu diejenigen Mengen der Pflanzen der III bei der Gerste in dem Verhältnisse von 1 : 1.5, während dieses Verhältniss beim Weizen 1 : 2.7 und beim Hafer sogar 1 : 3.3 betrug.

Bei einem weiteren Vergleich der aufgenommenen P_2O_5-Mengen, erkennt man, dass die Absorptionsfähigkeit für P_2O_5 beim Weizen am stärksten war. Der Gehalt der Asche an P_2O_5 betrug am Ende der III Periode: beim Weizen 3.6, beim Hafer 2.6 bei der Gerste nur 1.9%. Bei den Pflanzen der ersten Periode betrug der P_2O_5-Gehalt der Asche bei dem Weizen: 12.6, bei der Gerste 12.4 und beim Hafer 9.6%. Wenn wir alle diese Resultate zusammenfassen, können wir sagen: 1) 30 Gerstenpflanzen waren im Stande ungefähr dieselbe Menge N aufzunehmen als 30 Weizen resp. Haferpflanzen. 2) Obgleich

die Menge der Gesammtasche bei den Gerstenpflanzen am Ende des Versuches die grösste war, war die aufgenommene P_2O_5 am kleinsten. 3) Die in der Düngung gegebenen und im Boden vorhandenen Nährstoffe waren im Stande, die 30 Weizen- und Haferpflanzen zur völligen Entwickelung zu bringen, reichten jedoch nicht aus oder vielmehr waren nicht fähig dasselbe bei den Gerstenpflanzen zu vollbringen. In Tabelle II sind die in den oberirdischen Pflanzentheilen und in den Wurzeln enthaltenen N, P_2O_5 und Aschemengen zusammengefasst. Die Phosphorsäurezahlen sind bei den Weizen- und Haferpflanzen interressant und geben ein Bild von der fortschreitenden P_2O_5-Aufnahme. Was die Gerstenzahlen anbetrifft, so constatieren wir, dass am Ende der III Periode die Gesammtmenge von N in den oberen Pflanzentheilen die kleinste die in den Wurzeln durch die grösste Zahl ausgedrückt wird.

Der Prozentgehalt an N (Tabelle III) war bei den Gerstenpflanzen am Ende des Versuches am höchsten und zwar sowohl in den oberen Theilen als in den Wurzeln.

Wenn wir hierauf die Resultate der Raps- und Senfversuche betrachten, so fallen uns zuerst die Rapszahlen ins Auge. Die Zahlen für N und P_2O_5 (Tabelle I) zeigen vom Ende der II bis zum Ende der III Periode eine kleine Abnahme, die Zahlen für Trockensubstanz und Totalasche jedoch eine Zunahme. Beim Auswählen der drei Töpfe am Schluss der II Periode nahmen wir die Töpfe mit den best entwickelten Pflanzen, die Rapspflanzen befanden sich in einem Stadium kurz vor der Blüthe. Da bei dem Abernten und der weiteren Behandlung äusserst vorsichtig verfahren wurde, um so viel wie möglich, Substanzverluste zu verhüten, müssen wir annehmen, dass keine weitere Aufnahme von P_2O_5 und N in den so weit entwickelten Rapspflanzen stattfindet und dass die Zunahme von Trockensubstanz und Asche in den Pflanzen der III Periode durch die Aufnahme von andern, nicht für die Ernährung wesentlichen Stoffen verursacht sei.

Beim Vergleich der Raps- und Senfzahlen in Tabelle I sehen wir, dass das Wachsthum der Rapspflanzen bis zum Ende der II Periode kräftiger war, als das der Senfpflanzen. Die obwaltenden Verhältnisse, unter welchen unsere Versuche ausgeführt wurden, schienen von vorneherein günstiger für die Rapspflanzen als für die Senfpflanzen zu sein, infolgedessen die

Vegetation der ersteren beschleunigt, die der letzteren verzögert wurde.

Der Gehalt an P_2O_5 war in den oberen Pflanzentheilen beim Raps am Ende der II und III Periode höher als beim Senf und dasselbe war der Fall mit Aschengehalt am Ende des Versuches. Wenn wir jedoch die zu den Wurzeln gehörigen Zahlen betrachten, so constatieren wir das Gegentheil: Am Ende der II und ebenso am Ende der III Periode war der Gehalt an Asche sowohl wie an P_2O_5 gerösser in den Senfpflanzen.

Was den N-Gehalt anbetrifft, so ist in allen Fällen sowohl am Ende der I Periode (die Zahlen beziehen sich auf die gesammte Pflanzensubstanz) als auch am Ende der II und III Periode der Gehalt an N höher beim Senf als beim Raps und zwar in den oberen Pflanzentheilen und auch in den Wurzeln.

Die Zahl, welche die Gesammtmenge von N in den 30 Senfpflanzen am Ende des Versuches angiebt, ist nicht nur höher als die entsprechende Zahl beim Raps sondern auch höher als diejenigen der drei Cerealien. Auf der andern Seite zeigte jedoch der Raps die stärkste Absorptionskraft für P_2O_5.

Zum Schluss führen wir an, dass die Cerealien und die beiden Cruciferen während ihres ganzen Wachsthums ungefähr dieselbe Menge von N aufnahmen. Von P_2O_5 nahmen die Cerealien weniger auf als die andern Pflanzen, und dasselbe können wir von der Gesammtasche sagen. Die stärkste Absorptionsfähigkeit für P_2O_5 zeigte der Raps, dann folgte der Senf. Weizen besass von den 3 Cerealien für P_2O_5 die stärkste Absorptionskraft, die diesbezügliche Zahl für Hafer liegt zwischen Weizen und der Gerste.

In Beziehung der Aufnahme von Nährstoffen durch die Rapspflanzen zeigte unser Versuch, dass die stärkste Aufnahme stattfand als die Pflanzen die erste Entwickelung hinter sich hatten (Periode I) und andauert bis zu dem Stadium der Blüthe. Nach der Blüthe scheint keine weitere Aufnahme von Nährstoffen stattzufinden.

On the Rôle of Oxydase in the Preparation of Commercial Tea.

BY

K. Asō.

It is well known that fresh tea-leaves if steamed after being collected, will preserve their green color, while if exposed to a partial drying in the sun, they will turn gradually brown. The former is the first operation in the preparation of the green tea and the latter that of the black tea of commerce.

About ten years ago, Prof. Kozai[1] found that the tea-leaves if once sufficiently steamed do not undergo further change or the so-called fermentation, but that in the further preparation of black tea, the sacks of rolled and pressed tea-leaves showed a gradual rise of temperature to 34.°5 C at an air-temperature of 24° C.[2] He further observed that black tea contains considerably less tannin than green tea :

	In 100 parts of the dry matter,		
	Original leaves.	Green tea.	Black tea.
Tannin. (calculated as gallotannic acid)	12.91%	10.64%	4.89%

The development of the black color seemed to me to be due to the action of an oxydizing enzyme upon the tannin of the tea-leaves, and this has led me to the following tests.

The tea leaves were collected in the middle of September, taking only the five uppermost leaves of each branch. They were pulverised and extracted at the ordinary temperature with

[1] Kozai, Bull. College of Agric., Tōkyō. Vol. I. No. 7. 1890.

[2] The process of "fermenting" consists in pressing the rolled leaves into sacks, arranging them, side by side, under a white cloth and placing them in a sunny place.

dilute alcohol (30%) for 40 hours. The filtrate was mixed with strong alcohol (93%) and left to stand for several days, whereupon the precipitate was collected on an asbestos filter, dissolved in a small quantity of water and again precipitated with strong alcohol.

The product thus obtained from about 300 grams of the fresh leaves was dissolved in 150 c.c. water, and subjected to the following tests:

A freshly prepared guaiacum tincture added to the cold solution, gave at once a blue coloration, which soon increased in intensity, while the boiled solution failed to do so. This proves the presence of an oxidase. In order to observe the temperature at which this enzyme is destroyed some tests were made with the following result:

Temperature.	Time of heating.	Reaction with guaiac.
72° C	5 minutes.	Pale blue color after several seconds.
75° C	„	A pale blue color set in after about 10 minutes.
76° C	„	Slight coloration after about 13 minutes.

We may therefore infer, that the oxidase of the tea-leaves is destroyed at about 76°—77° C.

When tea-leaves are extracted directly with water, the solution does not give the blue reaction, since the tannin in the solution prevents it.

In order to test also the presence of peroxidase in the tea-leaves, I proceeded as follows:

Fresh tea-leaves were finely crushed with addition of some sand and extracted twice with strong alcohol (absolute) at the ordinary temperature in order to remove the tannin,[1] since it not only prevents the usual guaiac reaction of peroxidase, but in smaller quantities can also bleach out again the blue color after it has made its appearance. The residue was then extracted at the ordinary temperature with water, and this solution[2] was heated for 5 minutes at 76°C in order to destroy the oxidase. In this liquid the peroxidase reaction was obtained with

[1] Not only the guaiac reaction of oxidase and peroxidase, but also the action of myrosin and emulsin is prevented by tannin. (Reynolds Green: Fermentation, p. 154.)

[2] This solution produced a brownish coloration with hydroquinone.

tincture of guaiac and some hydrogen peroxide with great intensity.

In the following test, 60 c.c. of a cold prepared strong alcoholic extract of the tea-leaves were diluted with four times its own volume of water and separated into two portions. To one, some of the purified enzyme solution above mentioned was added in order to observe the action of the oxidase on the tannin of the tea-leaves while the other portion served for control. Both flasks were only half filled to admit air freely, and some ether was added to prevent bacterial growth and plugged with cotton.

The gradual change of color was very evident:

	Tannin solution containing enzyme.	Tannin solution without enzyme.
After 24 hours.	Somewhat brown.	Green.
,, 48 ,,	Brown.	Yellowish green.[1]
,, 72 ,,	Dark brown.	Brownish green.

Hence, an action of the oxidase of tea on its tannin is evident, and there can be no further doubt, that the so-called "fermentation" of the black tea leading to the formation of the black color is due to the action of the oxidizing enzymes in the tea-leaf.

Finally also a test for catalase was made, not only with fresh tea-leaves, but also with the residue after extracting leaves at the ordinary temperature with alcohol and water, and its presence recognised by the considerable development of oxygen from dilute hydrogen peroxide solution.

Some tests for oxidase and peroxidase were also made with the green as well as the black tea of commerce,[2] but none of the three enzymes mentioned was found. This is not surprising since in the manufacture of black tea, the leaves are finally heated up to 100° C or even higher. In the green tea of commerce, it is the steaming at the beginning of its preparation, that kills the enzymes.

Since Bertrand has observed a small percentage of manganese in oxidizing enzymes and attributes to this metal an essential rôle in their oxydizing actions, and since Lepinois

[1] This incomplete change of color is probably due to the acid reaction changing the chlorophyll.

[2] Prepared according to Japanese method.

found also some iron in one of them, and further, since it has been shown by Hofer, Macallum and others that the enzymes are secreted by the nuclei (at first in a state of zymogen), and moreover as Bunge, Macallum and Stoklasa have observed some nucleoproteids containing iron, I was led to the following tests, which show that there occur in tea-leaves nucleoproteids that contain not only iron but also manganese. Whether one and the same nucleoproteid contains both these metals, or whether there be two nucleoproteids of which one contains iron and the other manganese, was however not decided by my tests.

I proceeded as follows:

200 grams of air-dried tea-leaves were powdered and extracted first with ether and then with alcohol, then digested with artificial gastric juice for 24 hours at 38° C. The residue was treated with dilute ammonia and the solution precipitated by slightly acidifying it with acetic acid. The dark brown[1] gelatinous precipitate thus obtained was washed with dilute acetic acid, then with water, and dried and powdered. This substance was again dissolved in dilute ammonia and precipitated with absolute alcohol (a). The brown precipitate was thoroughly washed with alcohol and ether, and then subjected to digestion with artificial gastric juice for 30 hours at 37°-38° C. The residue was dissolved in dilute ammonia, and precipitated and well washed with alcohol. The dark brown substance thus obtained, contained phosphorus and yielded for 100 parts of dry matter:

N	4.91 %
Fe	0.22 %
Mn	0.04 %

The filtrate of the precipitate (a) was again precipitated with acetic acid, and the dark brown precipitate thus obtained was, after being washed with dilute acetic acid, subjected to artificial digestion for 24 hours at 38° C. The indigestible residue was dissolved in dilute ammonia and again precipitated with acetic acid, washed thoroughly with dilute acetic acid, alcohol, and ether, and dried. This substance contained:

[1] The brown color may have been partly due to traces of oxidised tannin.

In 100 parts of dry matter,

N	8.66
Fe	0.18
Mn	no reaction.

SUMMARY.

I. The black color of the commercial black tea is produced by the action of oxidase upon tannin.

II. The green variety of the commercial tea owes its green color to the destruction of oxidase in the first steps of preparation.

III. By the final steps of its preparation, the black tea also loses the oxidizing enzyms.

IV. In tea leaves occur proteids containing iron and manganese.

On the Occurrence of Organic Iron Compounds in Plants.

BY

U. Suzuki.

Although iron is indispensable for the formation of chlorophyll, yet according to Gautier and to Molisch,[1] it is not contained in this colouring matter itself. Further J. Stoklasa[2] found that it is not present in his "Chlor lecithin." Bunge and others had already proved that in plants iron never exists as inorganic compounds. Bunge[3] succeeded in isolating an iron-nuclein compound from the yolk of the hen's egg which he called haematogen to indicate its close relation to haemoglobin. Stoklasa[4] obtained from the bulb of Allium cepa and from the seed of Pisum sativum, a similar compound. Haematogen contains, besides iron, a small quantity of calcium, magnesium, chlorine and much phosphoric acid. The presence of these is common in nuclein. It was hitherto supposed that the common nuclein does not contain iron; this may be due to the usual mode of its preparation which consists in dissolving it several times in alkaline solution and subsequently precipitating it.

The "haematogen" of Bunge and of Stoklasa has the following composition:—

We see that the haematogen of animal and vegetable origin closely resemble in their chemical nature. The only difference being the higher percentage of iron in the vegetable haematogen.

Zaleski obtained Bunge's haematogen from the liver of animals. Macallum also found iron in the nucleus of plant cells.

[1] H. Molisch;—Eisen und ihre Beziehungen zu den Pflanzen,—Jena 1892.

[2] J. Stoklasa;—Ueber die physiologische Funktion des Lecithins in der Pflanze (Akademie der Wissenschaften, Wien 1896).

[3] J. Bunge;—Ueber die Assimilation des Eisens (Zeitschrift für physiologische Chemie); Strassburg 1885.

[4] J. Stoklasa;—Comptes rendus d. l'Acad. des sciences 127. 282-83. Chem. Labor. des Polytechnikums. Prag.

	Nuclein.	Bunge's Haematogen from egg yolk.	Haemoglobin.	Stoklasa's Haematogen from plants.
C	40.81	42.11	54.26	43.05
H	5.38	6.08	7.10	5.56
N	15.98	14.73	16.21	15.13
S	0.38	0.55	0.54	0.28
P	6.19	5.19	0.77(P_2O_5)	6.21
Fe	—	0.29	0.43	1.68
O	31.26	31.05	20.69	28.08

We see how closely nuclein and haematogen agree in composition.[1] Both chemical and microscopical examination of seeds by Bunge has shown that the iron is localized in the embryo and endosperm only in an organic combination. Most probably the haematogen is used for the formation of the cellular nucleus. Stoklasa tried in vain to isolate haematogen from young maize plants cultivated in a solution containing no iron while those cultivated in a solution containing iron, gave a moderate quantity of it. Lower plants containing no chlorophyll require nevertheless, according to Molisch, iron just as chlorophyll bearing plants do. Thus, Mucor mucedo or Bacillus megatherium never develop well in a solution free from iron. Certain fungi contain also haematogen; thus from 1000g. of Boletus edulis 3.5g. haematogen was isolated by Stoklasa, and he therefore suggested that iron is an essential constituent of nuclein like phosphoric acid. K.Aso[2] has shown that the spores of Aspergillus oryzae contain relatively much iron (about 5% Fe_2O_3 in the ash). On treating the spores with dilute alkali and neutralizing the alkaline extract with acetic acid, a protein substance was obtained, which, after being subjected to artificial pepsin digestion, yielded an insoluble residue containing iron. This compound was again dissolved in ammonia, the ammoniacal solution again neutralized with hydrochloric acid, and even after this purification process the nuclein obtained was tolerably rich in iron. It is especially interesting that this

[1] Recently Häusermann found a small quantity of iron in the serum of blood, (1 mg. in 100 c.c.). Dried fibrin from the dogs blood contained 0.01% iron.

[2] Bul. Coll. of Agriculture Vol. III No. 1.

fungus grown on a substratum poor in iron like rice (0.005% Fe_2O_3) store up so much iron in the spores.

Since the question whether iron forms a regular constituent of nucleins is of considerable importance, I have made some further investigations with vegetable materials. My attention was first drawn to the richness in iron of the seed of Polygonum tinctorium, and this has led me to analyse more fully the ashes of the seeds and leaves of two indigo plants.

	Polygonum tinctorium		Indigofera tinctoria	
	Seeds.	Leaves.	Seeds.	Leaves.
In 100 parts of dry matter :				
Crude ash	2.84	15.5	4.0	4.3
In 100 parts of ash.				
SiO_2	0.35	1.75	3.1	6.25
SO_3	4.10	4.77	2.4	3.80
P_2O_5	41.1	4.13	34.2	5.50
K_2O	21.5	21.40	18.7	17.80
Na_2O	2.4	6.00	4.2	2.00
Fe_2O_3	12.1	3.11	12.0	4.8
CaO	5.1	39.10	11.0	37.0
MgO	15.2	12.30	9.9	6.4
Total	101.85	83.56	95.50	80.55

We see that about 12% of the ashes, corresponding to 0.34% of the dry matter, of the seeds consists of Fe_2O_3. Similar results were obtained with other samples. Such a high percentage of iron content in seeds has to my knowledge not yet been observed. On looking through Wolffs tables, I found that of the twenty two species of seeds examined, the richest in iron contains only 2.5% in the ash and 0.08% in the dry matter. Occasionally plants absorb by accident unnecessary substances from the soil and deposit them in the leaves and stems, but this can hardly be the case with the seeds.

In the following pages several experiments to isolate the iron compounds from the seeds[1] are described.

(1). Seeds of Polygonum tinctorium were dried and finely pulverized, and treated with ether, absolute alcohol and water. *These Extracts* contained no iron. Further the water extract

[1] All the materials used in this investigation were carefully tested for traces of iron and were found free of them.

contained very little proteids. The residue from the water extract, was now treated with 1% sodium chlorid solution. This extract was also almost free from iron. The residue now left, was treated with 0.2% caustic potash solution at the ordinary temperature, frequently stirred, and after standing for one day, the clear supernatant solution was decanted, since it was difficult to filter, and mixed with dilute acetic acid until a moderately acid reaction was obtained. Hereby a greyish white flocculent precipitate was abundantly produced. This precipitate, consisting of several proteids, was washed with water, at first by decantation, then collected on a filter, washed with water, alcohol and ether, dried and pulverized. It contained :—

N = 10.4% Fe = 0.5% N : Fe = 100 : 4.9.

Repeated tests have shown that nearly 60—70% of the iron contained in the seeds may thus be obtained in a form soluble in dilute potash and separable from this solution with acetic acid.[1]

(2). In this test I applied the method of Stoklasa which consists in extracting the seeds with a large quantity of 0.1% hydrochloric acid, evaporating this extract at 30—36°C and subjecting the product thus obtained to artificial pepsin digestion. The residue thus obtained was, however, so little (from 100 grams of the seeds only 0.1—0.2 gram was obtained) that further examination was impossibble.

(3). 100 g. of the dry seeds were extracted with ether and alcohol and the residue divided into two portions :—

One portion (a) was boiled with water for half an hour cooled to 60°C and a small quantity of malt extract added to saccharify the starch. The residue from filtration was washed with water and then subjected to artificial pepsin digestion, whereby a large part of the proteids was dissolved. The remaining residue was now treated with dilute caustic potash

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[1] Fearing that the extraction with potash might injure the original iron compounds to some extent I used in another case dilute ammonia for extraction ; but I observed that the iron compound is very imperfectly soluble in it.

a). Crude ferro-nuclein of Polygonum tinctorium was extracted with dilute ammonia and precipitated with alcohol, subjected to artificial digestion the residue once more dissolved in ammonia and precipitated with alcohol, it contained only N = 5.8%.

b). Crude ferro-nuclein of Indigofera tinctoria thus treated, yielded

N = 6.6% Fe = 0.51%.

and the alkaline extract was acidified with acetic acid whereby the crude ferro-nuclein was obtained :—

Ash	4.9%
Fe_2O_3	1.3%
Fe	0.91%

This precipitate was however probably not yet pure.

The other portion (*b*) was not previously deprived of its starch content, but directly subjected to artificial pepsin digestion, the insoluble residue treated with 0.1% caustic potash, acidified with acetic acid and the precipitate thus produced, was washed with water, alcohol and ether. Nearly 7 grams of impure ferro-nuclein resembling closely that of (*a*) was thus obtained.

Thus from 100 grams of dry sample I obtained nearly 10 grams of impure ferro-nuclein with about 0.9% iron.

(4). 0.2 grams of the crude ferro-nuclein (containing 0.5% iron and 5% nitrogen) of Polygonum seeds[1] was treated with 100 cc. of 0.2% hydrochloric acid, and divided into two parts. To one part, was added some pepsin and both were kept for twenty hours at the temperature of 37—40°, whereby a small portion of the iron was evidently splitt off by the action of the acid from the original compound, as the ferro-cyanide test clearly proved. It was further observed that the albumoses and peptones formed by the action of pepsin contained iron in organic combination.

These experiments were repeated with several other samples with essentially the same result. Since the haematogens of Bunge and of Stoklasa do not undergo any change by artificial digestion and as they do not so easily splitt off some iron by the action of 0.2% hydrochloric acid, my ferro-nuclein differs evidently from the former, especially since it is not easily soluble in dilute ammonia.

The existence of apparently the same ferro-nuclein in the seeds of Indigofera tinctoria, etiolated shoots of Polygonum tinctorium, and in the full grown leaves of Indigofera and Polygonum was shown by my further experiments. Similar

[1] and 0.1 grams of that of indigofera seed.

ferro-nuclein exists evidently also in other plants, as tests with mulberry leaves[1] and tea leaves[2] have shown.

Summary of Results.

1). Seeds of Polygonum tinctorium and Indigofera tinctoria are exceedingly rich in iron, as are the leaves of the same plants. The iron does not exist in these plants as inorganic salts.

2). Ethereal, alcoholic and aqueous extracts of the dried and pulverized seeds or leaves contain no iron. Also the sodium chlorid extract contains no iron compound or only traces of it. However the dilute alkali extract contains a nuclein like substance which can be precipitated with dilute acetic acid. This contains the greater part of the iron of the original material. This precipitate was subjected to artificial pepsin digestion, whereby a portion of the proteids dissolved, and if this solution is again precipitated with absolute alcohol the products formed still contained iron. The insoluble residue obtained from artificial digestion chiefly consists of a nuclein like substance and contains 0.5—1.0% iron and 5—10% nitrogen according to the methods of preparation. A small portion of iron was liberated during the digestion process.

3). Tests made to isolate the so called haematogen by the methods of Bunge and of Stoklasa from plant seeds, yielded unsatisfactory results. Stoklasa obtained 1.9 grams haematogen from 1500 grams of dry Allium cepa, that is to say nearly 0.002% of iron in the dry matter of the original material was found to be in the form of haematogen. With Pisum sativum his result was similar. As these vegetable materials contain on the average more than 0.02% of iron in the dry matter, only about one tenth of the iron in the original materials was obtained in the form of "haematogen." Therefore we may infer that the greater part of the iron in these cases also exists in other forms than "haematogen."

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[1] About 300 grams of dry powder were extracted with dilute caustic potash, but the precipitate obtained with acetic acid was exceedingly gelatinous, which made further purification difficult. It could however be easily shown that this precipitate contained iron.

[2] Tea leaves are very rich in iron, sometimes 12% of the ash consisting of the oxid. Compare the article of Mr. Aso in this Bulletin.

4). The iron compound which I obtained is evidently different from the so called "haematogen," since the former is partly soluble by artificial pepsin digestion, and both the residue and the dissolved portion (precipitable with absolute alcohol) contain iron in organic combination and liberate a small quantity of the iron by the action of 0.2% hydrochloric acid while the so called "haematogen" does not undergo any change by artificial digestion or by the action of 0.2% hydrochloric acid for a short time at the ordinary temperature. The substance obtained by me is also much more difficultly soluble in dilute ammonia.

5). A similar iron compound exists in many other plants according to my examination, and its distribution seems to be very wide.

Investigations on the Mulberry Dwarf Troubles, a Disease widely spread in Japan.

BY

U. Suzuki.

In a former number of this Bulletin (Vol. IV, No. 3) I have described how widely distributed this disease is and how much damage is caused by it. The general diagnosis was given, numerous analyses were carried on to find out the differences in chemical composition of the healthy and the diseased plants; the relation of reserve materials to the disease was especially investigated, and extensive field experiments were further made and many facts observed with plants from different provinces. From my studies I came to the conclusion that the disease was not caused by any parasite, but the primary cause was to be sought in the practice now in vogue of subjecting the mulberry tree to repeated cuttings in the growing season. During winter a considerable amount of reserve materials (especially nitrogen compounds and starch) is stored up in the bark of stems and roots, while in spring, when the development of new leaves commences, the greater part of it is transported to the growing shoots. The assimilation products in the leaves again migrate to the stems and roots in late autumn, when the leaves begin to fall. Therefore the stems and roots are rather poor in reserve materials during the growing season. Now it will be at once evident that the cutting of the plants in the growing season must have a very bad effect on the shoots that develop later on, since the supply of reserve materials upon which they depend is but very scanty. Moreover, it is not impossible that the stored materials may be entirely exhausted before the new shoots have attained a certain height and have become able to prepare their own organic food by assimilation. In such a case

the normal growth of the shoots will be seriously impaired and starvation must set in ; the leaves remain small and their tissues develop only partly. For the same reason, the disease must be induced by the frequent plucking of the leaves. This view was comfirmed by numerous observations and no doubt could further be entertained about the correctness of my inferences. But there remain still many points to be investigated, in regard to the various pathological phenomena, accompanying the deficiency of reserve materials, and also in regard to other secondary causes which accelerate the disease. Finally methods of checking or preventing the disease should be found. The following investigations were carried out with the intention to throw more light upon the pathology of the disease.

OXIDIZING ENZYMES.

The wide distribution of oxidizing enzymes in the vegetable kingdom has recently attracted the notice of many plant physiologists and various investigations have been made upon the nature and the physiological actions of these interesting substances. Albert F. Woods[1] has propounded the view that the change of chlorophyll in the autumnal yellowing of leaves is chiefly due to their action, a view which seems to agree well with the fact that the oxidizing enzymes are abunduntly produced in autumn when the activity of the cells in the leaves decreases. If such leaves are ground in a mortar with addition of some water, the green colour will soon be changed to reddish brown, while generally the green of the leaves in full vigour is but slowly changed. Woods has also made very interesting observations on the influence of the oxidizing enzymes upon various diseases of plants. Thus he found that the plants diseased either by some physiological causes or by the attacks of insects and fungi or by some mechanical disturbances, produce always an abnormal quantity of oxidizing enzymes. He made especially careful examinations of albinism or variegation of many plants, especially of the so-called "Mosaic disease" of tobacco : In the light green parts of the diseased leaves, always much of the oxidizing enzymes has been found, sometimes even five or six times more than in the normal leaves. Further those discolored

[1] Centr.-Bltt. für Bakt. II. Abt. V. Band 1899 No. 22.

regions contained, in spite of the imperfect developement of chlorophyll, always many starch granules. He found that the oxidizing enzymes can inhibit the diastatic action, and by making the saccharification of the starch in the cells much more difficult than in the healthy leaves, prevent the migration of it into other parts of the plants.[1] To prove the correctness of this view, he prepared a concentrated solution of tobacco oxidase, mixed it together with a small quantity of "Taka-diastase" or malt extract with some diluted starch paste and kept the mixture at 45°C. At the same time a control mixture was prepared, in which the oxidase solution was previously boiled for a few minutes; After thirty minutes a very remarkable difference was observed. In the former case starch was not at all saccharified, erythrodextrin being the final product, while in the latter (control case) the entire starch was saccharified. Such phenomena may also occur in the diseased leaves. Beijerinck[2] supposes that there exists a so-called "Contagium vivum fluidum" in the extracted and filtered juice of the tobacco leaves suffering from the mosaic disease. This fluid contains no visible organism, still it produces the same disease by injection. Woods rejects this view and thinks that the so called "contagium vivum fluidum" is identical with the oxidizing enzymes. He made several experiments to produce the disease by injecting solutions of oxidizing enzymes into the healthy plants and succeeded in some cases, though not always.

The question why oxidizing enzymes are produced in an abnormally large quantity in the diseased leaves has not yet been satisfactorily answered, but it is very probable that the decrease of vital activity and the deficiency of nutriment in the cells has some intimate relation with their production. Woods succeeded in producting the mosaic disease artificially by cutting back rapidly growing tobacco plants. Loew also holds the view that partial starvation can cause an increase of these

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[1] Dr. Smith also observed an increase of the amount of the oxidizing enzymes in the diseased leaves in the "peach yellow"-disease.

[1] Science. N. S. Vol. XI., No. 262, page, (January 5, 1900).

Beijerinck. Verhandelingen der Koninklijke Akademie van Wetenschappen to Amsterdan. Tweede sect. Deel. VI. 1898, No. 5.

enzymes. Brown and Morris[1] proved that the secretion of diastase is much enhanced by partial starvation. Weak lily plants containing much of oxidizing enzymes, especially peroxidase, are always those which have a very poor root system and are suffering from partial starvation. Church analyzed the albino leaves of *Ilex aquifolium*. *Hedera helix* and *Acer negundo* and always found less organic matter, especially nitrogen compounds. Thus in *Acer negundo* the following differences were observed :—

	Albino leaves.	Green leaves.
Water.	82.83	72.70
Organic substances.	15.15	24.22
Ash.	2.02	3.08

By the puncture of aphides, scale insects, leaf hoppers, etc., the attacked parts show partial starvation, sugar and nitrogenous matter being removed, and thus, as Woods found again, an increase of oxidizing enzymes results. In short, there is no doubt that the oxidizing enzymes stand in close relation with many diseases of plants. This has led me to examine whether in the mulberry dwarf disease, these enzymes are also produced in abnormal quantities and stand in causal relation to it. This will prove of interest not only for further explanations of the phenomena of the mulberry dwarf disease, but also as a further contribution to the knowledge of the oxidizing enzymes.

The tests for oxidizing enzymes in the mulberry plant were made exactly after Loew and Woods. The details are as follows :

a) Oxidase.

Oct. 2. Several samples were gathered at Komaba, and tested in the fresh as well as in the air dry condition. The diseased leaves contained always more oxidase, sometimes four or five times as much as the healthy ones. The air dry and powdered leaves were at first treated with alcohol and ether, the residue dried, ground in a mortar, and made into a fine paste, with addition of some water. The paste was then diluted and filtered, and to the clear filtrate were added a few drops of guaiac tincture. A difference was at once observed between

[1] Journal of the Chemical Society Vol. XIII. London 1893.

the healthy and diseased leaves, thus the filtrate obtained from the latter producing at once a dark blue color, while in the filtrate obtained from the healthy leaves the coloration set in much more slowly and increased but gradually.

2. Oct. 29. *Roso* variety, gathered at Komaba. The diseased leaves contained nearly two and a half times as much oxidase as the healthy ones. As the leaves were still healthy and the assimilation process was still energetically going on and as there was no noticeable quantity of tannin and albumin, the colorimetric comparison must be considered reliable. It is also to be noticed here that the diseased leaves turned very quickly reddish brown on being ground in a mortar with addition of some water, chlorophyll and other organic substances being changed in but a few minutes, while in the case of the healthy leaves, the change of color was very gradual, the green color of chlorophyll being retained for a long time. This evidently shows the strong oxidizing power of the diseased leaves. It was generally observed that the growing leaves in full activity and full assimilation energy, contained always less oxidase than the old leaves. Very young plants, however, contained sometimes much more oxidase than the full grown ones. So it is very probable that the activity of oxidizing enzymes is inversely proportional to the energy of assimilation.

3. Oct. 5. *Akagi*-variety at Nishigahara. The diseased leaves contained much oxidase but the difference from the healthy ones was not so marked here.

4. Oct. 27. Other varieties behaved as follows :

Yeijiwase	Diseased leaves contained 2-3 times as much oxidase.					
Ichihei	,,	,,	,,	6-7	,,	,,
Roso	,,	,,	,,	2-25	,,	,,
Tsuruta	,,	,,	,,	a little more but the difference not very marked.		
Nezumigayeshi	,,	,,	,,	2	,,	,,
Shimanouchi	,,	,,	,,	2	,,	,,
Jūmonji	,,	,,	,,	2	,,	,,
Akagi	,,	,,	,,	a little more but the difference not very marked.		
Yanagita	,,	,,	,,	difference not very marked.		
Takasuke	,,	,,	,,	difference not very marked.		

Sometimes the difference was not very marked since the extract was too concentrated, but after a proper dilution, the

difference became very noticeable, sometimes the difference was heightened when the extract was heated to 70°C for five minutes, thus retarding the activity of oxidase. But except in a few cases, this procedure was hardly necessary, the differences being plainly noticeable.

b) Peroxidase.

Peroxidase, as already mentioned, does not produce a blue colour on simple addition of guaiac tincture, but it requires also some hydrogen peroxid. It always accompanies oxidase in the mulberry leaves. Its resisting power against heat is, however greater than that of the latter. After destroying the oxidase at 75—80°C, the peroxidase can still produce a distinct blue coloration with hydrogen peroxid and guaiac tincture. The coloration was always much darker than that of the oxidase and somewhat greenish. We can also prove the presence of peroxidase when mixed with oxidase by properly diluting the extract. Thus, the extract was divided into two parts, to one portion was added only guaiac and to the other guaiac and hydrogen peroxide. After a few minutes, a very marked difference was observed; the blue coloration being several times more intense in the latter. Further, by heating to 70°C, the power of oxidase is largely weakened and the subsequent addition of hydrogen peroxid produces a distinct difference after a few minutes. The opinion of Spitzer, that peroxidase can cause the developement of oxygen from hydrogen peroxide is erroneous, since the activity of catalase, i.e., the power of decomposing hydrogen peroxid, is entirely lost by heating to 70—75°C for five minutes, while still a distinct blue coloration may be produced by the addition of guaiac and hydrogen peroxid.[1] In the mulberry leaves I found always a good deal of peroxidase. The comparison of the peroxidase reaction in healthy and diseased leaves showed the following result:

Oct. 27. gathered at Nishigahara.

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[1] Hydrogen peroxide is for our purpose conveniently prepared by dissolving 6 grams of sodium peroxid in 100 c.c. water and exactly neutralizing this solution with dilute sulphuric acid.

Takasuke	Diseased leaves contained 2 times as much or more peroxidase.				
Akagi	„	„	„	„	„
Roso	„	„	4-5 times more, healthy leaves contained only traces.		
Jumonji	„	„	2	„	„
Nezumigayeshi	„	„	2	„	„
Tsuruta	„	„	2	„	„
Shimanouchi	„	„	much, while the healthy contained only traces.		
Yanagita	„	„	more		
Yeijiwase	„	„	very much		
Ichihei	„	„	„	„	

Oct. 29. *Roso.* At Komaba. Diseased leaves contained very much.

We see from the above results that the diseased leaves contain always much more peroxidase than the healthy ones; but here I must mention that all the above samples were taken in October and never earlier in the year. It will be necessary to repeat these experiments once more with the leaves of early summer, though I have no doubt that essentially the same results will be obtained. The root bark of the diseased plants also contains sometimes much more oxidase than that of the healthy ones.

Some experiments were also made to see whether zymogens of the oxidizing enzymes exist in the plant cells, but no decisive result has thus far been obtained.

As we have already stated, the transportation of starch in the diseased leaves is very slow and sometimes becomes almost insignificant and much of it is therefore retained in the leaves. *This is one of the most characteristic phenomena of the mulberry dwarf disease.* This fact was repeatedly observed by Prof. Miyoshi by means of the jodine test. I have also stated in my former report that much of the assimilation products in the diseased leaves remains unchanged there. Miyoshi gathered the leaves in the early morning and at 3 o'clock in the afternoon, and compared the amount of starch. He found in the healthy leaves almost always no starch in the morning but very much in the afternoon; in the diseased leaves, however, he found no essential difference in the starch content at different hours of the day. This shows that the starch formed in the healthy leaves during the day time is completely dissolved during the night and for the most part transported to other organs, while one portion is consumed by the respiration process. The migration of starch

in the healthy leaves is remarkably rapid; thus, I have very often observed in October that in rainy or cloudy weather or even in the evening shortly after the sun had set, starch was no longer visible, and only in fine weather when the leaves were exposed to strong sun light, the accumulation of starch in the leaves was plainly observed. The following table also shows very marked differences between healthy and diseased leaves:

Average composition of healthy and diseased leaves (15 samples).

In 100 parts of dry matter:

	Healthy.	Diseased.	Ratio.
Crude protein	31.47	25.76	100 : 81.8
Crude fat	4.42	3.80	100 : 86.0
Crude fibre	10.00	8.14	100 : 81.4
Other compounds, chiefly starch	47.97	57.60	100 : 120.0
Crude ash	8.52	7.75	100 : 91.0

We see from this table that the starch content is markedly increased in the diseased leaves. I have also repeated the experiment of Prof. Miyoshi; 0.1—0.2 grams of dried and pulverized leaves were boiled for a few minutes with 10 c.c. water to pastify the starch. Upon addition of a few drops of iodine tincture, the following results were obtained.

Starch content.

		Healthy.	Diseased.	
Oct. 2	Roso	None	Much	
„ 6	Jumonji	„	„	
„ 5-6	Akagi			
	Morning 6 o'clock,	„	„	
	Noon 12 „	much	„	
	Evening 6 „	Almost none	„	
„ 27.	Ichihei	none	„	gathered 10 a.m. cloudy day.
„ „	Nezumigayeshi	very little	„	
„ „	Takasuke	Almost none	„	
„ „	Akagi	none	„	
„ „	Yanagita	„	„	
„ „	Jumonji	„	„	
„ „	Shimanouchi	„	„	
„ „	Tsuruta	„	„	
„ „	Roso	„	„	

The same experiment was repeated with several other samples, always with the same result. I tried to see how long it would take to use up the starch deposited in the diseased leaves when kept in the dark, the assimilation process

being completely prevented. For this purpose, a healthy and a diseased plant were selected. A portion of the leaves was taken from each plant for control (*a*) (Oct. 11) while the entire plants were then covered with a large black paper cylinder and thus kept deprived of light for two weeks, when leaves were again gathered (*b*). The iodine test gave the following results :

		(*a*)	(*b*)
Nezumigayeshi (1)	Healthy	very little	none
	Diseased	very much	Almost none
,, (2)	Healthy	Almost none	none
	Diseased	very much	Almost none
Ogon (Normal condition) (Not kept in the dark)	Healthy	very little	none
	Diseased	very much	very much

Thus even after two weeks in darkness, traces of starch were still present, while in the healthy leaves, the starch disappeared completely in one night.

I have also endeavored to find out whether the migration of nitrogenous compounds in the diseased leaves is also retarded like that of starch. It is a well known fact that the nitrogen salts absorbed from the soil, are converted chiefly in the leaves into proteids. According to my observation[1] the synthesis of proteids chiefly goes on in the leaves during the day time, whereas during the night they are again split up into soluble amido-compounds which are transported to the other organs, like starch is in the form of sugar. I have also observed that in the young growing leaves the migration of these amido-compounds during one night can clearly be shown by analysis ; the absolute quantity of nitrogen in the leaves gathered in the morning is considerably less than in the evening. As to the mulberry tree my experiment was made a little too late in the season, and the migration intensity was too weak to show a sufficient difference after one night. Hence I adopted the following way : In the late autumn, when the activity of the cells decreased and the absorption of nitrogen compounds became rather slow, a healthy and a diseased plant were selected ; a portion of the leaves was taken from each plant and dried directly in the air bath at 50°C, while another

[1] Bulletin of the Coll. of Agriculture. Komaba, Tokyo. Vol. III, No. 3.

portion of the leaves was gathered from the same branches 10—14 days later. Both samples were now carefully compared in regard to their nitrogen contents. As in this period nitrogen compounds migrate from the leaves into other organs in larger quantities and more quickly than they are formed by synthesis, the leaves must show a considerable decrease in the amount of nitrogen, and this decrease must be greater if the migration is quicker. This method would, of course not be applicable in the growing season, when the synthesis of proteids in the leaves surpasses the intensity of transportation. Another method of comparison was as follows: In late autumn, portions of leaves from a healthy and a diseased plant were taken and directly dried at 50°C, while the plants were covered with large black paper cylinders and kept thus in the dark for 10—14 days. Hereby the synthesis of proteid, was suppressed, since glucose, the product of carbonic acid assimilation is hereby absolutely required. The results were as follows :—

Oct. 15—26. Takasuke. Kept normal, not in dark.

	Healthy.		Diseased.	
	Oct. 15	Oct. 26	Oct. 15	Oct. 26
Number of leaves.	26	25	25	25
Fresh weight.	48.8	41.3	18.80	20.6
Dry weight.	19.711	16.309	6.948	6.816
Moisture.....	59.6%	60.5	63.1	66.9
Dry matter.	40.4%	39.5	36.9	33.1
Fresh weight of one laef. ...	1.88	1.65	0.75	0.82
Dry weight „ „ „ ...	0.76	0.65	0.28	0.27
Ratio of dry matter „ „ ...	100.0	85.5	100.0	96.4
Total nitrogen „ „ ...	0.0293	0.0223	0 00997	0.0103

This table clearly shows that the nitrogen compounds are transported far more quickly from the healthy leaves than from the diseased ones. In the former case nearly 14.5% of the dry matter and 23.9% of the nitrogen disappeared from the leaves during 11 days, while in the latter case only a decrease of 4% of dry matter and no decrease of nitrogen was observed.

b). Oct. 11. Leaves from another variety served for a similar experiment.

Control (*a*) ; Leaves 14 days in darkness (*b*).
Nezumigayeshi (I).

	Healthy.		Diseased.	
	(*a*)	(*b*)	(*a*)	(*b*)
Number of leaves	25	25	25	25
Dry weight of one leaf	0.78	0.552	0.46	0.381
Ratio of dry matter in one leaf	100.0	70.8	100.0	82.8
Total nitrogen	4.64	4.92	4.04	4.39
Total nitrogen in one leaf	0.0362	0.02706	0.01858	0.01678
Ratio of total nitrogen in one leaf	100.0	75.1	100.0	90.0

Decrease of nitrogen during 14 days ; Healthy **100** : Diseased **40.1.**

Nezumigayeshi (2). Kept in dark 11—25.

	Healthy.		Diseased.	
	Oct. 11	Oct. 25	Oct. 11	Oct. 25
Number of leaves	30	30	30	30
Dry weight of one leaf	0.81	0 637	0.32	0.29
Ratio of dry matter in one leaf..	100.0	78.7	100.0	90.5
Total nitrogen	4.53	4.78	3.61	3.76
Total nitrogen in one leaf.....	0.036g	0.03014	0.01155	0.01090
Ratio of total nitrogen in one leaf	100.0	83.0	100.0	94.3

Decrease of nitrogen during 14 days ; Healthy **100**: Diseased **33.5.**

c). Ōgon. Kept normal, not in the dark. 11—25.

	Healthy.		Diseased.	
	Oct. 11	Oct. 25	Oct. 11	Oct. 25
Number of leaves	30	30	30	30
Dry weight of one leaf.....	0.83	0.60	0 30	0.26
Ratio of dry matter in one leaf..	100.0	72.2	100.0	81.8
Total nitrogen	4 03	4.05	3 32	3.86
Total nitrogen in one leaf.....	0.003345	0.00243	0 001c6	0.001004
Ratio of total nitrogen in one leaf	100.0	72.6	100.0	95.2

Decrease of nitrogen during 14 days ; Healthy **100** : Diseased **27.6.**

These experiments clearly show that the migration of nitrogen compounds in the diseased leaves is very much slower than in the healthy ones.[1] These observations perfectly agree with the fact that the stems, roots and dormant buds in the diseased plants contain always very little reserve materials during the winter.

On the existence of diastase in mulberry leaves.

On Oct. 5, leaves gathered at Komaba were dried at 30—40°C, pulverized and ground well in a mortar with some water, and then, after addition of some more water, filtered after 2—3 hours. The extract was devoid of reducing substances. A few c.c. of this extract was added to a highly diluted starch paste and kept at 50—55°C for half an hour.

A small amount of reducing sugar was now clearly recognized in the extract of the diseased leaves, but only a trace in that of the healthy ones (The healthy leaves contained no starch, while the diseassd contained much.)

Oct. 6. Jumonji, at Nishigahara, dired at room temperature and powdered. Here neither the healthy nor the diseased leaves showed any diastatic power (Here the healthy leaves contained no starch, while the diseased contained it moderately.)

Oct. 6. Akagi at Nishigahara.

	Diastase.		Starch content.	
	Healthy.	Diseased.	Healthy.	Diseased.
Morning 6 o'clock	none	much	none	much
Noon 12 „	none	much	very much	much
Evening 6 „	somewhat	somewhat	somewhat	much

As the boiled extract in both cases produced no sugar, the presence of diastatic ferment in the diseased leaves is most probable.

Oct. 2. Roso at Komaba. 5 grams air dry sample were finely pulverized, ground with addition of water, diluted to 100 c.c., let stand for 24 hours and filtered. 2 c.c. of the filtrate was added to 10 c.c. of starch paste and kept for one and a half hour at 50°C. The extract of the diseased leaves reduced nearly 10 c.c. of Fehling's solution, while that of the healthy leaves reduced only half as much. In the control test, where

[1] I have not yet proved the existence of a proteolytic enzyme in the mulberry leaves, yet its presence is very probable and the oxidizing enzymes may probably inhibit also the action of it, as it is the case with diastatic ferments.

the extract was boiled before the addition of starch paste no reducing sugar was produced. The presence of the diastatic ferment was beyond doubt.

Oct. 12. Akagi (cut in autumn) at Nishigahara. The root bark of both, healthy and diseased plant, contained originally some reducing sugar. That of the healthy one however contained considerably more diastase than that of the diseased.

Oct. 12. Takasuke at Nishigahara (cut twice in a year and deprived of the young leaves and hence diseased). The root bark, of both, the healthy and diseased plants contained very little diastase.

Oct. 12. Akagi (cut in summer) at Nishigahara. The root bark, both of the healthy and diseased plants contained little diastase. We see from the above experiments that the diseased leaves contain generally more of diastatic ferment than the healthy ones, but no distinct relation was observed with the root bark.[1]

Woods has observed that oxidase can inhibit the action of diastase; This depends of course much upon the relative quantities of oxidase and diastase. In the cases above described the oxidase was probably too diluted to prevent entirely the diastatic action.

On the existence of catalase in the mulberry leaves.

Catalase was found by O. Loew in 1900 in the leaves of the tobacco plant; He first observed that the tobacco leaves contain an insoluble enzym which liberates oxygen gas from hydrogen peroxid. Physiologists have until now attributed this action on hydrogen peroxid to every enzym. Loew confirmed by careful investigation the different and independent nature of the enzym and proved that diastase or any other enzym in their purest state never possess this property. He extended his observation to several other plants and at last reached the conclusion that the enzym is contained in every living cell of plants. It has decidedly the important function to destroy every trace of the

[1] The apparent contradiction between the existence of diastase and that of much starch in the diseased leaves is easily explained on the ground that the sugar formed by diastatic action is not transported to the trunk and hence that it is always reconverted into starch by the chloroplasts. We further have to concede that there may be in the diseased leaves, as long as they are fresh, only the zymogen of diastase, which in my experiments just mentioned, generally was transformed into diastase itself.

poisonous hydrogen peroxid that may be formed as a by-product in the process of respiration. I thought it of some interest to investigate whether there is an abnormal increase of this enzym in the diseased mulberry leaves. I took at first 1 gram of air dried and powdered leaves, mixed with 30 c.c. water and added 5 c.c. of freshly prepared hydrogen peroxid of 2—3% strength. The development of oxygen gas was here so energetic that I used in the following tests only 0.1 gram of the dried leaves. Temperature and the reaction of the sample have much influence upon the development of oxygen, 30—40°C and neutral or slightly acid reaction being preferable.[1] Great care was taken to have exactly the same conditions in all tests. The following table shows the results.

1. Akagi leaves gathered on Oct. 5, 0.1 g. air dry.

	Morning. 6 o'clock.		Noon. 12 o'clock.		Evening. 6 o'clock.	
Minutes.	Healthy. (c.c. Oxygen.)	Diseased.	Healthy. (c.c. Oxygen.)	Diseased.	Healthy. (c.c. Oxygen.)	Diseased.
5	2.3	2.0	2.7	3.7	2.5	4.0
10	3.4	4.2	4.0	6.8	4.7	6.6
20	5.0	9.0	8.0	11.5	9.2	11.2
30	6.8	11.2	10.7	14.5	11.1	14.0

2. Roso leaves, fresh 0.5 grams. Oct. 21.

Minutes.	Healthy.	Diseased.
5	7.0 c.c.	2.
10	10.0	4.
20	14.5	6.2
30	16.0	7.7

[1] It is to be mentioned that by frequent shaking, the development of oxygen is very much accelerated. In this experiment, the mixture was shaken on the addition of hydrogen peroxid and afterwards left fo itself. In some cases the mixture was shaken at the last reading.

3. Shimanouchi, Oct. 27.

Minutes.	Healthy.	Diseased.
5	1.5	3.0
10	2.5	3.5
20	4.2	4.0
30	4.9	4.3
40	5.5	5.0

4. Takasuke, Oct. 27.

Minutes.	Healthy.	Diseased.
5	2.6	2.3
10	3.3	3.6
20	3.8	4.3
30	4.0	5.0

5. Jumonji, Oct. 27, air day 0.1 gram.

Minutes.	Healthy.	Diseased.
5	2.0	3.3
10	2.7	4.2
20	2.7	4.5
30	2.8	5.2
After frequent shaking	5 0	7.2

6. Akagi, Oct. 27, air dry 0.1 gram.

Minutes.	Healthy.	Diseased.
5	4.4	0.8
10	4.5	1.0
20	4 5	1.6
After shaking.	4.3	5.3

[1] The healthy leaves were here still very active, but the diseased ones were in a rather poor condition.

7. Roso, Oct. 27, air dry 0.1 gram.

Minutes.	Healthy.	Diseased.
5	1.0	0.9
10	1.7	1.6
20	2.7	2.7
After shaking.	5.7	5.5

8. Tsuruta, Oct. 27, air dry 0.1 gram.

Minutes.	Healthy.	Diseased.
5	0.5	1.3
10	1.5	2.7
20	2.8	3.7
30	3.5	4.4
After shaking.	6.5	8.0

9. Yanagita, Oct. 27, air dry 0.1 gram. (Temp. 14°.)

Minutes.	Healthy.	Diseased.
5	0.4	1.5
10	1.0	2.6
20	1.7	3.4
30	——	——
40	2.2	4.2
60	2.4	4.7
After shaking.	3.4	6.7

10. Nezumigayeshi, Oct. 27, air dry 0.1 gram. (Temp. 14°).

Minutes.	Healthy.	Diseased.
5	3.7	1.0
10	4.3	1.8
After shaking.	no more.	no more.

11. Yeji wase, Oct. 27 air dry 0.1 gram.

Minutes.	Healthy.	Diseased.
5	1.0	6.3
10	1.6	6.7
After shaking.	2.3	9.7

12. Ichihei, Oct. 27, air dry 0.1 gram.

Minutes.	Healthy.	Difeased.
5	3.0	3.7
10	5.1	5.7
20	6.9	7.2
30	7.6	7.8
After shaking.	8.9	8.9

13. Jumonji, Oct. 27.

Minutes.	Healthy.	Diseased.
5	7.0	6.5
10	8.0	12.0

14. Akagi, root bark, Oct. 24, air dry 0.5 gram. (Temp. 21°).

Minutes.	Healthy.	Diseased.
5	4.5	2.9
10	4.6	3.3
30	4.6	3.7
After shaking.	7.7	7.7

15. Akagi, (cut in Autumn) root bark, Oct. 12, air dry 0.5 gram.

	Healthy.	Diseased.
After 2 hours.	1.2	6 0

16. Jumonji, root bark, air dry 0.5 gram.

	Healthy.	Diseased.
After 2 hours.	6.0	4.0

17. Akagi, (cut in summer) root bark, air dry 0.5 gram.

	Healthy.	Diseased.
After 2 hours.	1.0	4.0

We see from the above tables that the diseased leaves contain generally much catalase, but there are still some exceptions; and further the air dried leaves develop oxygen always less energetically than the fresh ones, hence I can not draw any safe conclusion until further experiments can be made next summer.

According to Loew, there are two kinds of catalase, one soluble, and the other insoluble in water, the latter being perhaps a kind of nucleo-proteid. In the leaves of the mulberry there exist also these two kinds. The soluble catalase develops oxygen very quickly but stops very soon. On the contrary, the insoluble catalase develops oxygen slowly but continues longer. The action of soluble catalase is almost destroyed by 5 minutes heating to 70°C, but after standing for 2-3 hours, the activity somewhat returns and from 0.1 gram., 5 cc. of oxygen were developed in two hours by frequent shaking. In another experiment the pulverized leaves were heated to 70°C for five minutes and filtered, the filtrate at that time had no activity, which however after standing for 48 hours developed about 5 c.c. oxygen in two hours. This was also the case when some chloroform was added as an antiseptic.

Influence of cutting upon the decay of roots.

The decaying of small rootlets and finally of the entire root system is one of the most remarkable phenomena of the mulberry dwarf disease, and many have believed it to be due to parasites. But as I have already discussed in my former report,

the principal cause of the disease is never of parasitic nature. It is exclusively the cutting in the growing season that causes the disease. Small rootlets which develop vigorously in the growing season can only support their activity by the supply of organic nutriments furnished by the leaves. Therefore the cutting off of the branches and leaves in the growing season stops the supply of food to the roots. At first the small rootlets starve and die off. New rootlets however come forth again after the new shoots had reached a certain height and acquired power of assimilation. I have calculated that the new shoots coming from the cut stocks, rely upon the reserve material in the roots even as long as 40 days after cutting and until they have reached the height of 50—60 cm. Therefore it is very evident that if the new shoots had used up all the reserve materials in the roots before the development of new rootlets which can absorb the soil nutriments freely, then the new shoots must naturally suffer from starvation and disease must be the final result. I have proved this fact experimentally by examining the state of root development during the last summer. In the beginning of June, when the leaves were in full development, the growth of roots was very marked and small rootlets (so called white roots) were well developed forming a net work. After cutting in June (June 5—10) the growth of these rootlets was completely stopped and the white fresh appearance gradually turned yellow and reddish brown and finally the roots decayed. Even 30 days after cutting, no development of new rootlets was yet observed, while the development of the rootlets of the plants not cut was more and more advanced. In two weeks from cutting new shoots had developed and reached 50—60 centimeter in four or five weeks, but new rootlets were not yet visible. Later on they gradually appeared but were very scanty and until autumn they failed to achieve the normal development. These facts were observed with many varieties and under different conditions and agree perfectly well with my former assumption that the new shoots developing almost directly from the roots rely upon the reserve materials in the roots even after reaching the height of 50—60 cm. Thus it is very evident that the exhaustion of reserve materials may very often take place before the new shoots can be provided with new mineral nutriments from the soil and this circumstance again retards

the development of the entire root system, finally causing its starvation and death, the death being followed by decay which spreads gradually into the larger roots. I have observed very remarkable cases which furnish a convincing proof. The rows of a mulberry plantation were alternately cut at the beginning of June; a remarkable difference was here already observed at the end of July when the roots were examined. The normal plants had white rootlets like net works while the plants of the cut rows had no white rootlets whatever. Also the thickness of the root bark showed a considerable difference. Thus it is very evident that the power of developing new rootlets is more and more retarded in consequence of the successive cuttings in the growing season.

On the quantity of reserve starch.

I have already shown by careful analysis that the diseased leaves and stems contain always less nitrogen, and Miyoshi has also proved that the diseased stems and buds contain considerably less starch than the healthy ones. As the migration of starch and nitrogenous compounds from the diseased leaves is but very slow, these phenomena are naturally to be expected. But according to my assumption, the deficiency of reserve materials is not only the effect of the disease, but it must become the cause of its further development In other words, those plants which had used up the reserve materials must first show the disease. The following observations were made to test this view.

1). Jumonji, Sept. 30. The root bark of the healthy plants contained much starch but that of the diseased contained none.

2). Akagi, Sept. 30. The root bark of healthy plant contained some starch, but that of the diseased not. As this plant was cut at the end of August and the new shoots were still very young, no new rootlets had yet developed. Sufficient time has not yet elapsed for the new shoots to prepare the reserve starch, on the contrary they were still depending upon the reserve materials which had been prepared before cutting. We can say that the first appearance of the disease is caused by the exhaustion of the reserve materials in the roots.

3). Agaki (cut in autumn) was examined on Oct. 20, the

disease had appeared shortly before the examination was commenced.

The healthy roots contained much more starch than the diseased ones and further there was a remarkable difference in the thickness of the rooot bark, that of the diseased one being only $\frac{1}{3}-\frac{1}{2}$ as thick as that of the healthy one.

4). Takasuke.

Some of the plants were subjected to cutting twice in summer and the new shoots coming were repeatedly deprived of the new leaves. Upon the appearance of the signs of a severe disease they were examined on Oct. 12. Those which remained still healthy under the same treatment, contained some starch, while the diseased ones were almost entirely deficient of it, a proof that the plants which used up the reserve materials first became also first diseased.

5). Akagi, Oct. 12.

	Total carbohydrates.		Total nitrogen.	
Root bark of.	Healthy.	Diseased.	Healthy.	Diseased.
Akagi (1) (cut in autumn)	17.0	10.3	—	—
Akagi (2)	14.5	13.7	—	—
Takasuke (twice cut)	16.2	15.1	1.48	0.92

The above experiments suffice to show that the disease is evidently due to the exhaustion of the reserve materials in the roots.

Summary and Conclusions.

My former view on the primary cause of the disease was further confirmed by the experiments carried on in the last year. It was experimentally proved that the bad development and finally the decaying of roots in the diseased plants is simply caused by the cutting in the growing season and is not due to parasite attacks. The small rootlets which had grown considerably, before cutting, lose their activity soon after cutting and go gradually to decay. They do not develop anew until 40–50 days after cutting when the new shoots had reached 50–60 cm. in height. The new shoots depend upon the reserve materials in the roots until that time. It was further shown that the root bark of those plants which had just commenced to show the signs of the disease contained always less reserve material than that of the healthy ones. The deficiency of reserve

materials in the roots is not only the result of the disease but also the principal cause of its further development.

Abnormal increase of oxidase and peroxidase in the diseased leaves is one of the most striking phenomena. At the same time, we observe that the migrationof starch and nitrogenous compounds is remarkably retardedin the diseased trees. According to the investigation of Woods, it is very probable that the oxidizing enzymes not only destroy chlorophyll but also exert an inhibiting action upon the diastatic and proteolytic enzyme and thus may become one of the principal causes of the retardation of migration power and consequently of the retardation of growth in the diseased plants. A second cause has been found recently by Prof. Miyoshi in the insufficient development of the transportation tissues. These phenomena are closely analogous to those of the mosaic disease of tobacco and the variegation or albinism of other plants, and further with the parasitic diseases caused by fungi and insects. In the latter case also the abnormal production of oxidizing enzyms and the retardation of starch migration are the principal phenomena. Although we have no definite opinion on the cause why oxidizing enzyms are produced in such abnormal quantities in the diseased plants, yet the partial starvation of the cells seems to be the probable cause of it. As the cutting of mulberry plants in the growing season causes deficiency of nutriments for the new developing shoots, this seems to afford a new and strong support for the above assumption.

Further investigations on this mulberry disease are intended.

Contributions to the Physiological Knowledge of The Tea Plant.

BY

U. Suzuki.

I. Is theine contained in the seeds of the tea plant ?

There can hardly be any doubt, that the production of theine takes place in the tea plant in the green leaves, but as to the presence of theine in other organs and its variation under different influences, but little is known. Kellner examined the seeds and his conclusion that the seeds are free from theine agrees well with my recent investigation. Theine therefore does not follow the current of other nitrogenous compounds, as asparagin, tyrosin, leucin, probably for the reason that it is not changed in the seeds into other compounds.

I have subjected the seeds to a complete analysis which yielded results very similar to those of Kellner.

1. Composition of the seed.

The seed was shelled, dried, pulverized and analyzed.

	In 100 parts of dry matter.	
	Suzuki.	Kellner.(1)
Crude protein.	10.68	11.00
Crude fat.	32.70	37.41
Crude fibre.	5.90	2.80
Total carbohydrates.	31.00	—
Crude ash.	3.30	2.90
Nitrogen free extract.	47.42	45.89
Total nitrogen.	1.71	1.73
Albuminoid nitrogen.	1.48	1.50
Non-albuminoid nitrogen.	0.23	0.23

	In 100 parts of ash. (including CO_2)
$Si O_2$	0.10
SO_3	3.66
$P_2 O_5$	15.48
$K_2 O$	41.63
$Na_2 O$	1.67
$Fe_2 O_3$	2.00
$Ca O$	7.50
$Mg O$	9.80

(1) Kellner ;—Bull. Agric. Coll. Tokyo, Komaba. Vol I.

The tables show that the greater part of the nitrogen in the seed is present in the form of protein compounds and only a very small portion in the form of non-albuminoid compounds.

To see whether the protein of the seeds would yield theine by decomposition with acids it was prepared after Ritthausen's method and boiled with hydrochloric acid of the specific gravity of 1.10, with addition of some stannous chlorid for 20 hours. The extract after removal of the dissolved tin showed the following proportion of basic nitrogen.

Total dissolved nitrogen.	0.2250	100.0
Nitrogen in phospho-tungstic precipitate.	0.0900	40.0
Nitrogen in ammonia.	0.0300	13.4
Nitrogen in organic bases.	0.0600	26.6

A portion of the extract was carefully tested for theine by the usual method after treatment with basic acetate of lead and shaking with chloroform, but no trace of it was found.

II. Is theine found in the germinating seedlings of the tea plant?

(1) Etiolated seedlings.

The seeds were first soaked in water for several days and kept in pure sea sand in perfect darkness at the temperature of 15-30°C. for several months. When the seedlings had reached 10-15 cm. and had two or three leaves, a portion (*a*) was analysed after washing; the rest (*b*) was analysed after a further period of 17 days.

Seedlings (*a*) freed from cotyledons had small white leaves of 3-6 cm diameter, stems 6-10 cm, root 6-12 cm, dry weight=130 grams. Seedlings (*b*) 2-3 leaves opened, stems 13-16 cm, roots 6-12 cm, number of seedlings 228. dry weight=21.85 grams. (Dry weight of 100 seedlings=9.58 grams).

In 100 parts of dry matter.	(*a*)	(*b*)
Total nitrogen.	3.95	3.49
Albuminoid nitrogen.	1.54	1.33
Theine nitrogen.	0.127	0.127
Nitrogen in other forms.	2.28	2.03
(Theine)	0.48	0.48
Of 100 parts of total nitrogen.		
Total nitrogen.	100.0	100.0
Albuminoid nitrogen.	38.9	38.1

Theine nitrogen.	3.2	3.6
Nitrogen in other forms.	57.9	58.3

Every 100 seedlings contain 0.046. g theine.

(2) Seedlings grown in day light.

Average length of stem 10 cm, average length of roots 10 cm, 3-4 leaves opened, the largest leaves 3 cm in length and 2.5 cm in width. Number of seedlings 235. Dry weight of seedlings (free from cotyledons)=20.44 g. (Dry weight of 100 seedlings=9.1 grams). Dry weight of cotyledons=71.2 (Dry weight of cotyledons of 100 seedlings=31.6 grams)

	(*a*) Seedlings free from cotyledons.	(*b*) Cotyledons.
In 100 parts of dry matter.		
Total nitrogen.	3.38	1.47
Albuminoid nitrogen.	1.78	0.79
Theine nitrogen.	0.174	0.013
Nitrogen in other forms.	1.43	0.67
(Theine).	0.66	0.05
In 100 parts of total nitrogen.		
Total nitrogen.	100.0	100.0
Albuminoid nitrogen.	52.7	53.7
Theine nitrogen.	5.2	0.9
Nitrogen in other forms.	42.1	45.4
Every 100 seedlings contain.		
Total nitrogen.	0.307	0.465
Albuminoid nitrogen.	0.161	0.250
Theine nitrogen.	0.158	0.0041
Nitrogen in other forms.	0.130	0.211 (*)

In another experiment the effect of sodium nitrate was tested.

(3) Seedlings were kept in darkness until they reached 10-15 cm and afterwards exposed to day light for 16 days keeping them in half saturated gypsum solution.

Control seedlings: (*a*) Number of seedlings 200. Dry weight of leaves 4.11 g. Dry weight of leaves of 100 seedlings= 2.06 g. Dry weight of stems and roots 23.5 g. Dry weight of stems and roots of 100 seedlings=11.8 g. Nitrate seedlings (*b*) were watered with a 0.2% sodium nitrate and half saturated with gypsum. Number of seedlings 340. Dry weight of leaves 6.50 g. (Dry weight of leaves of 100 shoots=1.91 g). Dry weight of

(*) This agrees essentially with the observations of Clautriau: Nature et Signification des Alcaloides Végétaux Brussels 1900. p. 79.

stems and roots = 36.5 g (Dry weight of stems and roots of 100 seedlings = 10.7 g).

In 100 parts of dry matter.

	(*a*) Watered with half sat. Ca SO_4			(*b*) Watered with 0.2% Na NO_3 half saturated with Ca SO_4			(*c*) Control entire seedlings dried on May 16.
	Entire seedlings (free from cotyledons).	Leaves.	Stems and roots.	Entire seedlings (free from cotyledons).	Leaves.	Stems and roots.	
Total nitrogen	3.23	6.04	2.76	3.32	5.90	2.86	3.49
Albuminoid nitrogen	1.58	4.06	1.15	1.61	3.83	1.22	1.33
Theine nitrogen.....	0.193	0.824	0.082	0.17	0.752	0.066	0.127
Nitrogen in other forms	1.48	1.16	1.53	1.54	1.32	1.57	2.03
(Theine)	0.73	3.12	0.31	0.646	2.85	0.25	0.48

In 100 parts of total nitrogen.

Total nitrogen	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Albuminoid nitrogen	48.6	67.2	41.6	48.5	64.9	42.6	38.1
Theine nitrogen.....	5.93	13.6	3.0	5.1	12.7	2.3	3.6
Nitrogen in other forms	45.5	19.2	55.4	46.4	22.4	55.1	58.3

Every 100 seedlings contain

Total nitrogen	0.4484	0.1241	0.3243	0.4198	0.1128	0.307	0.3344
Albuminoid nitrogen	0.2192	0.0840	0.1352	0.2043	0.0733	0.131	0.1274
Theine nitrogen.....	0.0266	0.0169	0.0097	0.0215	0.0144	0.0071	0.0121
Nitrogen in other forms	0.2032	0.0237	0.1795	0.194	0.0250	0.169	0.1950

(3) Experiments with full grown tea plants.

(*a*) On April 17. tea plants of the college farm about ten years old were deprived of all the old leaves, leaving only small buds. The leaves developed from the buds in full day light, were analyzed on May 16.

In 100 parts of dry matter.

	Stem bark.		Buds.	Leaves.	
	April 17.	May 16 old leaves taken off on April.	April 17.	May 16 old leaves deprived on April 20.	May 16 normal growth.
Total nitrogen	1.09	1.23	5.3	5.94	5.77
Albuminoid nitrogen.....	1.06	1.15	4.02	4.60	4.44
Theine nitrogen.....	0.024	0.045	0.763	0.707	0.723
Nitrogen in other forms ...	0.01	0.03	0.53	0.54	0.61
(Theine)	0.09	0.17	2.89	3.02	2.74

In 100 parts of total nitrogen.

Total nitrogen	100.0	100.0	100.0	100.0	100.0
Albuminoid nitrogen	97.2	93.5	75.7	77.4	77.0
Theine nitrogen.....	2.2	3.7	14.4	13.4	12.5
Nitrogen in other forms ...	0.6	2.8	9.9	9.2	10.5

(*b*) On May 1. tea plants about fifteen years old were deprived of the old leaves. Some of these plants were covered with a box to shut out day light while others remained uncovered

In 100 parts of dry matter.

	Buds May 1.	Leaves May 16 day light.	Leaves May 16 dark.
Total nitrogen.	5.47	5.54	5.31
Albuminoid nitrogen.	4.42	4.18	4.01
Theine nitrogen.	0.562	0.634	1.032
Nitrogen in other forms.	0.49	0.73	0.27
(Theine).	2.13	2.40	3.91

In 100 parts of total nitrogen.

Total nitrogen.	100.0	100.0	100.0
Albuminoid nitrogen.	80.8	75.4	75.5
Theine nitrogen.	10.3	11.4	19.4
Nitrogen in other forms.	8.9	13.2	5.1

Leaves developed in the dark contain a greater percentage of theine than those grown in day light, but here it must be remarked that the former are very poor in other organic matters. Theine does not increase directly by the application of nitrates as above mentioned, hence it is not a synthetical product analogous to asparagin; The following table shows the various amounts of theine-nitrogen in % of total nitrogen in different parts of the tea plants:

Seed.	Bark of stems of full grown plants.	Cotyledons of young seedlings.	Stems and roots of young seedlings.	Etiolated seedlings.	Leaves of young seedlings.	Leaves of full grown plants.
none	doubtful trace.	0.9	2.3-3.0	3.2-3.6	12.7-13.6	12.5-13.4

Further theine does not stand in any relation to protein production. An observation of Miyachi [1] also supports this view. He found that theine is a poor source of nitrogen for fungi and that in old leaves theine increases by the process of starvation, that is by keeping them in a dark place, thus:—

Theine-nitrogen in % of total nitrogen:

Original leaves.	Starved leaves.
11.15	13.74

Here the processes of metabolism show more connection with theine production than those of assimilation and synthesis do. It remained however to be decided whether in the presence of a large amount of carbohydrates and in the absence of any other suitable source of nitrogen, theine may be used as a source of nitrogen for building up proteids in the tea leaves. Here the following points have to be mentioned.

According to Kellner [2] the quantity of theine in the leaves gradually decreases as they become older.

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(1) Bull. College of Agr. Komaba. Tokyo. vol II. No. 7. p. 460.

(2) Versuchs-Stationen 39. Bull. Coll. Agr. Tokyo. vol I.

Leaves gathered on	Theine nitrogen in % of total nitrogen.
May 15	16.5
July 15	22.1
November 30	10.2
Old leaves of May 15	8.6

Hence a gradual decrease of theine takes place from July to November not only relatively but absolutely. Since in autumn various products migrate toward the seeds or stems and roots, the decrease of total and theine nitrogen in the leaves in autumn might of course be expected ; and further, as we have found no theine in the seeds and only doubtful traces of it in the stems and roots of the tea plant, and as also there is only very little non-albuminoid nitrogen in the seeds as well as in the stems and roots, we may safely conclude that the theine once formed in the leaves may later again be transformed gradually into proteids. However it is not probable that it is directly used for this purpose, as we have already pointed out, but it seems that the process is an indirect one. Perhaps theine is firse destroyed and its nitrogen is liberated as ammonia, befort protein formation from that source can take place.

My investigation permits the following conclusion :—

1) Seeds of the tea plant contain originally no theine. Neither do the proteids of the seeds yield theine by the action of hydrochloric acid. Hence the formation of theine during the germination process can not be due to a mere splitting off from the proteids, but must be due to a far reaching transformation of the products of metabolism.

2) Light seems to have no direct influence upon the formation of theine. Since etiolated shoots as well as the shoots grown in day light, contain it in nearly the same quantities.

3) Cotyledons of germinating seedlings contain also some theine (though very little).

4) Stems and roots also contain a moderate amount of theine though its percentage is considerably lower than in the leaves.

5) Leaves contain the largest amount of theine, its quantity being nearly proportional to the development of the leaves.

6) No essential increase of theine was noticed by the application of sodium nitrate, making it also very probable that

theine is not like asparagine a product of synthesis, but one of katabolism.

7) The bark of the trunk of the tea plant contains only doubtful traces of theine. But the dormant buds are moderately rich in it.

On the Localization of theine in the tea leaves.

By

U. Suzuki.

Thus far nobody has succeeded in localizing the theine in the tea leaves, while in regard to many other alcaloids, the investigations of Errera,[1] Maistriau and Clautriau, have shown that they are localized 1st. in the very active tissues: the vegetative tissues and the embryo. 2nd. Along the fibrovascular bundles (sourtout près de la région libérienne et dans cette région mème ;) 3rd. in the epidermis: cortical layers and the shell of fruits etc. and generally all the outer tissues for protection. 4th. in the special secretary organs. In regard to theine, Clautriau has not arrived at any satisfactory conclusion.

This author says[2] " J'ai en recours d'abord à la microchimie, et j'ai tenté de déterminer la localisation de la caféine dans le caféier ou le Thé. Malgré de nombreux essais sur un matériel très abondant, je ne suis pas arrivé á mon but." and he says again " Il n'en résulte pas que je considère la localisation microchimique de la caféine comme impossible, mais elle présente de grandes difficultés et demande encore de longues études préliminairès." I have undertaken the same question as Clautriau and have solved it satisfactority in the following manner: At first I applied phosphotungstic acid upon sections of the leaves, but this produced more or less precipitate in all the cells, but decidely more in the epidermal cells. Since proteids are thus precipitated just as well as alkaloids, this reaction gave no decisive answer, I afterwards applied a concentrated solution of iodine in potassium iodide, but also in this case no decision could be reached. This reagent however acts decidely better

(1) Errera: Akademie Royale de Belgique. Bull. No. 3. 1887.

(2) Nature et signification des Alkaloids Végétaux Brussells 1900. p. 55 & 56.

on theine after the addition of a little hydrochloric or sulphuric acid and on application of this acidulated solution the epidermal cells gave decidedly a much more intensely brown color than the others which remained merely slightly yellowish. The most decisive answer however was reached by the following test. When a section of the leaf was placed in a 0.5% theine solution, the cells of the spongy and palisade tissues exhibited a very marked formation of proteosomes.[1] This fact appeared rather strange to me, since the coffeine content of the fresh leaves is certainly more than 0.5% and thus the formation of proteosomes ought to take place in the fresh leaves without any application of coffeine.[2] Since however no proteosomes were observed in the epidermal cells (which are in other plants often very rich in the stored up active albumen) by the action of the 0.5% coffeine solution, there remain no other conclusion than this, that these cells contain no active albumin and at the same time contain all the theine of the leaves. A further test with tannin proved this conclusion to be correct. A section of the leaf was left for two days in a tannin solution of about 3-4%; hereby a voluminous precipitate consisting of minute globules was produced in the epidermal cells, while the other tissues of the leaves showed only a slight turbidity. To prove that this precipitate consisted of tannate of theine was furnished by the application of highly diluted ammonia, which dissolved it at once.[3] This affords an easy way of distinguishing the precipitate from minute proteosomes, which latter solidify on the absorption of ammonia, and do not dissolve at all in it.

Hence there can be no longer any doubt that *the theine* in *the tea leaves* is *localized in the epidermis*.

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(1) Compare: IX and X of Chemische Energie der Lebenden Zellen by O. Loew Munich. 1899.

(2) This led me at first to the supposition that the theine in the fresh leaves, was present not in the free state but in a loose combination with some other substance. Hence I extracted the green leaves, after crushing them, directly with chloroform, but I obtained about the same quantity of theine for the dry matter as with the tea of commerce. The theine is therefore contained in the leaves in the free state.

(3) It is well known that some authors made the great mistake of confounding tannate of theine with true proteosomes.

明治三十四年六月十七日印刷
明治三十四年六月二十日發行

農科大學

編輯兼發行者　佐久間衡治
東京市牛込區市ヶ谷加賀町一丁目十二番地

印刷者　株式會社秀英舍第一工場
東京市牛込區市ヶ谷加賀町一丁目十二番地

印刷所

Über die Bekämpfung der Mäuseplage durch den Mereshkowsky'schen Mäusetyphusbacillus.

VON

Y. Kozai.

Eine der grössten Plagen der Landwirthschaft ist bekanntlich die Feldmaus. Dieselbe verrursacht wegen ihrer enormen Fortpflanzungsfähigkeit[1] und ihrer staunenswerthen Gefrässigkeit oft einen so grossen Schaden auf den Kulturfeldern, dass das Bebauen der letzteren überhaupt nicht möglich ist. Die Feldmäuse, die in diesem Frühling in der Provinz Ibaraki, nordöstlich von Tokio, in ungezählter Menge auftraten, zeigen in mancher Hinsicht Unterschiede von den in Europa verbreiteten (*Arvicola arvalis*) und gehören nach Herrn Prof. *Sasaki*[2] zu einer neuen Rasse, der dieselbe mit dem Namen „*Arvicola hatanesumi*"[3] belegt. Dieselbe ist nach dem genannten Autor[4] 10–13 cm. in Körperlänge und besitzt einen behaarten Schwanz von 3.2–4.2 cm. Der Leib ist untersetzt, der Hals dick, der Kopf etwas rund, die häutigen Ohren sind kurz, die Augen klein und dunkelbraun, die Füsse und Zehen fein, die Krallen kurz, der Schwanz ist ziemlich abgerundet. Die dicht anliegende und sanft anzufühlende Behaarung ist auf dem Oberkörper grau bis rothbraun, auf der Unterseite dagegen weisslich. Der Bau, in welchem unsere Feldmaus ihr Leben führt, ist sehr

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[1] Nach *Ritzema-Bos* (Thierische Schädlinge u. Nützlinge, Berlin 1891.) kann ein Mäusepaar unter günstigsten Bedingungen in einem Jahre über 200, nach *Brehm* (Thierleben, Bd. II.) und *Danysz* (Maladies contagieuses des animaux nuisibles, Paris 1895.) über 350, und nach *Rüdiger* (Wien. Landwirthschaftliche Zeitung, 1892, S. 737.) sogar über 20000 Nachkommen haben. Dies bezieht sich natürlich auf die europäische Feldmaus (*Arvicola arvalis*).

[3] Hatanesumi bedeutet Feldmaus.

[2] u. [4] Bericht der japanischen landwirthschaftlichen Gesellschaft 1901.

geschickt construirt. Das mit fein zerbissenen Pflanzentheilen sorgfältig präparirte Nest liegt gewöhnlich in den tieferen und sicheren Stellen des Feldes, besonders aber an Fusswegen und unter Gebüschen ; von dort ab laufen Abzweigungen in allen Richtungen, so dass das Thierchen nötigensfalls sich gut verbergen kann. Diese Abzweigungen oder Seitengänge liegen ca. 20 cm. unter der Erde und stehen durch zahlreiche, schräg gegrabene Öffnungen mit der Aussenwelt in Verbindung. Gewöhnlich befindet sich in der Nähe des Nestes eine Kammer, in welcher Nahrungsvorräthe wie Getreideähren, Erbsen, kurz zerbissene Wurzelfrüchte u. s. w. massenhaft aufgespeichert werden. In der oben erwähnten Provinz wurden nicht weniger als 5000 Hectar Felder von diesem Tierchen mehr oder weniger arg heimgesucht und alle Massregeln um dasselbe auszurotten, so z.B. Vergiftung u. Fangen, erwiesen sich als nicht genügend wirksam.

Bei dieser Sachlage wird man natürlich auf den Gedanken kommen, ob nicht der seit *Löffler* mit Erfolg zur Mäusevertilgung benützte *Mäusetyphusbacillus* auch in unserem Fall mit Vortheil verwandt werden könnte. In der That sind einige Laboratoriums- sowohl wie auch Feldversuche, angestellt von Herrn Dr. *Onuki* und Herrn *Sanui*[1] mit dem *Mäusetyphusbacillus*, als sehr gelungen zu bezeichnen, denn die im Käfig gehaltenen Feldmäuse verendeten durch *Infection per os* in 5–14 Tagen und auch auf den inficirten Versuchsfeldern fand eine starke Abnahme der Mäuse nach einiger Zeit statt.

Inzwischen erhielten wir von der Bezirksbehörde Ibaraki den Auftrag, die Wirksamkeit des Mäusetyphusbacillus gegen unsere Feldmaus genau zu prüfen und die praktische Verwendung desselben zur Mäusevertilgung zu beaufsichtigen. Leider war der in unserem Besitz gewesene *Löffler*'sche Bacillus abgestorben und eine frische Kultur konnten wir nicht auftreiben. So waren wir gezwungen, die von den oben erwähnten Herren benützte Kultur von unbekannter Herkunft in Anwendung zu bringen. Dieselbe zeigte aber bei näherer Prüfung in ihren kulturellen und physiologischen Merkmalen eine grössere Ähnlichkeit mit dem *Mereshkowsky*'schen Bacillus als mit dem *Löffler*'schen. Später theilte mir Herr Dr. *Haschimoto* in Sapporo mit,

[1] Bericht der japanischen landwirthschaftlichen Gesellschaft 1900.

dass die betreffende Kultur in der That von *Mereshkowsky* selbst seinem Institut überwiesen worden war. Da dieser Bacillus im Gegensatz zu dem *Löffler*'schen hauptsächlich *nur* von seinem Entdecker genau erforscht worden ist, so entschlossen wir uns, um seine Verwendbarkeit zu constatiren, denselben einer eingehenden Untersuchung zu unterwerfen. Zu diesem Zwecke haben wir nicht nur eine ganze Reihe von Infectionsversuchen sowohl im Laboratorium als auch auf freiem Feld ausgeführt, sondern auch den nämlichen Bacillus in Bezug auf seine kulturellen und physiologischen Eigenschaften studirt. Die dabei gewonnenen Ergebnisse theilen wir im Folgenden mit. Dabei möchte ich nicht unterlassen, den Herren Dr. *S. Kusuhara*, *K. Nakamura* und *G. Sasada* in Ibaraki für die freundliche Unterstützung bei der Ausführung der Feldversuche meinen aufrichtigen Dank auszusprechen. Ebenso bin ich Herrn *J. Susuki*, dem Assistenten am Institut, für seine eifrige Hilfe zum grossen Dank verpflichtet.

I. Kulturelle und Physiologische Eigenschaften des Bacillus.

Dieser Organismus stellt im hängenden Tropfen ein ziemlich *plumpes Stäbchen mit lebhafter Eigenbewegung* dar und lässt sich in dieser Beziehung von dem *Löffler*'schen Bacillus kaum unterscheiden. Doch ist der erstere in der Regel etwas *länger* in Form als der letztere und wächst *häufiger* zu *Scheinfäden* aus. Sein Verhalten den üblichen basischen Anilinfarbstoffen gegenüber bietet nichts Besonderes. Dagegen fällt die Färbung nach der *Gram*'schen Methode ***negativ*** aus.

In *gewöhnlicher Bouillon* ist das Wachsthum bei Brütwärme ein sehr kräftiges. Innerhalb der ersten 24 Stunden tritt eine starke Trübung ein und auf der Oberfläche der Flüssigkeit entwickelt sich eine zarte weisse Deckhaut, die beim Schütteln in Fetzen zerfällt und leicht zu Boden sinkt. Nach 5–6 Wochen wird die Kulturflüssigkeit ganz klar. Die chemische Reaction wird durch das Bacterienwachsthum *alkalisch*. In älteren Bouillonkulturen zeigt sich eine *schwache Indolreaction*. Reduction von Nitrat zu Nitrit tritt aber nicht ein. Ein eigenthümlicher Geruch, etwa an frisch gelassenen Pferdharn

erinnernd, lässt sich bei der Öffnung des Kulturkolbens deutlich wahrnehmen. In *Traubenzuckerbouillon* ist die Entwickelung anfangs ebenso üppig wie in der gewöhnlichen, bald kommt sie aber zum Stillstand, ohne Zweifel weil die von dem Bacillus erzeugten Säuren das weitere Wachsthum verhindern. Die zuckerhaltige Bouillon schäumt beim Schütteln, da unter andern etwas Kohlensäure gebildet wurde.

In *Traubenzuckerbouillon im Gärungskölbchen* findet bei Brütwärme innerhalb 24 Stunden eine starke Trübung statt. Etwa die Hälfte des geschlossenen Schenkels ist mit Gas gefüllt, das sich bei näherer Prüfung grossentheils als CO_2 zu erkennen giebt[1]; zugleich wird die Kulturflüssigkeit stark sauer. Bei Zimmerwärme geht die Entwickelung langsamer vor sich.

Bei den *Plattenkulturen* in gewöhnlicher Gelatine erscheinen am zweiten Tage feine Pünktchen, die nur sehr langsam an Grösse zunnehmen. Bei schwacher Vergrösserung stellen sich die Oberflächencolonieen als rundliche, ziemlich glattrandige, hellgraue feinkörnige Scheiben dar. Die kleineren Tiefencolonieen erscheinen bei schwacher Vergrösserung als unregelmässig runde, graue Scheiben. Der Nährboden wird nicht verflüssigt.

Bei *Gelatinestichkulturen* entsteht nach 24 Stunden dem Stichkanal entlang ein weisser dünner Streifen, der ziemlich gleichmässig bis zum Boden fortschreitet. An der Einstichstelle bildet sich eine weisse, dünne Auflagerung, die später concentrische Ringe erkennen lässt.

Auf der *gewöhnlichen Gelatine* erscheint am zweiten Tage längs des Impfstriches ein weisser, dünner Streifen, der sich langsam weiter entwickelt. Der Rand des Rasens buchtet sich mit der Zeit aus.

Bei *Agarplattenkulturen* entwickeln sich bei Brütwärme innerhalb 24 Stunden ziemlich grosse Colonieen, die auf der Oberfläche der Verdünnungsplatten einen Durchmesser von 2–3 mm. annehmen. Makroscopisch stellen sich die Oberflächencolonieen als rundliche, hellgraue, das Licht stark brechende, etwas erhöhte Scheiben dar. Allmälich gewinnen dieselben ein charakteristisches Aussehen, indem um den dichten Kern sich

[1] Nach *Mereshkowsky* soll sein Bacillus kein Gas entwickeln. (Centrabl. für Bakteriologie, Abth. I. Bd. XVII. 1895. S. 742.)

eine Anzahl concentrisch geschichteter Zonen lagert. Der Saum ist ziemlich regelmässig gelappt. Bei schwacher Vergrösserung stellen sich die Oberflächencolonieen als runde, fein gelappte grobkörnige Scheiben dar. In den centralen Bezirken zeigen sich oft faltige oder runzelige Figuren. Die Tiefencolonieen bieten mit unbewaffnetem Auge betrachtet, hellgraue Pünktchen dar: bei schwacher Vergrösserung erscheinen sie als unregelmässig runde, grob körnige Scheiben.

Bei den *Traubenzuckeragarstichkulturen* zeigt sich auf der Einstichstelle schon nach 24 Stunden bei Brütwärme eine weisse, dünne gelappte Auflagerung, die sich allmälich über die Agaroberfläche hin ausbreitet. Längs des Stichkanals bildet sich ein feiner, weisser, bis zum Boden reichender Streifen. Stets findet ausgiebige Gasentwickelung statt.

Auf *Kartoffelstückchen* ist das Wachsthum ein geringfügiges. Nach 24 Stunden ist längs des Impfstriches ein kaum wahrnehmbarer Belag, der jedoch nach langer Zeit keine wesentlichen weiteren Fortschritte zeigt.

In *Milch* wächst der Bacillus ziemlich gut. Keine Koagulation tritt ein, selbst nach mehreren Wochen bei Brütwärme nicht. Die Milch wird aber durch das Bakterienwachsthum deutlich sauer. Es findet auch eine schwache *Gasentwickelung* statt.

Bei *Abschluss des Sauerstoffs*, d. h. in einer Wasserstoff-oder Stickstoffatmosphäre sowohl auf den Agarplatten, als auch in Bouillon entwickelt sich der Bacillus viel *langsamer* und *geringfügiger* als bei Zutritt des Sauerstoffes.[1] Die entstandenen Colonieen resp. Bodensätze erreichen nie die Dimensionen der aëroben Vergleichsculturen.

Gegen *höhere Temperatur* besitzt der Bacillus keine besondere Widerstandsfähigkeit. Dieselbe wurde in der Weise festgestellt, dass aus einer 1–3 tägigen Bouillonkultur jedesmal 4 Oesen in frische, vorher im Wasserbade auf die gewünschte Temperatur erhitzte Bouillon übertragen wurden. Nach Ablauf von 5 Minuten wurden die Röhrchen unter dem Strahle der Wasserleitung rasch abgekühlt und dann in den Brütschrank gebracht. Es ergab sich dabei, dass eine Temperatur von 70°

[1] Nach Angaben von *Mereshkowsky* (A. a. O.) soll der Bacillus bei Abschluss des Sauerstoffes sich gar nicht entwickeln.

nach 5 Minuten den Organismus mit Sicherheit abtötete, während bei 65° C. der Erfolg kein gleichmässiger war.

Gegen *Licht* ist der Bacillus ziemlich widerstandsfähig. Ganz dünne frisch ausgegossene Agarplatten, die zur Hälfte mit undurchsichtigen schwarzen Karten bedeckt waren, wurden Ende Juni nachmittags dem Sonnenlicht ausgesetzt. Erst eine 8 Stunden dauernde Aussetzung tötete alle Organismen ab, während nach 7 Stunden noch viele Colonieen nachträglich zur Entwickelung gelangten. Die in dünner Schichte auf sterilen Deckgläschen übertragenen Bacillen werden dagegen nach einer 30 Minuten dauernden Insolation in der Regel vernichtet. Die Austrocknung wird in diesem Falle dadurch ausgeschlossen, dass die Deckgläschen während des Versuchs in einer mit feuchtem Filtrirpapier versehenen *Petri*'schen Schale gehalten werden.

Gegen *Austrocknung* erweist sich der Bacillus ebenfalls ziemlich resistent. Bei einer Reihe von Versuchen, in denen mehrere, mit einer leicht trüben wässerigen Bakterienaufschwemmung beschickte Deckgläschen im Exiccator bei Zimmerwärme aufbewahrt und nach verschiedenen Zeiträumen auf ihre Sterilität geprüft wurden, stellte sich heraus, dass der Bacillus erst nach einem 30 Stunden langen Liegenlassen mit Sicherheit abgetötet war. Bei ähnlichen Versuchen mit einer hoch verdünnten Aufschwemmung, in der sich keine sichtbare Trübung zeigte, erwiesen sich die inficirten Deckgläschen nach einem 26-stündigen Aufenthalt im Exiccator vollständig steril.

In *feuchtem Zustande* vermag der Bacillus ferner lange Zeit seine Virulenz zu erhalten. Der aus trocknem Buchweizenmehl und einer 48-stündigen Bouillonkultur angemachte Teig—die Form, in welcher der Bacillus zur praktischen Inficirung verwendet wird—zeigte nach einem 30-tägigen Aufenthalt in einem kalten feuchten Keller sich, wie Fütterungsversuche mit Feldmäusen ergaben, noch völlig infectionsfähig.

Endlich bildet der Bacillus in einer traubenzuckerhaltigen Nährflüssigkeit ausser etwas *Aethylalkohol* nicht unbedeutende Mengen von *Essig-* und *Bernsteinsäure*, ferner Spuren von *Ameisen-* und *Milchsäure* auch *Ammoniak* besonders in älterer Kulturflüssigkeit. Hierüber hoffen wir in Bälde mehr mittheilen zu können.

II. Infectionsversuche im Laboratorium.

Die sämmtlichen zu diesen Versuchen benützten Feldmäuse stammten von der oben erwähnten Provinz. Sie wurden jedesmal nach dem Einfangen eine Zeit lang in grossen aus Zinkblech und Drahtnetz bestehenden, mit trockener Erde und sauberem Stroh versehenen Kästen unter scharfer Beobachtung aufbewahrt. Nach Ablauf einer 14–21 Tage dauernden Quarantaine[1] wurden die Thierchen aus dem Behälter herausgenommen, in ähnliche aber kleinere Kästen gebracht und der Infection unterzogen.

Als Infectionsmaterial diente, wo nicht speciell anderes bemerkt wird, eine *24.-stündige Bouillonkultur*.[2] Mit 5–6 c.c. dieser Kulturflüssigkeit wurde ein Teig aus trockenem Buchweizenmehl angemacht und für 24 Stunden jenen Mäusen, jedesmal 4 Stück betragend, bei denen eine *Infection per os* eintreten sollte, als alleinige Nahrung gereicht. Nach dieser Frist, binnen welcher die inficirte Speise in den meisten Fällen vollständig verzehrt worden war, erhielten die Thierchen gewöhnliche, d. h. aus Reis oder Weizen und etwas Rettig bestehende Nahrung. Subcutan behandelte Thierchen bekamen 0.1–0.5 c.c. derselben Kultur und wurden in einem besonderen Kasten mit normalem, nicht inficirtem Futter ernährt. Auf diese Weise haben wir eine grosse Anzahl von Feldmäusen inficirt, wobei gleich bemerkt sei, dass alle der Infection erlagen.

Von dem allgemeinen Verhalten der erkrankten Thierchen ist zunächst zu bemerken, dass früher oder später sich eine immer zunehmende Apathie bemerklich macht. Die schwer Kranken kauern nämlich mit halbgeöffneten Augen und gesträubtem Haare zusammen und geben auf mechanische Stösse nur eine schwache Reaction. Ferner lässt sich, worauf schon *Mereshkowsky*[3] aufmerksam gemacht hat, an den hinteren Extremitäten eine immer zunehmende Parese wahrnehmen, so

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[1] Während dieser Zeit bekamen die Thierchen in der Regel abgerundete Figuren und glänzendes Fell.

[2] Die ursprüngliche Kultur war vor dem Versuche durch Verimpfen von Maus zu Maus in ihrer Virulenz gesteigert worden.

[3] A. a. O.

dass die inficirten Thierchen vor Todeseintritt nur noch auf den Vorderfüssen sich langsam fortschleppen können.

Bei der Obduction der verendeten Thierchen und zwar der *per os* inficirten lassen sich in den inneren Organen dieselben Veränderungen wahrnehmen, wie sie schon von *Mereshkowsky* beschrieben worden sind.[1] Am auffallendsten ist die Vergrösserung der Leber und Milz. Die letztere zeichnet sich ferner besonders durch dunkelrothe Färbung und derbe Beschaffenheit aus. Die Leber ist zumeist parenchymatös getrübt und weist mehrere kleine gelbe Flecken auf. Auch der Verdauungskanal lässt mehr oder weniger wesentliche Veränderungen wahrnehmen und ist nicht selten stark mit Gas gefüllt. Der Inhalt der Harnblase ist etwas dunkel und zeigt zuweilen eine mässige Trübung.

Was nun die bakteriologischen Untersuchungen der verendeten Thiere anbetrifft, so haben wir in der Regel einige Blutstropfen aus dem Herzen ferner Milz- und Leberstückchen unter den nötigen Cautelen in Bouillonröhrchen übertragen und diese im Brütschrank gehalten. Ausserdem haben wir in 6 Fällen auch den Inhalt sowohl der Harnblase wie auch des Darmkanals bakteriologisch untersucht. Die hierbei erzielten Ergebnisse lassen sich in folgenden Sätzen zusammenfassen. Die mit Leber Milz und Harn beschickte Bouillon zeigte *jedesmal* eine mässige *Trübung* innerhalb 24 Stunden und die Kulturen im hängenden Tropfen sowohl wie auch Plattenkulturen wiesen stets Reinkulturen des nämlichen Bacillus auf. Auch der Darminhalt enthielt in sämmtlichen von uns untersuchten Fällen den nämlichen Bacillus. Der Beweis dafür konnte aber wegen der Anwesenheit anderer Bakterienarten nur durch die subcutane Injection der Bouillonkultur mit Sicherheit erbracht werden.

Was nun die *letale* Wirkung des Bacillus betrifft, so trat dieselbe in unserem Falle, wie aus nachfolgender Aufstellung ersichtlich, in folgenden Zeitfristen ein :

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[1] A. a. O.

5	Tage	nach	der	Inficirung	starben	4	Feldmäuse
6	,,	,,	,,	,,	,,	4	,,
7	,,	,,	,,	,,	,,	6	,,
8	,,	,,	,,	,,	,,	6	,,
9	,,	,,	,,	,,	,,	7	,,
10	,,	,,	,,	,,	,,	5	,,
11	,,	,,	,,	,,	,,	7	,,
12	,,	,,	,,	,,	,,	8	,,
13	,,	,,	,,	,,	,,	10	,,
14	,,	,,	,,	,,	,,	11	,,
15	,,	,,	,,	,,	,,	14	,,
16	,,	,,	,,	,,	,,	8	,,
18	,,	,,	,,	,,	,,	4	,,
20	,,	,,	,,	,,	,,	3	,,
21	,,	,,	,,	,,	,,	2	,,
22	,,	,,	,,	,,	,,	1	,,
23	,,	,,	,,	,,	,,	2	,,
25	,,	,,	,,	,,	,,	1	,,
27	,,	,,	,,	,,	,,	2	,,
30	,,	,,	,,	,,	,,	1	,,
35	,,	,,	,,	,,	,,	1	,,
38	,,	,,	,,	,,	,,	1	,,

Im Ganzen 108 Feldmäuse

Die Sterblichkeit tritt also schon am 5. Tage der Inficirung auf, steigt dann rasch und erreicht das Maximum am 15. Tage, um wieder allmälich herunterzugehen. Auffallend ist die Thatsache, dass in 2 Fällen die Krankheit erst nach 35 resp. 38 Tagen ablief. Jedenfalls ist es sicher, dass der *Mereshkowsky*'sche Bacillus auf unsere Feldmaus *hoch virulent* wirkt. Dieselbe scheint aber *nicht* so empfänglich zu sein, wie die in Europa verbreitete Art (*Arvicola arvalis*), denn die letztere soll nach *Mereshkowsky*[1] binnen 1–9 Tage nach der Infection sterben.

Die oben erwähnte Krankheitsdauer bezieht sich nur auf diejenigen Mäuse, die eine genügende Menge der inficirten Speise verzehrt haben. Bei den Thierchen dagegen, die nur eine ganz geringe Menge des Infectionsstoffs aufgenommen haben, verläuft die Krankheit in der Regel noch viel langsamer.

[1] A. a. O.

Von den 2 Feldmäusen, denen versuchsweise von der inficirten Speise ganz wenig gegeben war, starb die eine nach 40, die andere erst nach 75 Tagen. Um dieses experimentell zu prüfen, haben wir eine Anzahl von Mäusen mit *verschiedenen Mengen* einer 24-stündigen Bouillonkultur sowohl durch *Darminfection* wie auch durch *subcutane Einspritzung* inficirt und unter scharfer Beobachtung im Aufbewahrungskasten gehalten. Die dabei gewonnenen Ergebnisse lassen sich aus folgender Tabelle ersehen.

Darminfection.			Durchschnitt.	Subcutane Injection.			Durchschnitt.
Bouillonkultur angewandt c.c.	Zahl der Mäuse.	Verendet nach.[1]		Bouillonkultur angewandt c.c.	Zahl der Mäuse.	Verendet innerhalb.[1]	
1.0	9	7.,8.,12.,12.,13.,13., 14., 16., 30. Tagen	14	$\frac{1}{2}$	4	24. Stunden	
$\frac{1}{5}$	6	8., 14., 14., 15., 20., 35. Tagen	17,7	$\frac{1}{3}$	5	24. „	
$\frac{1}{10}$	5	4., 15., 15., 20., 30. Tagen	16,8	$\frac{1}{6}$	5	48. „	
$\frac{1}{20}$	5	5., 8., 20., 25., 30. Tagen	17,8	$\frac{1}{20}$	6	4., 6., 8., 8., 9., 10. Tagen	7,5
$\frac{1}{40}$	5	10., 20., 20., 32., 40. Tagen	24,4	$\frac{1}{40}$	4	8., 12., 12., 14. Tagen	11,5
$\frac{1}{80}$	5	10., 20., 40., 40., 52. Tagen	32,4	$\frac{1}{80}$	4	8., 12., 12., 14. Tagen	12

Es unterliegt also keinem Zweifel mehr, dass die Krankheitsdauer bis zum letalen Ausgange gewissermassen von der Menge der in den Körper gelangten Microbien abhängig ist. *Zupik*[2] kam zu einem ähnlichen Resultat mit dem *Löffler*schen Bacillus. Der Einfluss der individuellen Disposition des

[1] In allen Fällen liess sich der Bacillus in den inneren Organen der Leichen nachweisen.

[2] Centrabl. f Bakteriologie, Abth. I. Bd. XXI. 1897. S. 446.

Thierchens ist in dieser Beziehung auch nicht zu verkennen. Ob beim Einführen von ganz geringen Mengen des Infectionsmaterials in den Organismus jegliche Wirkung ausbleibt oder demselben gar Immunität ertheilt würde, konnten wir bis jetzt nicht entscheiden.

Ganz in derselben Weise wie das Einführen von geringem Infectionsmaterial verhält sich die Infection mit der durch lang fortwährende Züchtung auf künstlichem Nährboden abgeschwächten Kultur. 4 Feldmäuse, die mit einer 4 Monate alten Agarkultur *per os* inficirt waren, blieben nach Verlauf von 40 Tagen noch am Leben. Eine dieser Mäuse wurde mittelst Chloroform getötet und die Organe, die eine charakteristische Veränderung zeigten, bakteriologisch untersucht, wobei sich der *Bacillus* aus Milz Leber und Herzblut in Reinkultur gewinnen liess. Ferner zeigte das Blutserum desselben Thierchens eine *sehr starke agglutinierende Wirkung auf den Bacillus*. Nach Ablauf weiterer 15 Tage wurde die eine der überlebenden Mäuse zwecks bakteriologischer Untersuchung ebenfalls mittelst Chloroform getötet, während eine andere (a) *per os* und die letzte (b) *durch subcutane Injection* mit einer *hoch virulenten* 24 stündigen Bouillonkultur inficirt wurde.[1] Die durch Chloroform getötete Maus liess den *Bacillus* in Milz Leber und Herzblut nachweisen. Auch das Serum gab eine *starke Widal'sche Reaction*. Die *Anwesenheit des Bacillus im Blut bei Lebzeiten der Mäuse* wurde noch bei speciellen diesbezüglichen Prüfungen constatirt. Dieselbe Beobachtung hat *Mereshkowsky*[2] mit dem *Löffler*'schen Bacillus gemacht. Von den zum zweitenmale inficirten Mäusen starb die eine (a) nach **14**, die andere (b) nach **3** *Tagen*, während von den in derselben Weise behandelten Controllthierchen die *per os* inficirte nach **5** Tagen die subcutan injicirte nach **36** Stunden zu Grunde ging. Das Einführen des abgeschwächten Bacillus in den Organismus scheint also die Wirkung des hoch virulenten Bacillus einigermassen zu *paralysieren*, ohne aber in unserem Falle dieselbe aufzuheben. Nach *Mereshkowsky*[3] soll die Infection mit einer 7 monatlichen

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[1] Zur Darminfection wurde 1 c.c. und zur subcutanen Einspritzung 0.3 c.c. der Kultur benützt.

[2] u. [3] Centralbl. für Bakteriologie, Abth. I. Bd. XVI. 1894. S. 612.

Agarkultur des *Löffler*'schen Bacillus europäische Feldmäuse (*Arvicola arvalis*) *immun* machen.

Von grossem Interesse ist noch, die Frage nach der Wirkungsweise des Bacillus zu entscheiden. Es wurden zu diesem Zwecke sowohl *aërobe*, als auch *anaërobe* Bouillonkulturen des Bacillus hergestellt und nach einen 14 tägigen Aufenthalt im Brütschranke folgenderweise behandelt.

a) Filtration durch ein *Pukal*'sches Filter,
b) Eine 5 Minuten lange Erhitzung auf 70° C.,
c) Zusatz von 0.3% Trikresol.

Nachdem die so behandelten Kulturen durch specielle Prüfungen sich als steril erwiesen hatten, wurden je 2 Feldmäuse mit je 2 c.c. der Kultur *subcutan* inficirt. Ausserdem wurden je 2 Mäusen 0.2 c.c. der *aëroben* sowohl wie auch der *anaëroben* Kultur *ohne* jegliche Behandlung unter die Haut eingespritzt. Nach *2 Tagen* starben die mit *unbehandelter Kultur* inficirten Controllthierchen und zwar ohne wesentlichen Unterschied in der Krankheitsdauer, während *alle übrigen dauernd gesund blieben.* Daraus kann geschlossen werden, dass unter den bei unseren Versuchen obwaltenden Verhältnissen der Bacillus *keinerlei Bildung von Toxin* veranlasst und dass die pathogene Wirkung des Bacillus nur der *Invasion an sich* zuzuschreiben ist.

Es sei schliesslich noch die Thatsache erwähnt, dass die Dejectionen der kranken Individuen als Verbreiter der Krankheit in *hohem Maasse* fungieren können. 3 gesunde Feldmäuse, die wir versuchsweise in einem mit Dejectionen erkrankter Thierchen besudelten Käfig aufbewahrt hatten, starben sämmtlich nach 25–35 Tagen und zwar von der *Wirkung des Bacillus.* In einem anderen Fall erlagen 2 von 6 im besudelten Käfig gehaltenen Individuen binnen 35 Tagen. *Zupik*[1] fand in ähnlichen Fällen, aber mit dem *Löffler*'schen Bacillus, nur selten eine Infection.

III. Infectionsversuche auf freiem Felde und Praktische Inficirung.

Hatten die vorerwähnten Laboratoriumsversuche die Empfänglichkeit unserer Feldmäuse gegen den *Mereshkowsky*'schen

[1] A. a. O.

Bacillus sichergestellt, so bleibt noch übrig, den *praktischen Werth* des Infectionsstoffs d. h. die Wirksamkeit desselben auf freiem Felde zu erproben. Bei den ersten diesbezüglichen Versuchen wählten wir ein Feldstück aus, auf dem Tabakstengel zu Haufen aufgeschichtet waren. Es wurden 10 solche Haufen mit dem nämlichen Bacillus inficirt, indem man je 10 aus Buchweizenmehl und einer 24-stündigen Bouillonkultur angemachten Portionen von Teig in der Grösse von etwa einem Cubikcentimeter in dem Innern eines jeden Haufens vertheilte. Am folgenden Morgen waren die meisten Teigstückchen verschwunden und die wenigen zurückgebliebenen wiesen frische Bissspuren auf. Nach 5 Tagen nahmen wir die inficirten Haufen auseinander und gruben zahlreiche darunter befindliche Mäuselöcher sorgfältig aus.[1] Hierbei trafen wir aber im Ganzen nur 4 kranke Mäuse,[2] während nach den anderwärts gemachten Erfahrungen eine weit grössere Anzahl zu erwarten gewesen wäre. Wahrscheinlich hatten viele Thierchen während der Versuchszeit im kranken Zustande die Flucht ergriffen.

Aus diesem Grunde wählten wir beim zweiten Versuche auf breiten Flussdämmen und im freien Felde (im Dorf Oba) mehere, passende, zahlreiche Mäuselöcher enthaltende Parzellen aus, welche, um das Entrinnen kranker und Hereinkommen frischer Mäuse zu verhindern, mit starken tief in die Erde eingeschlagenen Bambuspfählen dicht umgeben wurden. Ausserdem wurden diese Umzäunungen, um Raubvögel abzuhalteu, mit Schnüren überspannt. Die so vorbereiteten Parzellen wurden sodann am 24. Januar dieses Jahres sorgfältig inficirt, indem je ein Teigstückchen, etwa 1 c.c. Bouillonkultur enthaltend, in die Mäuselöcher geschoben wurde. Bei der am folgenden Morgen vorgenommenen Besichtigung waren die gelegten Teigstückchen meist bereits verschwunden und die noch zurückgebliebenen liessen oft Spuren von Scheidezähnen der Mäuse erkennen. Am 29. Januar, also am 5 Tage nach der Inficirung schritten wir zur sorgfältigen Ausgrabung der Versuchsparzellen. Die dabei angetroffenen Mäuse, die lebenden sowohl wie auch die toten

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[1] Um ein Entrinnen von Mäusen durch die Seitengänge zu verhindern, wurde zuerst jedesmal in tiefer Graben um den Haufen hergestellt.

[2] Specielle bakteriologische Untersuchungen ergaben, dass diese Thierchen in der That von dem Bacillus angegriffen waren.

wurden der bakteriologischen Untersuchung unterworfen. Die so erzielten Ergebnisse sind in der folgenden Tabelle zusammengestellt.

Nr. der Mäuse in fortlaufender Reihenfolge.	Nr. der Versuchsparzelle.	Der Bacillus[1] in			Bemerkung.
		Milz.	Herzblut.	Leber.	
1	I.	+	+	+	Lebendig eingefangen.
2		+	+	+	„
3		+	+	+	„
4		+	+	+	„
5	II.	+	+	+	„
6		+	+	+	„
7		+	+	+	„
8		−	−	−	„
9		+	+	+	„
10		+	+	+	„
11		+	+	+	„
12		+	+	+	„
13		+	+	+	„
14	III.	+	+	+	„
15		+	+	+	„
16		+	+	+	Tot im Nest gefunden.
17		−	−	−	Lebendig eingefangen.
18		+	+	+	„
19		−	−	−	„
20		+	+	+	„

Von den 20 untersuchten Mäusen waren also 17 Individuen von dem Bacillus angegriffen, so dass der Prozentsatz der in-

[1] In dieser und folgenden Tabellen wird die Anwesenheit des Bacillus durch (+) die Abwesenheit dagegen durch (−) bezeichnet.

ficirten 85 betrug. Auch die drei gesunden Nager wären schwerlich dem Eindringen des Bacillus entgangen, wenn die Mäuselöcher nicht so zeitig ausgegraben worden wären, da mehere infectionsfähige Teigstückchen[1] noch darin vorhanden waren.

Der dritte Versuch wurde in der Nachbarschaft des zweiten Versuchsfeldes vorgenommen und zwar in ähnlicher Weise wie vorher. Die Parzellen I und II wurden nämlich mit dem üblichen Infectionsmaterial beschickt, während wir auf der Parzelle III 2 schwer *erkrankte Mäuse freilaufen liessen.* Die am folgenden Morgen vorgenommenen Besichtigungen ergaben, dass die erkrankten Mäuse sowohl wie auch die meisten Teigstückchen verschwunden waren. Vier Tage nach der Inficirung d. h. am 9. Februar wurden die Versuchsparzellen sorgfältig ausgegraben, die angetroffenen Mäuse gefangen, in den schon erwähnten Kästen unter scharfer Beobachtung aufbewahrt und zwar so lange bis die meisten Thierchen der Wirkung des Bacillus erlagen. Die dabei gewonnenen Ergebnisse bringt die folgende Tabelle zur Veranschaulichung.

[1] 2 Mäuse, denen diese Stückchen zum Fressen gegeben waren, starben binnen 12 Tagen und zwar von der Wirkung des Bacillus.

Nr. der Mäuse in fortlaufender Reihenfolge.	Nr. der Parzellen.	Infections-material.	Der Bacillus in		Bemerkung.
			Milz.	Herzblut.	
21	I.	Inficirte Teig-stückchen.	+	+	Verendet nach: 5 Tagen der Inficirung.
22			+	+	6 „ „ „
23			+	+	7 „ „ „
24			+	+	8 „ „ „
25			+	+	12 „ „ „
26			+	+	16 „ „ „
27			+	+	16 „ „ „
28			−	−	Getötet nach 24 Tagen.
29			−	−	„ „ 30 „
30	II.		+	+	Tot gefunden.
31			+	+	Verendet nach 12 Tagen.
32			+	+	„ „ 12 „
33			+	+	„ „ 14 „
34			+	+	„ „ 15 „
35	III.	Kranke Mäuse.	+	+	Verendet nach 10 Tagen.
36			+	+	„ „ 12 „
37			+	+	„ „ 13 „
38			+	+	„ „ 15 „
39			−	−	Getötet nach 30 Tagen.
40			−	−	„ „ 30 „

Das Resultat des dritten Versuches bestätigt also das beim zweiten erzielte und liefert einen recht bedeutenden Prozentsatz der inficirten Mäuse (80). Ferner geht daraus hervor, dass der Ablauf der Krankheit im Freien ebenso schnell stattfindet wie beim Laboratoriumsversuche. Hier wie dort fängt am 5 Tage nach der Inficirung das Absterben an, das am 10–16 Tage am stärksten auftritt. Endlich sei noch hinzugefügt, dass Feldmäuse in der That auch auf *freiem Feld* wie in der Gefangen-

schaft ihre kranken Genossen auffressen und so den tötlichen Bacillus in sich aufnehmen.

Setzten also die oben geschilderten Versuchsergebnisse die Wirksamkeit des Bacillus als Mäusevertilgungsmittel ausser Zweifel, so blieb doch noch festzustellen, ob die Infection in ebenso günstiger Weise stattfindet, wenn das Inficirmaterial in geringerer Menge in Verwendung kommt. Demzufolge wurden 3 Parzellen auf einem alten breiten Flussdamme im Dorf Oba in schon erwähnter Weise umzäunt und überspannt. Dieselben wurden dann mit Buchweizenteigstückchen beschickt, welche die Agarkultur des Bacillus in 3 *verschiedenen* Verdünnungen enthielten. Es wurden nämlich in einem Liter einer $\frac{1}{2}\%$-igen Kochsalzlösung gleichalterige und gleich gut entwickelte Bakterienrasen aus 1, resp. 3 und 5 Agarröhrchen auf das Feinste vertheilt und mit jeder so gewonnenen Aufschwemmung Teig bereitet. Die meisten der gelegten Stückchen waren am folgenden Morgen verschwunden. Am 7. Tage der Inficirung (am 4. März) wurden die Mäuselöcher ausgegraben und die gefangenen Thierchen der bakteriologischen Untersuchung unterworfen.

Die folgende Tabelle zeigt die so erzielten Resultate.

Nr. der Mäuse in fortlaufender Reihenfolge.	Nr. der Versuchs-Parzelle.	Zahl der Agarkultur in einem Liter der Kochsalzlösung.	Der Bacillus in			Bemerkung.
			Milz.	Leber.	Herzblut.	
41			+	+	+	Tot gefunden.
42			−	−	−	Lebendig eingefangen.
43			−	−	−	„
44			−	−	−	„
45			+	+	+	„
46			+	+	+	Tot gefunden.
47			−	−	−	Lebendig eingefangen.
48			+	+	+	„
49	I.	1	−	−	−	„
50			−	−	−	„
51			−	−	−	„
52			+	+	+	Lebendig eingefangen, schwer krank u. starb nach einigen Stunden.
53			+	+	+	Lebendig eingefangen.
54			−	−	−	„
55			−	−	−	„
56			+	+	+	„
57			−	−	−	„
58			−	−	−	„
59			+	+	+	Lebendig eingefangen, sehr krank u. starb am folgenden Morgen.
60			+	+	+	Tot gefunden.
61			+	+	+	Lebendig eingefangen.
62			−	−	−	„
63	II.	3	+	+	+	„
64			−	−	−	„
65			+	+	+	Tot gefunden.
66			−	−	−	Lebendig eingefangen.
67			+	+	+	„
68			+	+	+	Lebendig eingefangen, starb am folgenden Morgen.
69			+	+	+	Lebendig eingefangen.
70			+	+	+	Tot gefunden.
71			+	+	+	„
72			−	−	−	Lebendig eingefangen.
73	III.	5	+	+	+	„
74			+	+	+	Tot gefunden.
75			+	+	+	Lebendig eingefangen, sehr schwer krank und starb nach einigen Stunden.
76			+	+	+	
77			−	−	−	Lebendig eingefangen.
87			+	+	+	Tot gefunden.

Betrachten wir vorliegende Tabelle, so werden wir gewahr, dass die Verbreitung der Krankheit gewissermassen von der Menge der inficirenden Bakterienkultur abhängig ist. So betrug die Prozentzahl der inficirten Individuen in der Parzelle III, wo die stärkste Bakterienemulsion benützt worden war, 82 (9 inficirte u. 2 gesunde Mäuse), also fast gleich viel wie die beim zweiten und dritten Versuche erzielten (85 resp. 80), bei denen Bouillonkultur resp. kranke Mäuse in Verwendung kamen. Dagegen belief sich dieselbe in der Parzelle II auf 74 (6 inficirte u. 3 gesunde Mäuse), und in der Parzelle III, wo die geringste Bakterienkultur zur Inficirung verwendet worden war, erreichte dieselbe kaum 45 (7 inficirte u. 11 gesunde Mäuse). Es sei gleich bemerkt, dass die hier benützte Kultur nicht die geringste Abschwächung erfahren hatte. Denn 2 Mäuse, denen 0.3 c.c. einer 24-stündigen Bouillonkultur desselben Bacillus subcutan injicirt wurden, starben binnen 48 Stunden.

Ausser diesen Versuchen haben wir noch eine Anzahl von Feldexperimenten ausgeführt, aus denen ein abschliessendes Urtheil über die dabei erzielten Resultate gezogen werden konnte. Bei diesen Versuchen wurden solche Felder ausgewählt, die von allen Seiten mit irgend einer natürlichen Grenze, d. h. Bachufer, Fusswegen, Sumpffeld u. dergl. mehr, umgeben waren. Es wurden zuerst alle Mäuselöcher zugetreten und die nach 3 Tagen aufgefundenen, frisch aufgegrabenen Löcher mit dem Teig in üblicher Weise beschickt, der die Bakterienkultur in *verschiedener* Stärke enthielt. Nach 5–8 Tagen wurden auch diese Löcher gezählt uud wieder zugetreten. Dieselbe Operation wurde alle 7–10 Tage wiederholt und zwar so lange bis keine resp. sehr wenige Löcher auf's Neue aufgegraben worden waren. In einigen Fällen wurden die neuen Löcher zum zweiten Male mit dem Inficirmaterial behandelt und weiterer Beobachtung unterworfen.

Die Daten dieser Versuche sind in folgender Tabelle zusammengestellt.

Tage an welchen Mäuselöcher inficirt, resp. gezählt und zugetreten wurden.	Nr. und Grösse des Versuchsfeldes.	Inficirmaterial.	Zahl der frisch aufgegrabenen Löcher.
2. Februar gezählt und inficirt.	I. Weizenfeld. (0.4 Hectar)	Eine 24-stündige Bouillonkultur.	130
8. „ gezählt und zugetreten.			Aus Versehen nicht gezählt.
14. „ „ „			64
17. „ „ „			26
23. „ „ „			18
28. „ „ „			6
4. März „ „			3
13. „ „ „			1
23. „ „ „			0

			(a)	(b)
18. Februar gezählt und inficirt.	II. a. Weizenfeld (0,4 Hectar).	In 1 Liter einer 0.5%-igen Kochsalzlösung wurden 4 Agarkulturen beim Versuchsfeld (a) und 3 Agarkulturen beim Versuchsfeld (b) gut vertheilt.	37	42
25. „ gezählt und zugetreten.			39	45
28. „ „ „			13	13
3. März „ „			9	8
9. „ „ „	II. b. Weizenfeld (0,6 Hectar).		7	3
14. „ „ „			7	6
17. Nochmals inficirt mit entsprechenden Teigstückchen.			7	6
20. „ gezählt und zugetreten.			0	2
27. „ „ „			0	3

			(a)	(b)
3. April gezählt und inficirt.	III. a. Weizenfeld (1,3 Hectar).	III. a. 5 Agarkulturen in 1 Liter einer 0.5%-igen Kochsalzlösung.	85	72
9. „ gezählt und zugetreten.			191	183
12. „ „ „			49	57
24. „ „ „	III. b. Weizenfeld (1,4 Hectar).	III. b. 1 Agarkultur in derselben Menge der Lösung.	19	26
3. Mai „ „			10	20
13. „ „ „			0	15

Zur besseren Veranschaulichung dieser Ergebnisse wurde in folgender Tabelle die Prozentzahl der frisch aufgegrabenen Löcher in gewissen Zeiträumen ausgerechnet, wobei die am Tage der Inficirung aufgefundenen Mäuselöcher als 100 angenommen werden.

Nr. der Versuches.	I.	II.		III.	
Inficirmaterial.	Bouillonkultur.	Zahl der Agarkultur.			
		(a) 4	(b) 1	(a) 5	(b) 1
Am Tage der Inficirung.	100	100	100	109	100
Nach 3–7 Tagen.	—	105	107	225	254
„ 15 „	20	24	19	58	79
„ 21 „	14	19	7	22	36
„ 30 „	2	19	14	12	28
„ 40 „	0	0	7	0	21

Eine plötzliche Steigerung der Prozentzahl der frisch aufgegrabenen Mäuselöcher am Anfange des Versuches ist auf den Umstand zurückzuführen, dass die Aufzählung und die Inficirung derselben 3 Tage nach dem ersten Zutreten, d. h. zur Zeit, wo die Mäuse ihre Löcher noch nicht genug aufgegraben hatten, stattfand. Ferner scheint nach dem Zahlenverhältnisse der frisch aufgegrabenen Löcher zu urtheilen, die Sterblichkeit der Mäuse binnen 7–15 Tagen nach der Inficirung am intensivesten aufgetreten zu sein, eine Erscheinung, die mit den Ergebnissen der schon erwähnten Laboratoriums- sowohl wie auch der Feldversuche in gutem Einklange steht. In weiteren 15 Tagen liess sich noch eine Abnahme der frisch aufgegrabenen Löcher beobachten, viele Mäuse schienen während dieser Zeit erst zu Grunde gegangen zu sein. Ob diese Mäuse, bei denens die Krankheit sich lange hingezogen hatte, zu wenig von dem inficirten Teig aufgefressen hatten oder durch Benagen der Kadaver erst später inficirt worden sind, muss natürlich dahingestellt bleiben. Endlich sehen wir aus der Tabelle, dass in den Parzellen, die entweder von Anfang an stark oder zweimal inficirt worden waren, wie Versuche I. II. (a) u. III. (a), keine

frischen Löcher nach 40 Tagen mehr beobachtet wurden. Dagegen wurden beim Versuche II. (b) u. III. (b), frische Löcher, obgleich nicht zahlreich, doch nach dieser Zeitfrist immer noch beobachtet. Diese geringere Infection muss sicher durch die Anwendung der verhältnissmässig verdünnten Bakterienemulsion bedingt worden sein. Dies Resultat stimmt mit dem beim Laboratoriumsversuche erzielten und bestätigt gleichzeitig in vollem Umfange die von *Mereshkowsky*[1] gemachte Beobachtung, dass die Kultur seines Bacillus erst bei *genügend intensiver Inficirung* sich zwecks Mäusebekämpfung verwenden lässt.

Abgesehen von der Krankheitsverbreitung auf dem inficirten Feld wird die Infection auf die Nachbarschaft übertragen, worauf *Mereshkowsky*[2] schon aufmerksam gemacht hat. Wir trafen nämlich bei der Besichtigung der Versuchsfelder einige kranke, sich langsam fortschleppende Mäuse auf den benachbarten Feldern. Dieselben wurden in der That, wie specielle bakteriologische Untersuchung zeigte, von dem nämlichen Bacillus angegriffen. Ferner fanden wir in einem auf etwa 200 Schritte von dem Versuchsfeld II. (a) entferntem Felde gelegten Strohhaufen 2 am Hals und Bauch etwas abgenagte frische Leichen von Mäusen auf, die, wie bakteriologische Prüfung erwies, in der That der Wirkung des Bacillus erlegen waren. Endlich starben von 20 lebendig eingefangenen, zum Versuchszwecke aufbewahrten Mäusen nach 4 Tagen 4 Individuen ab und zwar von der Invasion des Bacillus. Da diese Thierchen von einem nicht inficirten Flussdamme (im Dorf Oba) eingefangen worden waren, so muss man annehmen, dass die Krankheitskeime von dem etwa 500 Schritte entfernten Versuchsfeld (Nr. I) fortgeschleppt worden waren.

Nachdem die oben erwähnten Versuche den *Mereshkowsky*schen Bacillus als Bekämpfungsmittel gegen unsere Feldmäuse bei richtiger Anwendung als sehr geeignet erwiesen hatten, schritten wir dazu, die geschädigten Felder der genannten Provinz zu inficiren. Zu diesem Zwecke wurde zuerst eine Strohabkochung mit Zusatz von etwas Kochsalz, Pferdfleischauszug und Natto, einem aus Sojabohnen hergestellten, an Peptonen reichen Nahrungsmittel, in grossen Mengen verfertigt. Die so bereitete, mit kohlensaurem Natron neutralisirte Flüssig-

[1] u. [2] A. a. O.

keit, in der, wie Vorversuche geigten, der Bacillus sich gut entwickelt, wurde in meheren, etwa 10 Liter enthaltenden, aus Weissblech hergestellten Töpfen vertheilt und durch dreistündiges Erhitzen im Dampftopf sterilisirt. Nachdem die Kulturflüssigkeit auf 30-40° C. abgekühlt worden war, wurde sie mit einer Reinkultur des Bacillus inficirt und im Brütschrank bei 25-37° C. 2 Tage lang aufbewahrt. Nach dieser Zeit zeigte die Flüssigkeit in der Regel eine starke Trübung und war fertig zum Versenden. Dazu wurde der Wattepfropf in kurzen Stutzen des Topfes glatt geschnitten, eine passende Blechkappe darauf gestülpt und die Fuge mit schmelzendem Siegellack dicht verstrichen. Beim Gebrauch wurde die Flüssigkeit theils ohne Verdünnung, theils aber mit der gleichen Menge einer sterilen ca. 0.5%-igen Kochsalzlösung verdünnt, mit Buchweizenmehl unter Zusatz von etwas Weizenmehl[1] zu einem dicken Teig geknetet. Derselbe wurde nun an die Arbeiter vertheilt, die sodann in Reihen über die Felder gingen und ein Teigstückchen in der Grösse von etwa einem Kubikcentimeter in jedes Mäuseloch hineinschoben. Ausserdem wurden benachbarte Wälder, Feldraine Fluss- u. Bachdämme, Gräben u. s. w. wo Mäuse besonderes gern ihr Nest bauen, ebenfalls sorgfältig inficirt. Auf diese Weise haben wir bis Ende Juni ca. 3000 Hectar bebauter Felder behandelt, die von Mäusen stark gefährdet waren. Bald liefen zahlreiche Berichte bei uns ein, welche einen glänzenden Erfolg der Inficirung mittheilten. In der That boten die Felder bei der Ende October von uns vorgenommenen Besichtigung ein ganz anderes Bild dar wie früher. In diesem Frühling als wir die Inficirung in grossem Maassstab vornahmen, zeichneten sich die Felder durch zahlreiche Mäuselöcher aus und Kulturgewächse jeder Art liessen das Zerstörungswerk des Nagers erkennen. Und jetzt fand man nur ausnahmsweise hie und da ein paar Mäuselöcher, und junge Pflanzen, wie Bohnen und Erbsen wuchsen üppig weiter. Die im Frühling in staunenswerthen Mengen auftretenden Feldmäuse sind wie durch Zauber verschwunden. Wie glänzend der Erfolg des Mittels war, zeigt auch die folgende Thatsache. Im Frühling pflegten die Bauern im Dorf Oba gegen kleine Zahlung die zum Versuche benützten Feldmäuse jeder Zeit in jeder Zahl uns zu liefern. Jetzt aber

[1] Dies geschah um die Zähigkeit des Teiges zu vermehren.

liefern sie keine Mäuse mehr, selbst wenn wir den Preis hoch ansetzten.

Auf Grund der oben erwähnten Ergebnisse nehmen wir keinen Anstand mehr, den *Mereshkowsky*'schen Bacillus an die Seite des vielfach erprobten *Löffler*'schen zu stellen und ihn ebenfalls als ein sicher wirkendes Mittel gegen die Mäuseplage zu erklären. Ob derselbe Bacillus eine mit dem *Löffler*'schen oder dem *Danysz*'schen gleiche Virulenz besitzt, wird den Gegenstand einer weiteren Mittheilung machen.

Über die Bildung des Pyocyanolysins unter verschiedenen Bedingungen.

VON

O. Loew und Y. Kozai.

Vor Kurzem haben *Bulloch* und *Hunter* beobachtet, dass Culturen des *Bac. pyocyaneus* einen Körper enthalten, das Pyocyanolysin, welcher die Blutkörperchen des Ochsen, des Schafes, des Kaninchens und anderer Thiere hämolysirt.[1] Die Menge dieses Körpers variirte in verschiedenen Fällen, ältere Culturen sind reicher daran als junge. Von Interesse ist ferner, dass 15 Minuten langes Erwärmen auf 100° die hämolytischen Eigenschaften der Culturen noch nicht aufhebt, und dass dieser Körper vorzugsweise in den Zellen bleibt, so dass Filtrate der Culturen weit schwächer wirken als sterilisirte, unfiltrirte Culturen.

Von einigem Interesse schien uns die Frage, ob das Pyocyanolysin in verschiedenen Nährloesungen gleich stark auftritt und ob das Maas des Luftzutritts einen Einfluss äussert, ferner ob jener Körper in mässigen Mengen tötlich auf Thiere wirkt. Die oben genannten Autoren verwendeten *lediglich Bouilloncultur*en, von denen (nach Entfernung oder Tötung der Bacillen durch 15 Minuten Erwärmen auf 60°[2]) 0,05 bis 2 c.c. mit 2 c.c. einer 5% Aufschwemmung von Blutkörperchen 18-20 Stunden lang bei 37° im Incubator gehalten wurde. Bei den stärkeren Graden der Hämolyse waren die Blutkörperchen völlig gelöst.

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[1] Centralbl. f. Bakt., Band 28, S. 866. Wie dem Einen von uns von Prof. *M. Nencki* mitgetheilt wurde, haben *Nencki* und *Sieber* schon vor einiger Zeit die gleiche Beobachtung gemacht.

[2] Unter gewissen Verhältnissen verträgt *B. Pyocyaneus* jedoch weit höhere Temperaturen. Die Tötung bei 60° wurde jedenfalls durch das bacteriolytische Enzym in der Cultur beschleunigt.

Wir verwendeten zu unsern Culturen drei Nährloesungen:

I. Bouillon

II. Pepton 1% + Glycerin 0.1%

III. Asparagin 0,5% + Glycose 0,5%

Jede dieser Loesungen wurde auf zwei je 200 c.c. fassende Erlenmeyerkolben vertheilt, der eine enthielt nur zu 2 cm. Höhe Nährloesung, was ausgiebigen Luftzutritt ermöglichte, der andere war bis zur Verengerung am Kolbenhalse gefüllt, der Luftzutritt war hier sehr beschränkt und anaërobes Leben in den unteren Schichten begünstigt. Loesungen II. und III. erhielten noch 0.2% secundäres Kaliumphosphat und 0.01% Magnesiumsulfat (letztres in sterilisirter Loesung erst bei der Infection zugegeben). Die am 20. März inficirten Loesungen zeigten (bei 36° gehalten) am 4. April folgende Erscheinungen:

I. *Bei reichlichem Luftzutritt:* Schleimige Loesung und schleimiger Bodensatz, braune Färbung.
Bei geringem Luftzutritt: Sehr schleimige Beschaffenheit von Loesung und Bodensatz, schwach bräunlich.

II. *Bei reichlichem Luftzutritt:* Dunkel braungrün, Vegetation abgelaufen, die anfänglich bedeutenden Bacterienmassen wieder gelöst. Bodensatz gering, zum Theil krystallinisch. Loesung kaum merklich schleimig.
Bei geringem Luftzutritt: Noch viele Bacterienballen am Boden. Loesung hellgrün, trübe.

III. *Bei reichlichem Luftzutritt:* Schwach bräunlich. Die vorher geringen Vegetationsmassen waren fast völlig wieder verschwunden. Geringer, zum Theil krystallinischer Bodensatz. Schwach bräunliche Färbung.
Bei geringem Luftzutritt: Bacterienballen am Boden. Schwach grünliche Färbung.

Es schien demnach, dass bei reichlichem Luftzutritt mehr Pyocyanase gebildet wird, als bei geringem, was mit Erfahrungen an anderen Enzymen übereinstimmt. Bei II. war das ganz ausser Zweifel. Die Loesungen blieben bei alltäglich fortgesetztem einmaligen Umschwenken bis zum 9. April bei gewöhnlicher Temperatur stehen und dienten dann zu folgenden Versuchen mit Rinderblut, welche im Wesentlichen wie von *Bulloch* und *Hunter* ausgeführt wurden. Blutkörperchen aus frischem Ochsenblut wurden in 5% tiger Aufschwemmung in physiologischer Kochsalzloesung verwendet und zwar wurden stets 2 c.c. dieser

Suspension mit 0.5, resp 2 c.c. der Culturen vermischt und bei 37° gehalten. Die abgemessenen Culturflüssigkeiten wurden vorher 15 Minuten auf 60° erhitzt. Das Resultat war nach 20 Stunden folgendes:

Hämolyse.

I. Cultur mit viel Luft. 0.5 c.c. Gering. 2 c.c. Mässig stark.
Cultur mit wenig Luft. 0.5 c.c. Spur. 2 c.c. Gering.

II. Cultur mit viel Luft. 0.5 c.c. Ziemlich stark. 2 c.c. Sehr stark. Dunkelrote Loesung.
Cultur mit wenig Luft. 0.5 c.c. Keine. 2 c.c. Schwach.

III. Cultur mit viel Luft; 0.5 c.c. Stark.
Cultur mit wenig Luft; 0.5 c.c. Stark.

Es ergab sich also, dass in Bouillon weniger Pyocyanolysin entwickelt wurde, als in den andern beiden Loesungen, dass ferner Luftzutritt die Bildung des Pyocyanolysins förderte bei Pepton und Bouillon als Nährstoff. Auffallend war das Resultat bei Asparagin und Glucose als Nährstoff, bei III. Zucker setzt in der Regel die Enzymbildung bei Bacterien herab, hier aber finden wir, dass nicht nur mehr gebildet wird als in Bouillon, sondern auch, dass der reichlichere Luftzutritt keinen Unterschied von dem geringeren Luftzutritt ergab.

Um nun zu beobachten, ob der Hämolysingehalt obiger Pyocyanusculturen Thieren schädlich sein würde, wurden die oben erwähnten Culturen zunächst mit 0.3% Tricresol versetzt und nach 1 Tag 0.5 c.c. von jeder Cultur weissen Mäusen injicirt. Bemerkt sei, dass bei der zwei Jahre lang fortgesetzten Cultur dieses Microben auf Agar die Virulenz verloren gegangen war. Das Resultat war;

I. Cultur mit viel Luft: Maus lebt noch munter nach 10 Tagen.
Cultur mit wenig Luft: Maus tot nach 3 Tagen.

II. Cultur mit viel Luft: Maus munter nach 10 Tagen.
Cultur mit wenig Luft: Maus munter nach 10 Tagen.

III. Cultur mit viel Luft: Maus tot nach 15 Stunden.
Cultur mit wenig Luft: Maus tot nach 4 Tagen.

Das Resultat steht somit in *gar keinem Zusammenhang* mit dem Pyocyanolysingehalt. Gerade die Loesung, welche am meisten Pyocyanolysin enthielt, die Peptoncultur bei reich-

lichem Luftzutritt, war ganz harmlos, während die Bouilloncultur bei geringem Luftzutritt, in der sich nur geringe Mengen Pyocyanolysin fanden, tötlich wirkte.[1] Loesung III., welche gleichstarken Hämolysingehalt zeigte, bei reichlichem sowohl wie bei geringem Luftzutritt, ergab einen sehr bedeutenden Unterschied in der tötlichen Wirkung in beiden Fällen. Es war aus dem Asparagin etwas kohlensaures Ammoniak entstanden, wie das *Nessler*'sche Reagens ergab und zwar mehr beim reichlichen Luftzutritt. Es wäre nicht unmöglich, dass dieser Ammoniakgehalt den Tod der Thiere bedingte. Es ergibt sich hieraus:

1. Bei reichlichem Luftzutritt wird mehr *Pyocyanase* gebildet als bei geringem.
2. In Bouillon und in Pepton-Nährloesung bedingt reichlicher Luftzutritt auch eine Vermehrung des *Pyocyanolysins*. Bei Anwesenheit von Zucker (bei III.) war dieser Einfluss reichlichen Luftzutritts nicht zu bemerken.
3. Der Pyocyanolysingehalt obiger Culturen ist bei Injection von 0.5 c.c. der abgetöteten Culturen weissen Mäusen nicht schädlich.

Nachschrift: Nachdem diese Versuche abgeschlossen waren, ersahen wir aus einem kürzlich erschienenen Artikel von *Weingeroff*,[2] (welcher die Beobachtungen von *Bulloch* und *Hunter* bestätigte), dass dieser ebenfalls zum Schlusse kam, dass das Toxin virulenter Pyocyaneusculturen nicht identisch mit dem Pyocyanolysin ist.[3] Dieser Autor verwendete Culturen von Pyocyaneus, dessen Virulenz durch öfteres Passiren durch Kaninchen so gesteigert war, dass 10 c.c. des Filtrats ein Kaninchen binnen drei Tagen tötete. Ferner ist hervorzuheben, dass *Weingeroff* ausschliesslich Bouillonculturen verwendete.

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[1] Vielleicht wird das Pyocyanolysin leicht im Thierkörper zerstört. Die Hämolysine anderer Bacterienarten (Erysipel) scheinen durchaus nicht so harmlos zu sein.

[2] Centralbl. Bakt. Bd. 29, S. 777 (1901).

[3] Pepsin und Trypsin zerstören in 24 Stunden das Haemolysin, aber nicht das Toxin; letzteres wird bei 100° selbst nach 30 Minuten noch nicht zerstört.

Ueber die coagulirende Wirkung des Chloroforms.

VON

O. Loew und K. Aso.

Vor Kurzem veröffentlichte *E. Salkowski*[1] einige interessante Beobachtungen über eine coagulirende Wirkung des Chloroforms auf Blut und Milch. Auch Eigelb wird allmälig coagulirt, aber nicht das Weisse von Eiern, ebenso wenig das Lactalbumin in der Milch. Der eine von uns (L) wurde dadurch an eine schon vor längerer Zeit gemachte Beobachtung erinnert. Stücke von der Pancreasdrüse des Schweines nehmen nämlich in Chloroformwasser aufbewahrt bald ein anderes Aussehen an als beim Aufbewahren in verdünntem Alkohol von 15%; in jenem Fall wird sie heller als in letzterem und die Flüssigkeit giebt dort weniger Gerinnsel wie hier. Dieses veranlasste uns zu einigen Versuchen mit Leber und Muskeln vom Rind. 59 g. frischer feinzertheilter Leber wurde einerseits mit etwas Chloroform,[2] andrerseits mit etwa dem doppelten Gewicht verdünnten Alkohols von 15% zwei Tage bei Zimmertemperatur stehen gelassen, hierauf mit 200 c.c. Wasser eine Stunde lang bei 50° extrahirt, die Filtrate mit Zusatz von etwas Salpetersäure zum Kochen erhitzt und das Gerinnsel nach dem Waschen und Trocknen bei 100° gewogen. Der mit der einen Leberprobe in Contact gewesene Alkohol von 15% wurde ebenfalls zum Kochen erhitzt, mit etwas Salpetersäure angesäuert und das Gerinnsel ebenfalls gewogen. Das Resultat war folgendes:

Es war gelöst worden nach der Behandluug mit:

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[1] Z. physiol. Chem. Bd. 31, S. 329.

[2] Das Chloroform reagirte vollständig neutral.

Chloroform.	Alkohol von 15%
0.574 g.	1.899 g. +0.476 g. (gelöst im 15% igen Alkohol.) 2.375 g.

Es war somit bei Abwesenheit des Chloroforms etwas über viermal so viel Proteinstoff gelöst worden. Dieses ist bei manchen physiologischen Arbeiten wohl zu beachten. Bemerkenswert ist noch, dass das Wasserextract nach der Behandlung mit Chloroform nur schwach gelblich war, während nach der mit verdünntem Alkohol rot von gelöstem Haemoglobin. Der in analoger Weise mit Muskelfleisch[1] ausgeführte Versuch ergab bei:

Chloroform.	Alkohol von 15%
0.938 g.	1.055 g. +0.446 g. 1.501 g.

Es ist also in beiden Fällen eine coagulirende Wirkung des Chloroforms ersichtlich. Es erinnert dieses an die katalytische Wirkung mancher Aetherarten, wobei wahrscheinlich eine Uebertragung gewisser Schwingungszustände (chemische Energie) anzunehmen ist.

Nachschrift. Wie wir soeben aus einem der neuesten Hefte der Zeitschr. Biol. ersehen, hat auch *Krüger* denselben Gegenstand bearbeitet und schon vor elf Jahren einen Niederschlag durch Chloroform in wässerigen Extracten verschiedener thierischer Organe beobachtet. Er fand auch, dass Haemoglobin vollständig dadurch gefällt wird.

[1] Die nachherige Extraction geschah hier nicht mit blosem Wasser sondern mit fünfprocentiger Loesung von Magnesiumsulfat.

On Kaki-Shibu, a Fruit Juice in Technical Application in Japan.

BY

M. Tsukamoto.

The name "Kaki-shibu," or more briefly "Shibu," is given in Japan to the juice of the unripe fruit of the Kaki tree (*Diospyros Kaki, L.*). This juice serves for the preservation of fish-nets and fish-lines which are soaked in the liquid and left to dry well in the sun. After this treatment the nets or lines are much more durable. It further serves as an application to packing papers,[1] especially such as are used for packing tea. Since such paper is less penetrable by moisture, the danger of mould development in the tea in warm and moist climates is diminished. Also tubs and other wooden vessels are treated with the juice to render them more durable. Further the juice is frequently mixed with India ink and the mixture is then used as a paint for the outer walls of wooden buildings and also for wooden fences.

Considering that the Kaki-shibu has such important technical applications it appeared desirable to subject the juice to an investigation. First of all it should be stated that not every kind of Kaki fruit yields suitable juice. There are found two varieties of the Kaki in Japan: one becomes very sweet when ripened, while the other remains astringent and is very rich in tannin,[2] yielding in great quantity the juice desirable for making Kaki-shibu. Such fruit when full grown is only 3–4 cm. in diameter. 1200 kg. of the fresh fruit when well crushed and mixed with about 2 hl. of water will yield nearly 7 hl. of Kaki-shibu. The mixture remains three or four days in

[1] Also other kinds of paper are thus treated and used for various household and commercial purposes.

[2] In order to make the fruit of some of this variety palatable, it is necessary either to treat it when well-ripened with a very diluted lye, or to dry it in the sun after taking off the skin.

large tubs when a certain kind of fermentation sets in, which is recognizable by the development of gas. The Kaki-shibu is prepared principally in the southern parts of Japan and the best quality of it comes from Kyoto, Osaka and Nara. The largest quantity of it comes from the vicinity of Hiroshima. The juice is generally prepared in August[1] and may be applied fresh or after standing for two or three years. Some persons claim that the juice is improved by long standing in a cool place. In contact with the air an amorphous film is formed on the surface, probably by oxidation, while on the bottom of the vessels some bacteria and yeast-like cells are deposited causing the characteristic smell of butyric acid. This development of microbes proves that the preserving properties of the Kaki-shibu do not consist in any peculiar antiseptic action, and therefore the beneficial effects have to be traced to another source. The Kaki-shibu leaves on evaporation a film insoluble in water, and this substance fills up the pores of fibres and wood, thus diminishing the water-holding capacity and the chances for destructive fungi. It is further very evident that the fibres of lines and papers are more closely united to each other so that they closely cohere. Thus the deterioration by mechanical wear and tear is considerably lessened.[2] One can easily convince himself of this peculiarity by dipping filter paper in Kaki-shibu and letting it dry. The difference in behaviour of this paper and ordinary filter paper when rubbed in the moist state with the fingers, is quite remarkable. The former offers considerable resistance to the separation of fibres, while the latter does not.

Chemical Examination.

The most characteristic constituent seems to be a kind of

[1] It is said by the practical Kaki-shibu-makers that the season for the preparation of the juice is not of long duration, being only about 10 days; both before and after that season the quality and quantity of the product is inferior.

[2] The view expressed by Prof. I. Ishikawa (Journ. of Tokyo Chem. Soc. Vol. III. (1882) Transactions p. 19.) on this same subject is a little different from that stated here: namely, that the effective power of the juice is due to the formation of the film, which is formed on the surface of the Shibu when allowed to stand for some length of time. It seems to me, however, that the film formed on standing is different in its nature from that formed on evaporation, and that the former can hardly be supposed to be formed so soon.

tannin; but this has some abnormal properties, as will be seen later on.

When water is mixed slowly with old Kaki-shibu it can be observed that the latter remains for sometime suspended in the water in the form of a reddish and slimy liquid and only on shaking well does a complete distribution result, but this solution remains opalescent. Fresh Kaki-shibu is whitish and nearly odourless; old Kaki-shibu is more or less reddish brown, and gives off the odour of butyric acid. Old commercial Kaki-shibu in the undiluted state will yield when exactly neutralized with sodium carbonate a flocculent precipitate, and soon afterwards a jelly which is almost insoluble in boiling water and alcohol. Boiling alcohol does not extract a tannin-like compound while the insoluble part turns black at once in contact with ferric chloride, which shows that the tannin compound has here exceptional properties. The jelly mentioned is, however, soluble in dilute acids, but it is again precipitated by the addition of an excess of strong acids, as whitish floccules. The acid solution also gives precipitates by ferric chloride and basic lead acetate, but not by phosphotungstic acid. The jelly is soluble in dilute sodium carbonate, which latter solution becomes very dark on contact with the air.

The fresh juice shows only a moderate acid reaction but this reaction increases on standing,[1] which is no doubt due to the moderate development of microbes. On distillation in a current of steam a considerable portion of the acid distills over.[2] I have determined by titration the total acidity, and further the amount of non-volatile acid after evaporation of 10 c.c. to dryness. It was thus found that the amount of volatile acid is almost equal to that of the non-volatile acid in new Kaki-shibu, while it is more than twice as great as the amount of non-volatile acid in the old Shibu.[3]

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[1] For my observation for the acidity served two kinds of Kaki-shibu prepared in the same place, one of which was made this year (1901) and the other last year. The total acidity per litre of the new and of the old corresponded respectively to 0.978 g. and 1.948 g. of SO_3.

[2] The nature of this volatile acid was investigated about twenty years ago by Prof. I. Ishikawa (l. c. p. 19–20) who found butyric acid in it.

[3] The samples that served for my analysis came from Awotani-mura, Tsuzuki county near Kyoto. The new Kaki-shibu was prepared this year (1901), and the old in the last year (1900).

Commercial Kaki-shibu even in high dilution gives precipitates with basic lead acetate, mercuric chloride, mercuric nitrate and copper acetate. Since dilute phosphotungstic acid yields no precipitate, proteins seem to be absent to a notable degree.[1]

One of the most striking properties of the Kaki-shibu is the production of an insoluble film on evaporation to dryness. Whether this film is exactly the same as the film formed on the surface of the liquid on long exposure to the air may be doubted, since there evidently takes place a considerable oxidation in the latter case. In the former case, however, the loss of solubility in water is only due to the loss of the greater part of the acid by volatilization.[2] Furthermore, these two films show the following differences in their behaviour: The former film swells somewhat when moistened, and is very easily broken. The latter film is elastic and more coherent. When treated with dilute acetic acid the former first swells up and gradually dissolves on standing, but the latter is almost insoluble and shows no perceptible change after long standing. On addition of concentrated sulphuric acid the former dissolves at once, while the latter only very slowly. On boiling with dilute nitric acid the former dissolves very easily giving a fine yellow solution, but the latter is dissolved only with difficulty. When boiled with caustic potash the former dissolves also much more easily than the latter. In order to determine the quantity of the film formed by evaporation, 10 c.c. of Kaki-shibu was evaporated to dryness and this residue was treated repeatedly with cold water until no acid reaction could be percieved in the washing. The insoluble film was dried and weighed, giving 0.351 g. or 3.420% for a sample of the new Kaki-shibu and 0.360 g. or 3.512% for a sample of the old ;[3] they contained respectively 0.349 g. or 99.5%

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[1] In the new Kaki-shibu a little turbidity is observed by phosphotungstic acid.

[2] Fresh juice is not a homogeneous solution but contains much fine suspended matter. This gradually goes in solution as the amount of acid is increased by bacterial action, since the suspended matter of the fresh juice gives a much stronger tannin reaction than the filtered liquid does.

[3] It appears that the film formed on evaporation from the new Kaki-shibu is more soluble than that of the old juice.

and 0.358 g. or 99.3% of organic matter. This film turns black with ferric chloride.[1]

When Kaki-shibu is mixed with an equal volume of alcohol no precipitate is observed, but on a further addition of alcohol and some ether an almost white flocculent precipitate was formed and this on standing gradually turned reddish brown. Very remarkable is the behaviour towards acids. A moderate quantity of mineral acids will give no precipitate; while on further addition much precipitate is produced,[2] which is again soluble in pure water and alcohol, and very easily in dilute acids. Of organic acids a larger quantity is required than of the mineral acids. 10 c.c. of old Kaki-shibu requires nearly 15 c.c. of concentrated sulphuric acid to produce complete precipitation. In this behaviour towards acids the tannin compound is like ordinary tannins, although in some other respects its behaviour is quite different.

I have carried out the tannin determination by the Lœwenthal method as improved by von Schröder, and have found the results calculated as both gallotannic acid and quercitannic acid; they were respectively 4.883 g. and 5.041 g. in 100 c.c. of the new Kaki-shibu; and 3.535 g. and 3.649 g. in 100 c.c. of the old. The former correspond respectively to 74.7% and 77.1% of its total solid matter; while the latter had 58.7% and 60.6%.

The commercial Kaki-shibu neither contains sugar, nor does it yield sugar on decomposition with sulphuric acid. The Kaki tannin, therefore, is no glucoside. These reactions for reducing sugar were always made after the tannin had been removed with basic lead acetate. In one experiment 500 c.c. of commercial Kaki-shibu were boiled on the addition of 15 c c. of concentrated sulphuric acid for three hours, and thereby a red precipitate was separated on cooling in the form of little globules. This precipitate, which increases on standing for twenty-four hours, was then collected on a filter and washed with water for a short time, since prolonged washing dissolved gradually a portion of it. This precipitate resembling oak-red (Eichenroth), was colored

[1] It may be mentioned that the freshly cut surface of the unripe Kaki fruit when moistened with ferric chloride gives no uniform black reaction, but merely in numerous isolated points, and it seems therefore that the tannin is limited to certain cells.

[2] The fresh Kaki juice behaves differently, since it gives no precipitate with an excess of acids.

violet black by ferric chloride; in cold alcohol dissolved easily with a fine red color; in caustic soda, with a brown red color. The aqueous solution is precipitated on a moderate addition of sulphuric acid. From genuine oak-red, however, our substance is distinguished by its solubility in cold water and cold alcohol.

The liquid from which the red precipitate was separated was of a dark reddish brown color, and contained evidently more of the red substance in solution. The sulphuric acid was at first removed by barium carbonate and then the rest of the tannin by basic lead acetate. The filtrate now obtained was treated with sulphuric acid to remove the lead, and the filtrate evaporated on the water bath with the addition of barium carbonate. The search for sugar or chitosamine was in vain. I intend to repeat this experiment with the insoluble film obtained by evaporation of Kaki-shibu.

Finally for analytical results we have :—

	New Kaki-shibu.	Old Kaki-shibu.
Specific gravity at 15°c	1.0230	1.0250
Total solid Matter.	6.391%	5.874%
Total fixed organic Matter.	5.993 ,,	5.566 ,,
Ash.	0.398 ,,	0.308 ,,
Insoluble film.	3.420 ,,	3.512 ,,
Tannin { as gallotannic acid.	4.773 ,,	3 449 ,,
Tannin { as quercitannic acid.	4.927 ,,	3.560 ,,
Volatile acid (as acetic acid.)	0.087 ,,	0.217 ,,
Fixed acid (as lactic acid.)	0.085 ,,	0.102 ,,
Nitrogen [1]	———	0.030 ,,

General Conclusion.

The industrial value of Kaki-shibu is due to its containing a peculiar tannin which in some respects differs from all other kinds of tannin known, since it is insoluble in alcohol and water, and soluble in dilute acids. This tannin becomes insoluble when the volatile acid of the Kaki-shibu evaporates, and the insoluble film thus formed protects fibrous objects against mechanical wear and tear. A partial oxidation in contact with

[1] This nitrogen is probably due to an admixture of small quantities of protein. The amount varies greatly.

air still improves the qualities of the film. This film also diminishes the water absorbing capacity of such fibrous materials as paper and strings, and thus diminishes the chances of attacks from fungi.

In conclusion I must express my thanks to Prof. O. Loew for his kindness in giving me the direction of this work, and to Messrs. T. Ono and K. Onodera for their assistance in the investigations.

Investigations on the Digestive Enzymes of Some Lepidoptera.

BY

S. Sawamura.

The digestive organs of *Vertebrata* consist of the mouth, stomach, and intestines. The saliva secreted in the mouth is alkaline; the gastric juice of the stomach, acid; and the pancreatic juice and bile secreted into the intestines, alkaline: thus the reaction of the fluids in the alimentary canal from mouth to rectum changes several times. But, as to *Lepidoptera*, the reaction of the fluids is alkaline throughout the digestive canal. Hence, there arises the question as to whether the digestive enzymes of these animals are different from those of the *Vertebrata*.

As the silk-worm (*Bombyx mori.*) is the most useful insect in this country, it has been much studied, and thus the morphological character of *Lepidoptera* has become comparatively well known; but our knowledge of its digestive process is still very scanty. *O. Kellner*[1] made an investigation on the digestibility of mulberry-leaves by silk-worms, and found that they could digest albumin, fat and carbohydrates except cellulose; but his investigation did not extend to the process of digestion itself.

Not only with *Lepidoptera*, but also in the whole division of *Insecta*, the digestive enzymes are very imperfectly understood. The first investigators on this subject were *Plateau* and *Jousset.*[2] The former concluded, from his investigations, that the fluid in the fore-intestine (now commonly called the fore-stomach) being neutral or alkaline, contained diastatic enzyme, which the investigator thought to be secreted by

[1] Landw. Versuchsstationen. 1886.

[2] Jahresbericht über die Fortschritte der Tierchemie. 1877.

salivary glands ; and that in the middle intestine (now generally called the stomach) the fluid being neutral or alkaline, had the power of decomposing albumen and saponifying fat (with *Hydrophilinen* and *Scarbaeiden* diastatic enzyme was also present); and finally that in the end-intestine (now generally called the intestine) there existed no digestive ferment. The conclusion obtained by *Jousset*, who studied *Blatta orientalis*, was nearly the same as that of *Plateau*, the only difference being that in *Blatta orientalis*, the fluid in the middle intestine was acid, and contained no diastatic enzyme.

The investigations made by *Krukenberg* [1] were much more extensive than those above mentioned, but as unfortunately the author of this paper has had no opportunity of reading his original reports, the details of his investigations can not here be mentioned. The chief points of the summary of his experiments are, however, as follows :—

I. In *Insecta* and in other *Arthropoda* trypsin predominates over pepsin.

II. The property of the tryptic enzyme of insects is different from that of *Vertebrata*, other *Arthropoda*, and *Mollusca*.

III. In *Invertebrata* there being no division of the intestines which is specialized as stomach, corresponding to the stomach of *Vertebrata*, a certain part of the intestines is commonly regarded as a stomach.

Krukenberg called the tryptic enzyme of insects "*Isotrypsin*," but in what respect it differs from the known trypsin is not explained.

Biedermann[2] studied the digestion of *Tenebrio molitor* (*Coleoptera*), and found that the secretion in the upper part of the middle intestine had an acid reaction, while in the lower part it was always alkaline ; and further that it acted upon starch, disaccharides, fat, and albumen, but not upon cellulose ; and finally that the albumen was split up into amido-compounds as in the case of tryptic digestion. Besides these investigations, the discovery of invertin in the head of honey-bees by *Erlenmeyer* and *Planta* [3] may be mentioned.

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[1] Fortschritte der Tierchemie. 1880.

[2] *Arendt*. Chemisches Central-Blatt. 1898.

[3] *Biedermanns* Central-Blatt für Agrikulturchemie. 1879.

Some general knowledge of the digestive enzymes of insects may be obtained from the investigations mentioned, but as there exist among insects both herbivora and carnivora, the character of the digestive enzymes may also be different. Further, since the investigations of *Krukenberg* and others hitherto made have related chiefly to proteolytic enzymes and not to amylolytic and lipatic enzymes, some further questions remain to be solved.

The author has, therefore, undertaken to investigate the digestive enzymes of *Lepidoptera*, especially those of the *silkworm*.

The insects used in the first experiment were the living larvae of *Dasychira lumulata* Butl.

They were dissected, and the expanded part of the digestive canal (stomach) was taken out, and freed from its contents by washing it in water, and 3 grs. of these stomachs were triturated in a mortar with the addition of powdered glass. The crushed mass was divided into two parts. One part was digested with 15 grms. of glycerin containing 10% of water, while the other part was made faintly acid with dilute acetic acid, according to the trypsin-extraction method of *Heidenhain*,[1] and exposed to the air for a short time, whereupon it was digested with glycerin like the other part. To furnish a control case for the method of extracting enzymes, the stomach and intestines of a snake were treated in the same manner as those of the insects. After three days the glycerin extract prepared without the acid treatment was filtered. To the filtrate strong alcohol was added till a precipitate was produced. The precipitate was collected on a filter, dissolved in water, filtered and again precipitated. But this precipitate was too insignificant to be separated again from the filter. It was, therefore, exposed to the air on the filter, and after the evaporation of the alcohol, the whole was divided into two parts. One part was dissolved in about 20 c.c. of water containing 0.1% of HCl, and the other in 20 c.c. of 0.3% solution of sodium carbonate, since, according to *Hammarsten*,[2] trypsin acts best in a fluid containing 0.3—0.4 % of sodium carbonate. The extract of the stomach of the snake was treated

[1] *Hammarsten*. Lehrbuch der Physiologischen Chemie. S. 171.

[2] Ibid, S. 172.

in quite the same manner. The enzyme-solution thus prepared, served for the following experiments.

The first experiment was made to ascertain the influence of the reaction on the behavior of the enzymes. The materials tested with the addition of some thymol at room-temperature, or at 36° C., were sharply cut slices of coagulated egg-albumen, fibrin and starch-solution.

The results were as follows :—

Solutions.	Materials used.	Temperature.	Intervals.	Results.
0.1 % HCl.	fibrin.	room-temperature.	7 days.	not dissolved.
Acid-solution of the enzymes of snake-stomach.	"	"	"	slightly dissolved.
"	egg-albumen.	"	"	corroded.
"	starch.	"	3 hours.	not changed.
Acid-solution of the enzymes of the insect-stomach.	fibrin.	36° C.	2 days.	not dissolved.
"	"	room-temperature.	7 days.	not dissolved.
"	egg-albumen.	"	"	not corroded.
"	starch.	"	3 hours.	not changed.
0.3 % solution of sodium carbonate.	fibrin.	"	7 days.	not dissolved.
Alkaline solution of the enzymes of snake-stomach.	"	"	"	"

Solutions.	Materials used.	Temperature.	Intervals.	Results.
Alkaline solution of the enzymes of the insect-stomach.	„	„	„	dissolved, and produced biuret reaction.
„	„	36° C.	2 days.	„
„	starch.	room-temperature.	3 hours.	starch completely disappeared.

The acid extract of the insect-stomach and that of the snake-intestines were examined in just the same way as the former. The results were the same as those obtained with the insect-stomach. I therefore infer:

I. That the enzymes of *Lepidoptera* act only in an alkaline, and not in an acid solution.

II. That in the digestive organs of *Lepidoptera* tryptic enzyme is present, while pepsin is absent.

Since, however, it might be objected that there was present pepsinogen, which failed to be converted by the highly diluted acetic acid into the active state, a second experiment was made, and this time with old silk-worms. 16 grs of stomachs and 3 grs of intestines were obtained from silk-worms in the same manner as in the former case, and these were divided into two portions. After adding to one part some HCl of 0.1% (according to *Podwysozki*[1] pepsinogen is easily transformed into pepsin by HCl), both portions were crushed as above and digested with glycerin as above. After three days it was filtered, and to the filtrate strong alcohol was added. The precipitate was collected on a filter, dried and divided into two parts. Since according to *Klug*,[2] pepsin acts best in a solution containing 0.5—0.6% of HCl, and according to Ewald[3] trypsin acts also in a solution containing 0.3% of HCl, one part was dissolved in about 30 c.c. of 0.4% HCl, and the other in about 40 c.c. of 0.3% solution of sodium carbonate. Both solutions were

[1] *Neumeister*. Lehrbuch der Physiologischen Chemie. S. 178.

[2] *Oppenheimer*. Fermente und ihre Wirkungen. S. 100.

[3] Ibid. S. 113.

used for experiments as in the former case. To test the digestion of fat, olive oil was used. For this purpose a little soda solution was added to the olive oil, and after the mixture was well shaken it was dissolved in ether. The ethereal solution was separated, washed with water, and the ether left to spontaneous evaporation. The neutral olive oil thus obtained was mixed with some alkaline enzyme-solution in a small glass tube, a little azolithmin solution being added to indicate the change of the reaction of the solution. After 8 days the results observed were as follows :—

Solutions.	Material used.	Temperature.	Results.
0.4% HCl.	fibrin.	36° C.	not dissolved.
Acid solution of the enzymes.	,,	,,	,,
,,	,,	room-temperature.	,,
,,	starch.	,,	not changed.
0.3% solution of sodium carbonate.	fibrin.	36° C.	not dissolved.
Alkaline solution of the enzymes.	,,	,,	dissolved.
,,	,,	room-temperature.	,,
	starch.	36° C.	starch disappeared.
,,	fat.	,,	acid set free.

Gelatine was liquefied on bringing it in contact with some of the alkaline solution of the enzymes in the presence of some thymol. Also freshly precipitated casein was easily dissolved by it.

From the results of the experiments above mentioned, we may conclude

I. That in the stomach of silk-worms *tryptic*, *diastatic* and *lipatic* enzymes are present, but that *pepsin* and *pepsinogen* are quite absent.

II. That the digestion of fat is effected by decomposition into fatty acids, as in the case of *Vertebrata*.

The third experiment was made to ascertain the presence of digestive enzymes in the intestine of insects. 3 grs. of the intestines of silk-worms, which had been carefully separated from the stomach and freed from their contents by washing in water and then crushed in a mortar together with glass powder, were digested with glycerin as in the former case. After three days the mixture was further treated as above. One of the portions was dissolved in about 20 c.c. of 0.4% HCl, and the other in 20 c.c. of 0.3% solution of sodium carbonate. With both of these solutions the experiment was performed as before, and after six days the results observed were as follows :—

Solutions.	Materials used.	Temperature.	Results.
Acid solution of the enzymes.	fibrin.	30° C.	not dissolved.
,,	,,	room-temperature.	,,
,,	starch.	30° C.	not changed.
,,	,,	room-temperature.	,,
Alkaline solution of the enzymes.	fibrin.	30° C.	dissolved.
,,	,,	room-temperature.	,,
,,	starch.	30° C.	not changed.
,,	,,	room-temperature.	,,
,,	fat.	30° C.	acid was not set free.

From these results it may be concluded, that there exists a difference between the enzyme production of the intestines and that of the stomach, no diastase and lipase being present in the former.

Another experiment was made with the intestines of *Caligula japonica* Moor. 6 grs. of the intestines were washed in water, crushed in a mortar together with glass powder, and digested with 40% alcohol. After seven days it was filtered, and to the filtrate ether-alcohol (1 part of ether and 3 parts of alcohol) was added. The precipitate collected on a filter was exposed to the air for a short time to evaporate the alcohol, and then divided into two parts. One part was dissolved in 20 c.c. of 0.1% HCl, and the other in 20 c.c. of 0.3% solution of sodium carbonate. The tests were made as in the former experiments, and the mixture kept in a thermostat for six days. The results observed were as follows:—

Solutions.	Materials used.	Results.
0.1% HCl.	coagulated egg-albumen.	not corroded.
Acid solution of the enzymes.	,,	,,
,,	fibrin.	not dissolved.
,,	starch.	not changed.
0.3% solution of sodium carbonate.	coagulated egg-albumen.	not corroded.
Alkaline solution of the enzymes.	,,	corroded and biuret reaction produced.
,,	fibrin.	dissolved and biuret reaction produced.
,,	starch.	not changed.
,,	fat.	acid was not set free.

We may therefore infer, that there exist enzymes in the intestines which act upon albumin, but that amylolytic and lipatic enzymes are completely absent. *Plateau's* opinion that there are no enzymes in the intestines, seems to be erroneous.

As regards the products of digestion I have made tests with the enzyme precipitates obtained from the stomach of the silk-worm and of *Caligula japonica*. These precipitates were dissolved in a 1% solution of sodium carbonate (cryst). Some fresh egg-albumen served for the test with the enzyme from the silk-worm (*a*), while coagulated albumen was used with that from *Caligula japonica* (*b*). After the addition of some crystals of thymol the mixtures were kept at 36° C. for eighteen days. Putrefaction had been successfully prevented by the presence of the thymol. There was still some unchanged albumen present, as the test with nitric acid showed. The undigested albumen was removed from (*a*) by lead acetate according to *Hammersten's* method,[1] and after freeing the filtrate from lead by H_2S, it was neutralized with NH_3 and evaporated. The residue thus obtained was extracted with boiling alcohol, but no crystals of leucin or tyrosin could be observed on slow evaporation of this extract. The part insoluble in alcohol gave no indication of tyrosin crystals on slow evaporation of its solution; it consisted of a syrupy mass that behaved towards the usual reagents in every respect like *peptone*. The test for tryptophan was, like that for leucin and tyrosin, ineffectual; a solution of chloride of lime failed to give the purple-red color.[2] Tryptophan is a characteristic product of the tryptic digestion and according to *Neumeister* the formation of leucin and tyrosin can directly be inferred, when the reaction for tryptophan gives a positive result.

We observe therefore that the end-products of digestion were peptones, but no further decomposition products. Hence, the proteolytic enzyme of the stomach of *Lepidoptera* is different from the pepsin as well as from the trypsin of *Vertebrata*; it agrees with the pepsin in so far as it produces as end-products of digestion *peptones*, but differs from it as to the reaction of the active solution; and on the other hand, while it agrees with the trypsin

[1] Physio. Chem. S. 175.

[2] The same results were obtained from (*b*) after it had stood seven days at 36° C.

as to the alkaline reaction of the active solution, it differs from it in so far as it does not produce any amido-acids and tryptophan, since the action stops with the production of peptones.

Similar tests with starch solution were made to determine the end-products of the action of the diastase of *Lepidoptera*. After six days at 36° C, the mixtures still contained some starch. After evaporation to dryness, and extracting the residue with water, a solution was obtained that gave a *red* reaction with iodine, showing the presence of *erythrodextrin*. The chief sugar formed was *maltose*, to judge from the properties of the osazone obtained. To decide whether traces of dextrose were present some *Saccharomyces apiculatus* Reess, which has the peculiarity of fermenting dextrose only, but not maltose, was added to the solution in a fermenting tube. After standing for a few days, the evolution of some carbon dioxide was observed, which shows that some dextrose must have been present. There was probably present also some maltase-like enzyme, that further transformed the maltose produced by the diastase, into dextrose.

Hence, the following conclusions may be drawn.

I. All the enzymes secreted in the digestive canal of *Lepidoptera* act in an alkaline solution, the action ceasing completely in an acid solution.

II. The proteolytic enzyme of *Lepidoptera* decomposes albumen into peptones but does not further decompose peptone into leucin and tyrosin. Therefore, this enzyme resembles trypsin only in the reaction of its active solution, while it resembles *pepsin* in regard to the end-product of digestion.

III. The amylolytic enzyme liquefies starch, forming dextrine and maltose. Possibly there is also present some maltase-like enzyme.

IV. The lipatic enzyme of *Lepidoptera* like that of *Vertebrata* decomposes fat into fatty acids.

V. While in the stomach these enzymes are present; in the *intestines* proper only proteolytic enzyme is found, the two other enzymes being absent.

VI. Though the expanded part of the intestine of *Lepidoptera* is commonly called the stomach, its physiological

function resembles rather that of the intestines of *Vertebrata*. *Krukenberg's* view that in *Invertebrata* there is no part of the intestines that is comparable with the stomach of *Vertebrata*, is true at least for *Lepidoptera*, since there exists no genuine acid gastric juice in them.

On the Occurrence of Cane Sugar in the Seeds of Gingko biloba and Camellia theifera.

BY

U. Suzuki.

Recent investigations have shown that cane sugar occurs very frequently in the vegetable kingdom, having been observed not only in seeds but also in stems, roots and leaves. It not only plays an important rôle as reserve material during the germination process, but it is also the principal form in which starch is transported, and therefore germinating shoots may contain it, even when the original seeds do not. In the following lines I shall describe its occurrence in the resting seed.

I. Seeds of *Gingko biloba.*

The hard shells of the seeds of the Gingko were removed and the content dried and finely powdered—630 grams—was at first freed from fat and then repeatedly extracted with hot alcohol of 90%. This extract yielded according to the method of *E. Schulze* for the isolation of cane sugar, nearly 10 grams of crystals which on minute examination proved to be identical with cane sugar.

The aqueous solution gave a strong red coloration with resorcin and hydrochloric acid; reduced *Fehling's* solution, not directly but after inversion, became brown and finally black by the action of concentrated sulphuric acid; and the optical rotatory power calculated from observation on *Soleil-Ventzkes* saccharimeter was found to be $[\alpha]_D = + 66.5.^\circ$

A quantitative determination showed that the seeds of *Gingko* contained nearly 6% of soluble sugars of which $\frac{5}{6}$ reduce *Fehling's* solution only after inversion. Further tests left no doubt that there were present also other sugars in small quantities, which have less rotatory power and are soluble in alcohol with greater difficulty than cane sugar.

II. Seeds of *Camellia theifera*.

The seeds were at first deprived of their shells. 600 grams of these dried and powdered seeds were, after extraction with petroleum ether, extracted with hot 90% alcohol. The alcoholic extract yielded on cooling a yellowish white precipitate which was removed by filtration. The filtrate was now evaporated to a syrupy consistency and then warmed with the addition of just as much 90% alcohol as was necessary to dissolve the whole mass. After standing a few hours, crystals of cane sugar were abundantly produced, which were recrystallized several times from 90% alcohol. Thus nearly 30 grams, or 5% of the dry matter of the seeds, were obtained.

As far as my knowledge goes, this is the first time that such a high percentage of cane sugar has been found in seeds. Thus *E. Schulze* found in those of:

Oats	¼ gram from 3 kilo	Earth nut	8.0 gram from 1 kilo.
Rye	0.15 „ „	Yellow Lupine	4.0 „ 1.5 „
Buckwheat	3.5 „ „	Sun flower	0.7 „ 500 gram.
		Coffee	2.5 „ 250 „

A high percentage, however, is also found in pollen, viz. 11–14% in that of the pine and of the hazel nut (*Planta*).

On the Formation of Asparagin in the Metabolism of Shoots.

BY

U. Suzuki.

It is a fact that while the various amido-compounds formed from protein during the germination process disappear gradually with the further development of the shoots, the asparagin increases to a certain phase whereupon also this disappears. The more carbohydrate is present in seeds, the quicker will the asparagin disappear in the following period. But with seeds rich in protein the gradual transformations of the nitrogen compounds can be much better traced; the accumulation of asparagin is more considerable, and it is here that the sugar is finally formed by assimilation in the young leaves which causes the consumption of the asparagin in the process of protein formation.

We owe to Prof. *E. Schulze* and his students many very valuable investigations in this line. But thus far it is not entirely clear how the primary amido-compounds such as leucin, tyrosin, arginin, etc. formed by the action of a proteolytic enzyme upon the reserve protein of the seed, are gradually transformed into asparagin. It seems to me that most probably the primary amido-compounds are destroyed more or less completely by an oxidation process, and that the nitrogen liberated thereby as ammonia serves for the synthesis of asparagin, since ammonia would act noxiously by its accumulation.[1] Besides, as I proved some years ago, ammonia offered to the roots in larger doses than is needed for the immediate protein formation, is transformed into asparagin and is stored up as such

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[1] Compare also the theory of protein formation of *O. Loew*, in Chapt. 8 of: Die Chemische Energie der Lebenden Zellen.

for further use.[1] The carbon for the asparagin is furnished either by the products of partial oxidation of the amido-compounds or by sugar.

Now if this view is correct, then the partial destruction of the primary amido-compounds, and hence also the formation of asparagin, would be stopped when air is withheld and oxidation is prevented. I therefore tested the behaviour of shoots in the absence of oxidation, which deprivation they can endure for a certain time. Such an experiment had already been made by *Palladin*,[2] who observed that in the absence of oxygen asparagin formation stops, which fact he, however, tried to explain in a manner quite different from that which we can accept.[3] *Clausen* repeated these experiments with less decisive results.[4]

My experiments, however, with barley and soy bean shoots confirm the observation of *Palladin*, and I infer therefore that *asparagin must be considered as a synthetic product formed with the aid of an oxidation process*, after partial destruction of the primary amido-compounds. In my experiment, the etiolated shoots were kept in an atomosphere free of oxygen [5] for 45—52 hours at a temperature of 15—20°.

I then compared the amount of ammonia and of asparagin with that of the control plants. In my first experiment with barley I had withheld the oxygen for too long a time and some shoots had died off; hence this experiment with barley shoots had to be repeated.

Experiment with barley.

Etiolated shoots of barley of an average length of 24 cm. were carefully deprived of their endosperm [6] and divided into three portions :

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[1] Bull. College of Agr., Imp. Univ. Tokyo. Vol. II. No. 7. (1897).

[2] Ber. D. Bost. Ges., vol. 6. (1888).

[3] He believed that the protein yields asparagin by direct oxydation.

[4] Landw. Jahrbücher, Vol. 19.

[5] The oxygen was removed by caustic soda and pyrogallol in the usual way. By measuring the decrease of the volume of the air in the bell jar, the test was made for the complete absorption of the oxygen.

[6] The presence of the endosperm would have rendered the result much less decisive. It was to be assumed from analogy that the young plant had absorbed a sufficient amount of the primary amido-compounds, formed previously in the endosperm from protein, to assure a proper answer to our question.

(*a*) Original, dried at the beginning of the experiment. Number of shoots 250, average length 24 cm., dry weight= 5.4126 g.

(*b*) Control. Kept 45 hours in distilled water in darkness Number of shoots 250, average length 24 cm., dry weight= 5.1355 g.

(*c*) Test shoots. Kept 45 hours in darkness, deprived of oxygen. Number of shoots 250, average length 24 cm., dry weight=4.678 g.

The analysis gave the following results :

	In 100 parts of dry matter			For 100 parts of total nitrogen		
	Original.	Control.	Test shoots.	Original.	Control.	Test shoots.
Total nitrogen	5.00	4.90	5.45	100.0	100.0	100.0
Albuminoid nitrogen ...	2.46	1.99	2.27	49.20	40.61	41.65
Asparagin nitrogen ...	**1.40**	**1.88**	**1.52**	**27.96**	**38.12**	**27.90**
Ammonia nitrogen ...	0.20	0.17	0.22	4.00	3.55	4.04
Amido nitrogen†...	**0.94**	**0.86**	**1.44**	**18.84**	**17.72**	**26.42**

	Asparagin.	
	In 100 parts of dry matter	Every 100 shoots contain
Control	8.85	0.1818
In absence of air...	7.16	0.1336
Original	6.59	0.1427

[1] The shoots were nearly dead, but the roots were still fresh.

† Amido-nitrogen means here nitrogen in the form of the primary amido-compounds formed from protein.

Experiment with the Soy Bean.

Since an experiment with shoots of the soy bean had convinced me that very small shoots of 9—10 cm. height do not give a decisive answer, larger shoots were selected for the chief test.

The shoots had an average length of 18 cm.

(*a*) Original, dried at the beginning of the experiment. Number of shoots 51, dry weight=5.197 g.

(*b*) Control. Kept 52 hours in distilled water in darkness. Number of shoots 51, dry weight=4.804 g.

(*c*) Test shoots. Kept 52 hours in darkness, deprived of oxygen. Number of shoots 50, dry weight=4.592 g.

Analysis :

	In 100 parts of dry matter			For 100 parts of total nitrogen		
	Original.	Control.	Test shoots.	Original.	Control.	Test shoots.
Total nitrogen	9.69	9.83	10.39	100.0	100.0	100.0
Protein nitrogen ...	2.49	2.44	2.50	25.70	24.93	24.07
Asparagin nitrogen ...	**5.80**	**6.20**	**5.90**	**59.70**	**62.95**	**57.41**
Ammonia nitrogen ...	0.23	0.26	0.31	2.35	2.61	3.01
Amido-nitrogen ...	**1.17**	**0.93**	**1.68**	**12.25**	**9.51**	**15.51**

	Asparagin.	
	In 100 parts of dry matter	Every 100 shoots contain
Control..... ...	29.20	2.751 g.
In absence of oxygen ...	27.79	2.552 g.

In addition we place here the amount of the primary amido-compounds in the presence, and in the absence of oxygen, showing that their decrease in the presence of oxygen is intimately connected with the increase of the asparagin.

Nitrogen in form of the primary amido-compounds.

	Barley.		Soy bean.	
	In 100 parts of dry matter	In 100 parts of total nitrogen	In 100 parts of dry matter	In 100 parts of total nitrogen
With oxygen	0.86	17.72	0.93	9.51
Without oxygen.....	1.44	26.42	1.68	15.51

The following conclusion may be drawn from these results :

In etiolated shoots, the decomposition of protein continues in the absence of oxygen as well as in its presence. This is in accordance with what we know of the action of enzymes. The shoots had remained apparently healthy for 45—52 hours when deprived of oxygen. Only some shoots had lost turgor, but these were quite free from any bacterial attack. The roots were in contact with but little water and retained their normal appearance. A very decisive difference in the production of *asparagin* was however noticeable :

No increase in the absence of oxygen, but an increase in its presence.

Just the reverse is observed with the primary amido-compounds, which decrease by the production of asparagin from them.

		Analytical Data.											
		Total nitrogen			Protein nitrogen			Asparagin nitrogen			Ammonia nitrogen		
		Dry matter used.	BaO replaced.	N. found.	Dry matter used.	BaO replaced.	N. found.	Dry matter used.	BaO replaced.	N. found.	Dry matter used.	BaO replaced.	N. found.
Barley Shoots.	(1)	0.465	9.1	0.02325	0.930	9.2	0.02288	0.465	1.3	0.00325	0.465	0.37	0.00093
		„	9.5		„	9.1		„	1.3		„	0.37	
	(2)	0.461	9.1	0.02263	0.922	7.4	0.01838	0.461	1.7	0.00431	0.461	0.37	0.00080
		„	9.0		„	7.3		„	1.75		„	0.27	
	(3)	0.465	10.2	0.02534	0.930	8.7	0.02106	0.465	1.4	0.00353	0.465	0.37	0.000988
		„	10.15		„	8.15		„	1.45		„	0.42	

		Analytical Data.											
		Total nitrogen			Protein nitrogen			Asparagin nitrogen			Ammonia nitrogen		
		Dry matter used.	BaO replaced.	N. found.	Dry matter used.	BaO replaced.	N. found.	Dry matter used.	BaO replaced.	N. found.	Dry matter used.	BaO replaced.	N. found.
Soy bean shoots.	(1)	0.460	17.8	0.04457	0.920	9.6	0.02291	0.460	5.3	0.01332	0.460	0.42	0.00105
		„	18.05		„	8.8		„	5.4		„	„	
	(2)	0.456	18.0	0.04482	0.912	9.0	0.02229	0.456	5.6	0.01407	0.456	0.47	0.01175
		„	17.9		„	8.9		„	5.7		„	0.48	
	(3)	0.453	19.3	0.04706	0.906	8.9	0.02266	0.453	5.4	0.01345	0.453	0.57	0.001425
		„	18.5		„	9.3		„	5.45		„	0.59	

(1) Original shoots. (2) Control shoots. (3) Shoots in absence of oxygen.
10 c.c. $H_2SO_4 = 0.303$ g $BaSO_4$. 10 c.c. $H_2SO_4 = 14.6$ c.c. $Ba(OH)_2$.
1 c.c. $Ba(OH)_2 = \mathbf{0.00249}$ g. Nitrogen.

For the determination of ammonia in the shoots, the air dried, fine powder was well mixed with some water and then put in a flask, 100 c.c. water and 1 c.c. of 1% MgO suspension was added, and then subjected to distillation, the escaping ammonia was absorbed by standard sulphuric acid and titrated as usual. The control test with pure asparagin solution shows that no noticeable amount of ammonia is developed by the above treatment. It therefore can safely be assumed that the developed ammonia is not derived from a decomposition of asparagin (or other amido-compounds) in the shoots, but that it was present as such in the shoots.

Asparagin nitrogen was determined by *Sacchsse's* method.

The Composition of the Nuts of *Gingko biloba*.

BY

U. Suzuki.

The analysis of the nuts of Gingko biloba, a tree of frequent occurrence in Japan, is naturally of some interest since they are extensively consumed as food.

Freed from the shells, the nuts showed the following composition ;

In 100 parts of dry matter,

Crude protein	11.3
Crude fat	2.6
Lecithin [1]	0.17
Crude fibre	1.2
Ash (free from carbon and insoluble matter)	3.0
Starch	62.4
Cane sugar	5.2
Reducing sugar	1.1
Total nitrogen	1.8
Albuminoid nitrogen	1.4
Nuclein and other non-digestible nitrogen	0.26
Non albuminoid nitrogen (mostly basic)	0.4

In 100 parts of pure ash (including CO_2).

K_2O	47.3
Na_2O	6.0
CaO	2.7
MgO	6.3
$Fe_2O_3 + Al_2O_3$	1.7
P_2O_5	26.4
SO_3	6.0
SiO_2 (Soluble)	0.5
Cl. Mn_3O_4 etc.	Trace
Total =	95.9

[1] Cholesterin was apparently wholly absent.

The Gingko seeds are exceedingly rich in starch, amounting nearly to 63% of the dry matter. Microscopical examination showed that the starch is oval and sometimes round, much resembling potato starch, but distinguished from it by striation and fissures.

Observations on the Mulberry Dwarf Troubles (Schrumpfkrankheit), a Disease Widely Spread in Japan.

BY

U. Suzuki.

In a former No. of these Bulletins (vol. IV., No. 4) I reported on the relations of the oxidising enzymes in healthy and diseased mulberry leaves. In that article I mentioned however only observations on leaves gathered in the late autumn, while here I will report on analogous observations with leaves gathered in the spring and summer.

Besides the usual tests with guaiac resin, I also made tests with guaiacol and hydrogen peroxid, whereby a red coloration is produced. Various vegetable objects yield this reaction, which I have also observed with mulberry leaves. Animal objects give this reaction also, which is of special interest in the case of the saliva, since this often fails to give any other oxidase reaction, and probably contains a special oxidase. This can however not be taken for granted in the case of plants, where the blue reaction for peroxidase with guaiac tincture and hydrogen peroxid shows a striking parallelism to the red reaction with guaiacol and hydrogen peroxid. Thus far I have not succeeded in finding any vegetable object that yields one of these two reactions alone.

I have made numerous colorimetric comparisons with healthy and diseased mulberry leaves in the manner described in my former article (l. c.), in regard to oxidase, peroxidase and the guaiacol reaction, further comparative tests in regard to catalase, 0.5 g. of the fresh leaves serving for the test with 10 c.c. neutralized hydrogen peroxid of nearly 2%. The volume of oxygen developed in twenty-five minutes, shaking the mixture before the final reading, ranged from 7 to 42 cc.

As the general result of these comparisons the following inferences can be drawn:

1) Oxidase and peroxidase are present in larger quantities in the diseased leaves, which generally yielded a reaction 3-5 times more intense than the healthy ones. Only a few exceptions were observed with leaves collected at the beginning of May. No exceptions, however, were found with the leaves of the new shoots developed after cutting. Such leaves exhibited a very pale and yellowish appearance, probably due to the action of the larger amount of oxidizing enzymes on the chlorophyll.

As I have already shown, the diseased leaves of the new shoots coming after cutting in the growing season, are always poorer in nitrogenous compounds, compared with those leaves gathered before cutting or in the late autumn. We may therefore infer that the production of oxidases has a very intimate relation to the deficiency of nutrients, especially of nitrogenous compounds in the plant cells. Further the fact that the diseased plants very often recover when they are left without cutting, naturally leads us to suppose a close relation of oxdizing enzymes with the disease.

2) Peroxidase is always much more prevalent than oxidase in the diseased leaves.

3) As to catalase no safe conclusion can be drawn, although in the majority of cases it seemed to be present in slightly greater quantities in the diseased leaves.

On the Influence of Different Ratios of Lime and Magnesia upon the Development of Plants.

BY

K. Asō.

The physiological functions of magnesia and lime have been discussed repeatedly by *O. Loew*,[1] whose theory explains on the one hand why a certain amount of magnesia in the soil can act injuriously on the crops, and on the other why the liming counteracts this injurious effect ; and also further why a certain excess of lime has no longer any beneficial effect, but even retards development and decreases the yield. In the following lines I describe some further experiments which illustrate the unfavorable effect of a certain excess of magnesia as well as of lime, and show that for certain crops there exists a well defined ratio between these two bases that is more favorable to growth than any other ratio. I will at first describe some experiments made with calcium and magnesium nitrate alone, in the absence of other nutrients.

I. Action of Magnesium and Calcium Nitrate.

Seeds of barley and wheat were soaked for three days in solutions of 0.5% of calcium and magnesium nitrate respectively, and after germination two of each kind of the young shoots of 6–7 cm. length were placed in 1% solutions of calcium nitrate and 1% magnesium nitrate. Two other shoots of each kind were kept in distilled water for control (Dec. 21.). The temperature of the room ranged during the following eighteen days from 7° to 14°.

[1] Flora, 1892 ; Landw. Versuchs-Stationen, vol. 41. Bull. No. 18. U. S. Department of Agriculture, Division of Vegetable Physiology and Pathology, Washington, 1899. pp. 35; 42; 47; 60. Die Chemische Energie der lebenden Zellen, München 1899. Chapt. IV.

On January 8th., the result was as follows:

Solution.	Barley.		Wheat.	
	Length of longest leaf.	Length of longest root.	Length of longest leaf.	Length of longest root.
Calcium nitrate ...	10.0 cm.	6.0 cm.	12.5 cm.	4.0 cm.
	10.5 „	5.0 „	12.2 „	4.6 „
Magnesium nitrate ...	7.8 cm.	3.0 cm.	8.0 cm.	3.0 cm.
	7.9 „	3.0 „	7.6 „	3.0 „
Control	8.8 cm.	7 cm.	12.5 cm.	5.8 cm.
	10.0 „	6.6 „	12.5 „	14.2 „

It follows from these measurements that the development in calcium nitrate did not differ much from that in distilled water. Evidently, the magnesia content stored up in the seeds sufficed to counteract the retarding influence of the lime salt of the solution, while in the case of the magnesia plants the poisonous effect of the magnesium salt of the solution became very evident, and would doubtless have been much earlier felt, if the lime of the reserve stores had not partially counteracted it; the leaflets in this case did not increase in number and became yellowish, while the plants in distilled water and in calcium nitrate, showed new leaflets, and in the *case of calcium nitrate also a deep green color in all the leaves;* some yellowing had however also taken place with the control plants in distilled water.

An examination of the roots in magnesium nitrate (Jan. 8.) showed that the cells were dead. Not only were all the cells soon deeply colored in a highly diluted aqueous rosanilin solution, but they also failed to show plasmolysis in a solution of 10% potassium nitrate. Another very noticeable phenomenon was the very profuse production of *large roothairs in the calcium nitrate solution;* these could not be found either in the case of the magnesium plants or of those in distilled water.

II. Influence of Varying Mixtures of Lime and Magnesia salts upon Wheat in the Absence of Other Mineral Nutrients.

In these experiments mixtures of calcium and magnesium nitrate were prepared in such proportions that the following ratios between lime and magnesia were obtained:

	CaO		MgO
a.	0.3	:	1
b.	0.6	:	1
c.	1	:	1
d.	2	:	1
e.	3	:	1
f.	Control in distilled water.		

The concentration of the culture solutions was 0.2%. In 100 c.c. of these solutions were placed wheat shoots on November 15th. It was soon noticed that in the solutions (c) and (d), the plants were developed much more vigorously than in (a), (b) and (e), and in the control case with distilled water. In (c) and (d), the leaves were longest and the branches most numerous.

On November 30th, a photograph was taken, (see plate XLII.) Although this experiment was now terminated, it had sufficiently demonstrated the great influence of different ratios of lime and magnesia. Further development would of course soon have ceased, since the solution applied contained neither sulphates nor phosphates nor potassa. The results obtained are seen in the following table:

	Length of the longest leaf.		Number of leaves.	Number of branches.
	Nov. 15.	Nov. 30.		
	cm.	cm.		
a	16.5	17.5	5	2
b	18.0	20.0	6	2
c	18.5	21.0	11	3
d	17.5	19.4	10	2
e	18.2	20.5	9	2
Control	18.3	18.5	4	1

This proves that the ratio CaO : MgO as 1 : 1 (c) was the most favorable for young wheat plants.

III. Cultures of Barley in the Presence of all Mineral Nutrients.

The standard solution contained :

KH_2PO_4	0.1%
Na_2SO_4	0.1%
$FeSO_4$	trace

The amounts of lime and magnesia varied here in the same manner as in the case just described ; they were also added in form of the nitrates, and the ratios were also designated here as in the former case by a, b, c, d, e.

But while in the former case with wheat the experiment lasted only 15 days and the culture solution was incomplete, this experiment lasted 90 days and the culture solution was complete.

On January 21st, two barley shoots were placed in each of these solutions ; on February 27th, March 18th, and April 1st, the solutions were renewed. On March 22nd, the tips of the main leaves in (a) had dried up ; gradually these leaves also became yellow and withered. The proportion in the seeds is on the average, CaO : MgO as 1 : 3.3. It might naturally be expected that the influence of the mineral matter that had been

stored up in the seeds would decrease with the age of the plants and that therefore the best ratio between lime and magnesia for the later age might differ from that for the young state of the plants.

Since a fungus made its appearance on the leaves, the experiment was terminated on April 11th. The average length of the branches was :

a.	17 cm.
b.	19.6 ,,
c.	22.2 ,,
d.	26.1 ,,
e.	24.1 ,,

The thickness of the branches differed considerably, hence also the weight ; the greatest thickness, as shown in the photograph, was observed in (c) ; the next, in (d) ; five of the thickest branches from each case were weighed together with the roots of the whole plants ; the result was :

a.	11.0 grm.
b.	26.0 ,,
c.	32.5 ,,
d.	32.0 ,,
e.	28.0 ,,

The branches in (d) and (c), that is with the proportions $CaO : MgO$, 2 : 1 and 1 : 1 were the heaviest.

Since the water lost by evaporation, can, other things being equal, be considered as relative in amount to the total surface of the leaves, a record was kept of the water used in replenishing the evaporated solutions. The quantity of water lost by transpiration from March 6th, up to April 8th, was :

a.	456 cc.
b.	1196 ,,
c.	1362 ,,
d.	1564 ,,
e.	1389 ,,

The largest surface of leaves was therefore developed in (d), that is, where the proportion of CaO : MgO was as 2 : 1.

The same proportion is obtained from numerous analyses when the average of the lime and magnesia content of barley straw is taken.

Cultures of Soy-beans in the Presence of all Mineral Nutrients.

On March 15th, shoots of the soy-bean 7—11 cm. high, were placed (two shoots in each case) in the same solutions as had served in the experiment with barley just described. Five days afterwards the cotyledons were removed [1] in order to exclude the further influence of the reserve mineral matter.

After four weeks a decisive difference in the development was observed which became now more marked day by day, and by May 7th, most of the leaves in (*a*), (*b*), and (*c*), showed a sickly appearance. In (*a*) the uppermost leaves had turned white, retaining some green only along the chief veins while in (*b*) and (*c*), the normal green had changed to a yellowish color. In regard however not only to the color, but also to the size, the balance was much in favor of the solutions (*d*) and (*e*).

During the first two weeks, the plants in (*b*) and (*c*) had made a better showing than those in the other solutions, but gradually as the further development of new leaves required more lime the balance changed in favor of the solutions (*d*) and (*e*). On April 29th, the solutions were renewed and on May 13th, a photograph was taken, which is reproduced on plate XLIV. This shows the differences in development very plainly.

A very unwelcome feature, was noticed in the first traces of a parasitic fungus on some leaves in (*b*) and (*c*), but this trouble set in when the experiment had already decided the question at issue. At this time the pale color of the leaves in (*a*), had already clearly proved the imperfect formation of the chloroplasts under the influence of an excess of magnesium nitrate.

On May 28th, the plants were weighed in the fresh state after the water adhering to the roots had been removed with filter paper, and the total length of the stems was measured. The rather slow development of these shoots even in the most

[1] The average weight of one pair of cotyledons was, in the fresh state=0.796 grm.; dried at 100°=0.131 grm.

favorable solution was due to the early removal of the organic foodstores of the cotyledons. The weights and measurements are shown in the following table :

	CaO : MgO	Total length of two shoots.		Total weight of two shoots.	Remarks.
		March 15.	May 28.		
a	0.3 : 1	18 cm.	55.0 cm.	6.6 grm.	The two plants in b were discarded since the fungus attack had, by May 28, spread considerably over the leaves.
c	1 : 1	17 „	50.5 „	7.7 „	
d	2 : 1	18 „	60.5 „	9.9 „	
e	3 : 1	18 „	62.3 „	10.3 „	

It can be inferred from this, that a good development of soy-beans is only secured, when the amount of lime exceeds that of magnesia from two to three fold, and that a diminished development becomes at once evident when the amount of lime sinks to an equal with the amount of magnesia.

V. Culture of Shoots of Onion-plants in the Presence of all Mineral Nutrients.

Shoots of onions were, on March 29th, put in the solutions mentioned in III., and IV.

How the different ratios of lime and magnesia gradually influenced the development of new branches is seen from the following table, in which only the number of the still living branches is given.

	March 29	April 26	May 7	May 13	May 21	May 28	Remark.
a	2	3	3	3	3	3	
b	2	4	4	5	5	5	
c	2	4	5	5	6	6	
d	2	4	5	5	6	6	
e	2	3	4	4	5	4	Branches were thinner than in d and c.

The accompanying illustration (Plate XLV.) is from a photograph taken on May 15th. At that time, in a and b, one branch had completely withered.

On May 28th, the plants were weighed in the fresh state, and the branches measured :

	CaO : MgO	Number of branches.		Total length of all branches combined.		Weight of each plant.
		March 29.	May 28.	March 29.	May 28.	May 28.
a	0.3 : 1	2	3	33.7 cm.	88.2 cm.	3.3 grm.
b	0.6 : 1	2	5	33.2 „	139.7 „	5.0 „
c	1 : 1	2	6	26.3 „	150.2 „	6.5 „
d	2 : 1	2	6	27.3 „	150.1 „	6.6 „
e	3 : 1	2	4	27.7 „	140.5 „	5.3 „

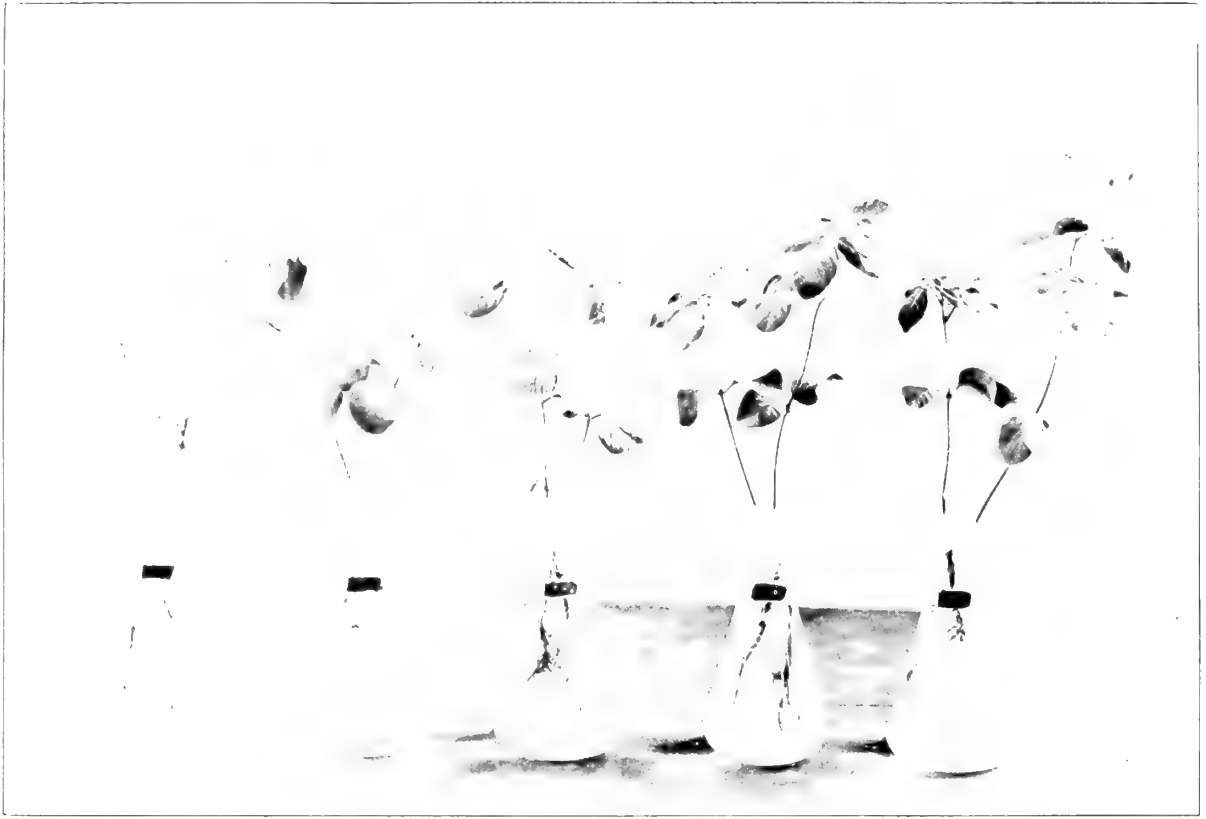
It will be observed, that the proportions between lime and magnesia, as 2 : 1 and 1 : 1 were much more favorable to the onion plant than the other ratios applied, which is in accord with the above mentioned results obtained with wheat and barley. An excess of magnesia depresses the yield more than an excess of lime, as a comparison of (c) with (a) and (e) plainly shows.

I also intend to make further studies of this kind with rice. It is an opinion widely held in Japan that the liming of paddy-fields is very injurious to the rice crop, and experiments by *Kellner* with slacked lime gave a decrease of protein in the grains. But we must here take into consideration that the slacked lime in such a soil rich in water remained long in the caustic condition and liberated ammonia from the salts which in a certain concentration is very injurious to the roots. Experiments with finely divided calcium carbonate might yield a different result when applied in very moderate quantities.

Gypsum might yield in such cases an increase of harvest only where there exists an excess of magnesia in the soil and a want of sulphates. As a general rule, however, paddy-fields which have been for hundreds of years under cultivation and have annually received manure in the form of excrements may

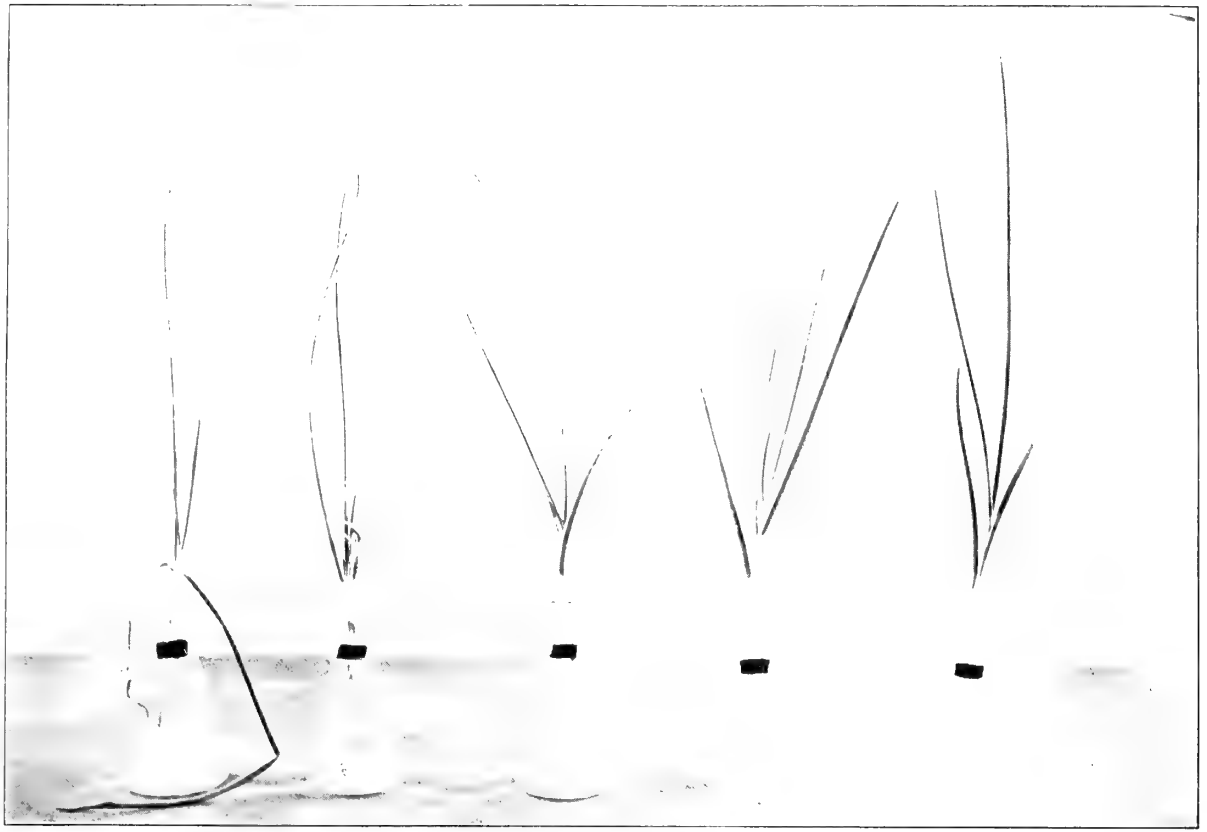
This plate shows the influence of different ratios of lime and magnesia upon wheat in absence of other mineral nutrients. See p. 363.

This plate shows the influence of different ratios of lime and magnesia upon barley in presence of all necessary mineral nutrients. See p. 365.



a *b* *c* *d* *e*

This plate shows the influence of different ratios of lime and magnesia upon soy beans in presence of all necessary mineral nutrients. See p. 366.



a *b* *c* *d* *e*

This plate shows the influence of different ratios of lime and magnesia upon onion plants in presence of all mineral nutrients. See p. 368.

$\frac{MgO}{CaO}$	2 / 1	1 / 1	1 / 2	1 / 3

$\frac{MgO}{CaO}$	1/3	1/2	1/1	2/1

MgO	2	1	1	1
CaO	1	1	2	3

be supposed to rarely require an addition of lime. Soils in Japan contain frequently more magnesia than lime, as the following table shows. Liming therefore is in all these cases in order.

In 100 parts of air-dry soil.
(Soluble in strong HCl.)

Locality	CaO	MgO
Asamiya (Omi)	0.13	0.62
Nihonmatsu (Iwashiro)	0.44	1.12
Yamashirodani (Awa)	0.24	0.57

In 100 parts of dry soil.
(Soluble in strong HCl.)

	CaO	MgO
Field soil near Tokyo	0.76	1.70
Udogori (Suruga)	0.48	1.38
Naritamachi (Shimosa)	1.73	3.33
Chitagori (Owari)	0.08	0.11

General Conclusions.

In comparing the results obtained with wheat, barley and onions, with those obtained with soy-beans, it will be noticed that the excess of lime over magnesia must be larger with the latter plant than with the others mentioned in order to produce the best results. This difference is evidently due to the greater leaf-surface developed in a given time by the soy-bean, every increase of leaf-surface requiring, above all, an increase in lime.

It is evidently of the greatest practical importance that more attention than heretofore should be paid to the proper ratio between lime and magnesia in the soil, [1] and also to fertilizing according to the laws of development in regard to lime and magnesia and not mainly according to the need of potassa, phosphoric acid and nitrogen. For the liming of a soil not merely the absolute quantity of lime found in it, but in a still higher degree the ratio between lime and magnesia in the soil must form the principal guide.

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[1] Especially to the ratio of lime and magnesia in the available forms.

Finally it may be pointed out that the poisonous action of magnesia in the absence of lime was again confirmed as well as the *great influence of lime on the production of roothairs*, as previously observed by *Loew*.

To what Extent should a Soil be Limed?

BY

T. Furuta.

The application of lime in agriculture can aim at two effects: 1. to improve the physical and chemical condition of the *soil;* 2. to provide the *plants* with a sufficient amount of lime which is for them an indispensable nutrient. It is the latter point alone which comes under discussion in this paper. Of course, liming will always be in order when a soil is exceedingly poor in lime, but this is not the only condition which necessitates liming for the nutrition of the plants. It is also in order in all cases where the soils contain an excess of magnesia over lime.[1] But to what extent will liming be necessary in such a case? The amount of lime to be incorporated with a soil to any given depth is a very important item since an excessive liming will depress the yield. The first requirement for a calculation is of course an analysis of the soil.

An ordinary soil analysis however will only show the amounts of lime and magnesia as a whole, but will not convey a proper idea of the extent to which these bases are easily available for the plants, since the roots can merely attack the surface of large particles of hydrous silicates, carbonates and phosphates, while very small particles can be wholly dissolved and absorbed by them. In order therefore to determine the extent of liming necessary for the next crop,[2] only the *assimilable* amounts of lime and magnesia need to be determined and these amounts, alone ought to be taken as a foundation.

Some consider 1000 kilo. of carbonate of lime per hectare a

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[1] Cf. O. Loew: On the physiological rôle of mineral nutrients, U. S. Dep't of Agriculture, Washington 1899.

[2] Sometimes even in limestone regions fields may be benefitted by liming. This is the case when the carbonate of lime has been washed out from the surface of the fields, or when the limestones contain also magnesia (dolomitic limestone).

small dose ; others have observed a considerable diminution of the yield on the application of 4000–5000 kilo. of carbonate of lime per hectare, especially with legumes.

Various methods have been employed in estimating the amount of lime necessary for the liming of the soil, but in many instances no attempt at all has been made to reach an approximate calculation, the amount of lime applied to a given extent of the field surface having been in fact arbitrary. Of course a reliable test in regard to the required dose of lime can be made from crops growing on the soil, limed in various degrees, but this empirical method would be very inconvenient and time consuming. In cases where merely the acidity of the soil has to be corrected with lime, the determination of the amount of lime to be applied would be simple. But such cases are exceptional and relate merely to soil improvement.

Recently an article was published by *D. Meyer*[1] on the lime compounds of the soil and the determination of the assimilable amount of lime. He found among other things that with the so-called light soils the solubility of the lime and magnesia in dilute acid was less than with heavy soils, and that the finest particles of the soil contained relatively more lime than the coarser particles. He further observed that the most favorable lime compound was the carbonate and the slaked lime, which latter yields carbonate in a finely divided condition.[2] It had a favorable action even in cases in which gypsum had proved unfavorable. He asserts that the assimilable lime can be extracted by digestion of the soil with 10% solution of ammonium chlorid for three hours at 100°C.[3] Thus the lime can be directly determined in the filtrate without the previous separation of the silica. The principal value of this method is according to the author that the lime content thus determined shows a much better correspondence with the yields of the harvest, relative to the quantities of lime which the crop could extract from the soil. He further concluded that a lime content of 0.25% in the soil can

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[1] Landwirthschaftliche Jahrbücher Vol 29, p. 914 (1900).

[2] Others, however, have observed that in certain cases gypsum is the most favorable form.

[3] *E. Hotter* proposes to determine the amount of assimilable lime by extraction with acetic acid of 20%. Z. landw. Vers. Oesterreich 1901.

still be considered as normal, and that liming becomes decidedly necessary when the assimilable lime content sinks below 0.20%.

Various authors place the minimum limit of lime in the soil for good returns at 1%. Others state that clay soils with less than 0.5% of lime are very much benefitted by liming. *Hilgard* considers the minimum percentage for the normal crop, 0.1% of lime in sandy soils, and 0.3% of lime in heavy clay soils.

D. Meyer did not take into consideration the necessity of liming in cases in which the *magnesia content of the soil was larger than the lime content. This is, however, a very important point.*

In order to furnish an illustration of the *importance of the ratio between the lime and the magnesia* content of the soil, the following experiments were made:

From the soil of a field that had not been manured for four years, all particles smaller than 0.25 mm. were separated in the air dry state by a sieve. The amount of fine earth thus obtained was 43.94%. The amounts of easily assimilable lime, magnesia and phosphoric acid in this fine earth were determined by treatment with hot concentrated hydrochloric acid. The analysis gave the following data:

Hygroscopic water	17.39%.
Humus	11.40 ,,
Lime	0.48 ,,
Magnesia	0.44 ,,
Phosphoric acid	0.16 ,,

Meyer's method for determining the amount of assimilable lime applied upon the same fine earth, yielded less than one-fourth of that obtained by the determination with hydrochloric acid. As regards the determination of magnesia by the application of *Meyer's* method, ammonium magnesium phosphate was obtained but in such small traces that it could not be weighed.

On the basis of the above result I prepared from the soil[1] three mixtures, in which the ratio of assimilable lime and magnesia[2] was as follows:—

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[1] This soil contained 0.27% potassa, as former analyses had shown.

[2] I had intended to apply powdered magnesite for increasing the amount of magnesia necessary for Pot No. IV., but unfortunately I could not procure that preparation and had to use the precipitated product of commerce, which consists of exceedingly fine particles, too fine for a critical comparison.

	CaO	:	MgO	
No. I.	3	:	1	
No. II.	2	:	1	
No. III.	1	:	1	(Original soil.)
No. IV.	1	:	2	

On April 3rd., sixteen porcelain pots received each 8 kilo. of our analysed air dry soil mixed with 16 gr. of commercial superphosphate, 10 gr. of potassium carbonate, and 16 gr. of sodium nitrate.

In order to reach the above mentioned ratios of assimilable lime and magnesia :

CaO : MgO

Pot No. I. (3 : 1) received 42.6 gr. quick lime,
Pot No. II. (2 : 1) received 32.5 gr. quick lime,
Pot No. III. (1 : 1) no further addition,
Pot No. IV. (1 : 1) received 89.5 gr. magnesium carbonate.

The pots were placed in the green house, and served now for cultures of cabbage, buckwheat and oats.

Experiment with Cabbage.

On April 20th., four pots received ten cabbage seeds each, the germination proceeded with different degrees of rapidity, as will be seen in the following table :—

Cabbage.	Number of young plants appearing.			
	CaO:MgO No. I. (3 : 1)	CaO:MgO No. II. (2 : 1)	CaO:MgO No. III. (1 : 1)	CaO:MgO No. IV. (1 : 2)
April 24.	1.	1.	2.	0.
„ 25.	2.	2.	5.	1.
„ 26.	2.	3.	6.	1.
„ 29.	2.	4.	6.	1.

Since some of the seeds failed to germinate, a number of young plants taken from the field served on May 3rd. to increase in each pot the number of shoots to seven. On May 30th., a

photograph was taken, see Plate XLV. which shows that the development of pot No. II., was the best, next came No. III., while that of No. IV. was exceedingly poor.

On June 18th., the plants were cut and weighed in the fresh state with the following results :—

Cabbage.	Rate of CaO : MgO.	Total weight of seven plants.	Weight of the largest plant.	Average weight.
No. I.	3 : 1.	375 gr.	130 gr.	54 gr.
No. II.	2 : 1.	475 gr.	140 gr.	68 gr.
No. III.	1 : 1.	390 gr.	100 gr.	56 gr.
No. IV.	1 : 2.	75 gr.	45 gr.	11 gr.

It follows therefore that for cabbage the ratio of 2 : 1 between the assimilable lime and the magnesia present in Pot No. II., was far superior to the other ratios.

Experiment with Buckwheat.

On April 10th., buckwheat was sown, there being two series of four pots each in eight of the pots, each pot receiving fifteen seeds. On May 3rd., the number of equally well developed shoots in both series, A and B, was reduced to eight. On May 5th., the flowers began to develop, but with different degrees of rapidity as is seen from the following table :—

Buckwheat.	Number of plants bearing flowers.			
	Pot No. I. CaO : MgO 3 : 1	Pot No. II. CaO : MgO 2 : 1	Pot No. III. CaO : MgO 1 : 1	Pot No. IV. CaO : MgO 1 : 2
May 9.	A 1 B 0	A 2 B 1	A 1 B 0	A 0 B 0
„ 11.	A 2 B 3	A 5 B 4	A 1 B 2	A 1 B 2

On May 12th., flowers were open on all the plants.

It will be seen that the flowers in Pot No. II. opened sooner than those in the other three pots.

On May 15th. a photograph was taken (see Plate XLVI.) which shows that pot No. I. had the most leaves, and that No. II. showed the greatest height of plants, while No. IV. was backward in development.

On May 30th., the seeds approached their ripening stage. On June 12th., white spots of a fungus appeared on several leaves, especially on leaves of No. II. A. On June 19th. the plants in the series A of these pots were cut and weighed with the following results :

Buckwheat.	No. I.	No. II.	No. III.	No. IV.
(8 plants in each pot.)	CaO : MgO 3 : 1	CaO : MgO 2 : 1	CaO : MgO 1 : 1	CaO : MgO 1 : 2
Total weight.	382 gr.	220 gr.	190 gr.	106 gr.
Average weight of one plant.	35.3 gr.	27.5 gr.	23.8 gr.	13.3 gr.
Average height.	72 cm.	68.3 cm.	57.4 gr.	48.6 cm.
Number of leaves.	199.	155.	181.[1]	118.
Number of the bundles of seeds. ripened.	112.	96.	75.	62.
Number of the bundles of seeds. unripened.	18.	10.	64.	19.
Weight of seeds. ripened.	55. gr.	45. gr.	34. gr.	20. gr.
Weight of seeds. unripened.	5.5 gr.	5.5 gr.	14.5 gr.	5.5 gr.
Weight of 100 ripened seeds.	4.53 gr.	4.35 gr.	3.92 gr.	3.86 gr.

Conclusion :—On glancing over this table it will at once be seen, that the yield from the pot No. I., was in every respect the richest. Tolerably close follows the yield of pot No. II., but with pot No. III., a very considerable falling off will be noticed

[1] Pot No. III., was rich in leaves, but many of these leaves were very small.

and still greater is the decrease from pot No. III., to No. IV. There can therefore be no doubt that the ratio of 3 parts of CaO to 1 part of MgO in the soil is the most favorable for buckwheat. A further increase of lime, to judge from observations with other crops, would in all probability depress the yield.

The second series, B, of buckwheat plants, (8 plants in each pot) was cut on June 28th, with the following results :

Buckwheat.	No. I. CaO : MgO 3 : 1	No. II. CaO : MgO 2 : 1	No. III. CaO : MgO 1 : 1	No. IV. CaO : MgO 1 : 2
Total weight.	340 gr.	275. gr.	281. gr.	240. gr.
Average weight.	42.5 gr.	34.4 gr.	35.1 gr.	30.0 gr.
Average height.	75.1 cm.	67.1 cm.	67.3 cm.	56.3 cm.
Number of leaves.	267.	182.	226.	173.
Weight of the seeds. { ripened.	64.5 gr.	58.0 gr.	58.5 gr.	56.0 gr.
Weight of the seeds. { unripened.	6.5 gr.	11.0 gr.	8.5 gr.	8.0 gr.
Weight of 100 ripened seeds.	4.31 gr.	5.70 gr.	4.12½ gr.	4.64 gr.

We observe from this comparison that also here the richest yield as to straw and seeds was obtained with the ratio 3 CaO to 1 MgO while on the other hand the single seeds were best formed with the ratio 2 parts CaO : 1 part MgO, since 100 seeds weighed here considerably more than in the other cases.

Experiment with Oats.

On April 10th, four pots were sown with oats, each receiving 15 seeds. The rate of germination was about equal in the four pots. On April 28th, the young plants were thinned out to eleven in each pot. On May 12th, it was noticed that in pot No. IV., some leaves were withering and becoming brown, which phenomenon had made considerable advance on May 30th.

On June 8th, buds began to appear on the plants except in No. IV., where they appeared later, as will be seen from the following table;

Oats.	Number of stalks bearing flowers.			
	No. I.	No. II.	No. III.	No. IV.
June 10.	8.	8.	4.	0.
„ 12.	10.	8.	7.	0.
„ 14.	12.	10.	10.	1.
„ 15.	13.	13.	12.	4.
„ 17.	18.	21.	21.	10.

On June 10th, black *Aphides* appeared on the leaves and stalks of the plants except on those of No. IV. On June 17th, a photograph was taken which is reproduced in plate No. XLVIII. At the same time the length of the tallest stalks was measured with the following result:

No. I.	140 cm.
No. II.	145 cm.
No. III.	145 cm.
No. IV.	108 cm.

The plants in pot No. II., were richest in leaves and stalks; then followed those in No. III., while those in No. IV., showed a rather poor development.

The results obtained with oats differed from the results obtained with buckwheat. The oats had ripened on July 22nd, and yielded on harvesting the following data:

Ratio of $\frac{CaO}{MgO}$.

	No. I. 3 : 1	No. II. 2 : 1	No. III. 1 : 1	No. IV. 1 : 2
Average height	113 cm.	116 cm.	120 cm.	91 cm.
Total weight	260 g.	280 g.	297 g.	219 g.
Fresh weight of grains with husk. ...	46 g.	51 g.	50 g.	15 g.
Fresh weight of straw...	214 g.	229 g.	247 g.	203 g.

As a general result we observe that No. I., and No. IV., gave a smaller yield than No. II., and No. III. An increase of lime beyond the proportion of $\frac{2\ CaO}{1\ MgO}$ brought on a moderate decrease, while an increase of magnesia in No. IV., caused a considerable decrease in the yield.

The most favorable ratios $\frac{CaO}{MgO}$ in my experiments were therefore :

Buckwheat = 3 : 1
Cabbage = 2 : 1
Oats = 1 : 1

In turning back now to our original question :

" To what extent should a soil be limed ? ", we would have to answer : *After the amount of easily assimilable lime and magnesia has been determined in the way above indicated by me, the ratio should be corrected by adding lime to such an extent that it becomes 3 : 1 to a given depth, when crops rich in foliage are to be grown, while the ratio 1 : 1 has to be prepared when oats and similar cereals are to be grown.*

On the Lime-factor for Different Crops.

BY

O. Loew.

Remarks on the foregoing communications of Mr. Aso *and Mr.* Furuta.

On reviewing the results of Mr. *Aso* and Mr. *Furuta* the fact that the greatest yield of a certain crop depends—other things being equal—upon a distinct ratio between lime and magnesia cannot be denied. Mr. *Aso* has operated with water cultures and restricted his observations to the period before flowering and fruiting, while Mr. *Furuta* has operated with soil-cultures and directed his observations to the ripened harvest. On comparing *Aso's* results with barley with *Furuta's* results with oats, the conclusion may be drawn that cereals before the fruiting stage require more lime relatively to magnesia than in the fruiting stage. This does not surprise us, since on the one hand the formation of the leaves requires much lime, while on the other hand the formation of grains, much magnesia. It is a fact that the grains of cereals contain more magnesiumphosphate than calciumphosphate.

The greater the leaf surface to be developed in a given time the greater will be the amount of lime required, hence we observe also in the above experiments that soy beans (*Aso*) and buckwheat (*Furuta*) require more lime relatively to magnesia than oats (*Furuta*). The best ratio of $\frac{CaO}{MgO}$ is according to

these experiments [1] for oats (ripened harvest) 1 : 1
for barley, before flowering, 2 : 1
cabbage 2 : 1
buckwheat 3 : 1

If we take the amount of magnesia as the unit we may call the relative amount of lime present the *limefactor* and express the result thus: The limefactor for buckwheat is 3; for cabbage, 2; for oats 1.

If we find the limefactor in a soil 1, the soil does not need liming for a crop of oats, but will need it when buckwheat or onions are to be grown. The beneficial influence of the rotation of crops is partly due to the variations of the limefactor in the soil caused by the growth of different crops.

Since the limefactor of cereals before flowering is larger than later on after the flowering stage, great benefit might accrue from the application of a highly diluted solution of calcium nitrate to the young plants. The limefactor for various crops may be calculated from the average of many ash analyses; and thus we find the limefactor by calculation from *Wolff's* tables of ash analyses, for

Wheat in the flowering stage.... 1.4
Clover ,, ,, ,, ,, .. 3.2
Lettuce 2.8
Sugar cane..... 1.05

But it is safer to determine the limefactor by actual test, since the analysed plants may have taken up more lime than necessary. Many plants will render this surplus partly or wholly innocuous by precipitating it as oxalate. Should plants, however, take up more magnesia than is required from a soil rich in magnesia but poor in lime, the injury cannot be repaired, the harvest will be very unsatisfactory. Such a soil is devoid of natural fertility, even if it be rich in potassa, phosphoric acid and nitrogen compounds.

One of the beneficial effects of liming is believed to consist in, "unlocking such potassa, as is at the time unavailable for

[1] With onions in water cultures the results were best with the ratio 2 : 1, but the results with the ratio 1 : 1 were nearly the same. These experiments have to be repeated with soilcultures as must also those with soybeans, since Aso's tests relate only to a very early stage. These figures relate as usual in agricultural relations to absolute quantities, not to the number of molecules.

plants"; but it may be objected that such potassa as can be unlocked in the soil by slaked lime or carbonate of lime, can surely also be unlocked by the rootlets themselves. The writer showed years ago that *one of the effects of increasing the lime consists in the rich development of roothairs* [1] and this explains satisfactorily the fact that on liming the soil, the plants become capable of absorbing an increased amount of potassa. A second very important physiological effect of lime is the rich production of dark green and normal chlorophyllbodies.

The retarding effect of an abnormal excess of lime over magnesia in the soil, or of an excessive liming, may be overcome by an application of powdered magnesite. Burnt magnesia or precipitated carbonate of magnesia should be avoided, being too finely divided and hence much more easily absorbed than the lime compounds.

It was in the year 1892 that I first called attention to the importance of a proper ratio between lime and magnesia in the soils, but nobody took notice of my deductions. I had said [2]: "Aus unseren Studien ergibt sich also, dass, ein so notwendiger Bestandteil der Pflanzennahrung auch Magnesia salze sind, sie doch bei gewissem Ueberschuss schädlich wirken, wie kein anderes Nährsalz. Ist zu viel Magnesia im Verhältniss zum Kalk vorhanden, so ist eine pathologische Wurzelentwickelung oder baldiger Tod der Wurzeln die Folge; ist aber zu wenig vorhanden, so wird die Entwickelung der Pflanzen verzögert. Dort treten Gift-, hier Hungersymptome auf. Es wäre nun von Interesse, festzustellen, welches Minimum von Magnesia im Boden bei gegebenem Kalkgehalt noch eine annehmbare Ernte zulässt, und andererseits wie viel Kalk bei gegebener Magnesia menge nöthig ist, pathologische Erscheinungen zu verhindern."

It was Mr. *May* in Washington D.C., who at my suggestion first took this problem up last year and who obtained very decisive results.[3] Among other things he found that in certain cases gypsum can better overcome the injurious action of an excess of magnesia in the soil than carbonate of lime can.

The writer's theory of the physiological functions of lime and magnesia was first published in Flora (1892) and later on

[1] Flora 1892, p. 384.

[2] Landw. Versuchsstationen, vol. 41, p. 474.

[3] Bulletin No. 1 of the Bureau of Plant Industry, Washington, 1901.

in English in Bulletin No. 18 of the Division of Vegetable Physiology and Pathology, U. S. Department of Agriculture 1899, under the title: The Physiological Rôle of Mineral Nutrients.[1] From this a few lines containing the main points may be extracted. The lime is according to this theory necessary for the formation of certain calcium compounds of nucleoproteids required in the organized structures of nuclei and chlorophyllbodies, while the magnesia serves for the assimilation of phosphoric acid, since magnesium phosphate can give up its phosphoric acid more easily than any other phosphate that occurs in plant juices. While calcium is *fixed* in the organized structure, magnesium is *movable*, since one and the same atom can in the form of secondary phosphate serve repeatedly the same purpose as a carrier of assimilable phosphoric acid in the formation of nucleoproteids and lecithin.

It follows therefore that in the case of an excess of lime being absorbed, the assimilation of phosphoric acid will be rendered more difficult, since this acid will then chiefly combine with the lime whereby the chances for the formation of magnesium phosphate will be diminished. The effect will then be the same as if the amount of available phosphoric acid in the soil were lessened, i. e., the growth of the plant will be retarded and even starvation phenomena will set in. The effect of this excess of lime will be still more marked with the decrease of the phosphoric acid present.

If on the other hand an excess of magnesia is entering the cells, the calcium nuclein compounds of the organized structures can not be formed or when previously formed, will be changed into the respective magnesium compounds, which are not suited for the same function, perhaps on account of a very different capacity of imbibition.

Hence nuclei and chlorophyllbodies will first suffer from the excess of magnesia, and this can be traced under the microscope on filaments of *Spirogyra*. Even in a 0,1 per cent. solution of magnesium nitrate these cells will die within five days, while on the addition of 0,3 per cent. calcium nitrate they will remain alive for a number of weeks, although on account of the absence

[1] Comp. especially pp. 28; 37; 42; 47; 60. and Bul. No. 1. Bureau of Plant Industry. That Bul. will be sent free of charge to any one who applies to the U. S. Dept. of Agriculture, Washington, D. C.

of other mineral nutrients the multiplication of cells will cease. The functions of lime and magnesia are intimately connected with each other, since the nuclei require lime and phosphoric acid as separate constituents. These inferences also hold good for the animal organism and the law inferred by the writer,—*the greater the nucler mass of an organ the greater is also its lime content* has been confirmed in a number of cases.[1]

[1] Cf the above cited Bul. No. 18, p. 57.

On the Lime Content of Phanerogamic Parasites.

BY

K. Asō.

Of phanerogamic parasites, only one, *Cuscuta europæa*, has thus far been examined with regard to the composition of the ash. When compared with other phanerogamic plants, the striking fact was revealed that this ash is exceedingly poor in lime, containing only about 2%; while its host, the clover, is rich in lime, yielding an ash containing about 30—36%. The fact that parasites being devoid of chlorophyll require less lime than green plants, is of much interest. Seedlings require less lime as long as they have no chlorophyll. Further, etiolated leaves of *Vicia faba* contain less lime than the green leaves.[1] According to Church,[2] also less lime (and more potassa) is present in albino leaves than in normal ones.

These facts agree well with the inference that not only the nuclei but also the chlorophyll-bodies, require lime. I had therefore believed it of interest to investigate in this respect another phanerogamic plant without chlorophyll, and selected *Gastrodia elata* Bl., an orchid.

This plant is characteristic of Asia and is not found in Europe. It occurs frequently on the main island of Japan.[3] The stemlike, brownish peduncle which has some scales comes forth from the ground in the spring and reaches 60—70 cm. in height; in June appear some yellowish brown flowers which yield capsules with numerous very small seeds. The underground part consists chiefly of a rhizome covered with scales

[1] Palladin, Ber. d. Deut. Bot. Ges., Vol. X., p. 179.

[2] Jour. Chem. Soc., 1878 and 1886.

[3] Near *Nikko* it is very frequent.

and is very rich in starch.[1] Whether this plant is parasitic or saprophytic is not yet quite decided.[2]

I collected the plant in the beginning of June and separated the rhizomes from the other parts.[3] The ash of each part was separately analyzed with the following result :

In 100 parts of dry matter.

Above-ground part	5.25 %
Under-ground part	3.94 %

In 100 parts of dry matter.

	Above-ground part.	Under-ground part.
SO_3	0.196	0.073
P_2O_5	1.500	0.679
K_2O	2.323	2.009
Na_2O	0.379	0.541
CaO	0.384	0.200
MgO	0.367	0.251
Fe_2O_3	0.095	0.102

In 100 parts of ash.

	Above-ground part.	Under-ground part.
SO_3	3.73	1.85
P_2O_5	28.57	17.24
K_2O	44.25	50.99
Na_2O	7.22	13.73
CaO	7.31	5.08
MgO	6.99	6.37
Fe_2O_3	1.81	2.59
SiO_2 and loss	0.12	2.15

When we compare these results, with the ash constituents of green plants, a striking fact is noticed in regard to the ratio of lime and magnesia. In the chlorophyll-bearing parts, the content of magnesia is always surpassed by that of lime; for instance, with cereals in the blossoming stage the ratio of CaO : MgO is 2 : 1 ; in lucerne before flowering 8 : 1 ; and in rape before flowering 5.5 : 1. *Church* compared the ash of albino leaves with that of green leaves of *Quercus rubra* and found in regard to lime and magnesia the following data :

[1] After fruiting, the plant dies having used up all the nutrients in the rhizome.

[2] According to Mr. *Shibata*, botanist in the College of Science, Imperial University, Tokio, this plant is probably a saprophyte, because no one has observed any connection between its rhizome and the root of other plants.

[3] I found only traces of calcium oxalate in this plant.

	Albino leaves.	Green leaves.
CaO	8.25 %	24.50 %
MgO	6.52 %	9.55 %

In the above-ground part of *Gastrodia* I found the ratio to be nearly 1 : 1.

This fact indicates very clearly again that the chlorophyll-content determines the ratio between lime and magnesia in the leaves. The more chlorophyll there is, the greater will be the proportion of lime in regard to magnesia.

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[1] In the root crops and tubers, the ratio $\frac{CaO}{MgO}$ is about 1 or less.

On the Amount of Soluble Albumin in Different Parts of Plants.

BY

H. Uno.

While the seeds of most plants have been repeatedly the subject of investigations in regard to their proteins,[1] the other parts have served but rarely for such studies.

It has long been known that the juices of plants on being heated, often yield a coagulation of albumin. This is, however, not due to the protoplasm present in all cells, since this is insoluble in water, but it is due to reserve albumin which may occur in the dissolved state not only in the cell sap but also in the juice of the protoplasm itself. This albumin plays the rôle of reserve material which may either be comsumed in the same cells by their further growth, or after transportation into other parts of the plants may be used for building up living matter in the flowers, fruit or roots.

It seemed to me that it would be of considerable interest to ascertain which parts of the plants contain most of this soluble reserve albumin [2] and I have therefore made a series of quantitative determinations of it in roots, leaves and flowers.

Of the subjects, in most cases, 50 grams fresh weight were crushed with the addition of some sand in a mortar, and pressed. The expressed residue was further washed with water, and expressed once more. The filtered juices thus obtained were heated, with the addition of some nitric acid, on the water bath nearly to the boiling point and the precipitate formed was collected on a weighed filter, dried at 100° C. and weighed.

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[1] Cf. especially the publications of *Ritthausen* and of *Osborne* and *Campbell*; further of *Bokorny*, *Pflüg. Arch.* 1900.

[2] The common, passive albumin alone is here considered. On the occurrence of the labile, active albumin, see these Bulletins vol., II., Nos. 1 ; 2 ; 4.

The results obtained are shown in the following table :

Plant.	Coagulated Albumin.				Stage.
	Roots.	Stems.	Leaves.	Flowers.	in which the flowers were collected.
Rape	0.0318%	0.0264%	0.5998%	0.2428%	Middle.
Radish (Japanese)	——	0.0296 „	0.4446 „	0.1780 „	Ending.
Radish (variety)	0.0776 „	0.0716 „	0.1760 „	0.1476 „	Beginning.
Cabbage.	0.0899 „	0.0940 „	0.6452 „	0.2952 „	Middle.
Linseed plant	0.2527 „	0.0294 „	0.1260 „	0.2182 „	Ending.
Barley.	0.1275 „	0.0124 „	0.1214 „	——	
Orchard grass	0.2300 „	0.0283 „	0.2516 „	——	
Buckwheat.	0.1150 „	0.0222 „	0.2182 „	0.2000 „	Middle.
Astragalus lotoides	0.1232 „	0.0112 „	0.3412 „	0.1534 „	Beginning.
Bean	0.3304 „	0.0444 „	0.2812 „	0.1049 „	Ending.
Pea (Japanese)	0.3310 „	0.0612 „	0.1924 „	0.1119 „	Ending.
Pea (French)	0.4000 „	0.0640 „	0.7362 „	0.0930 „	Ending.
Red clover (cultivated)	0.5884 „	0.0458 „	0.6228 „	0.0636 „	Middle.
Red clover (wild)	0.4220 „	0.1436 „	0.1052 „	0.0384 „	Middle.
Lathyrus palustris	0.5418 „	0.2742 „	0.6484 „	——	
Potato	0.1263 „	0.0316 „	0.0662 „	0.0812 „	Beginning.

It will be seen therefore, that in the majority of cases the leaves are richest in soluble albumin excepting those of the leguminous plants, which showed in three cases out of six more of it in the roots. Further the roots of the pea, bean and clover, Lathyrus palustris contain much more of it than those of other plants, an interesting fact, as this shows the protein forming influence of the *Rhizobium leguminosarum*, which exists in the form of nodules upon the roots ; these nodules had not been removed before pressing. A fact of interest is also the relatively high content of albumin in the roots of *Linum usitatissimum*, and the exceptionally low content in the leaves of that plant. The stalks generally contain less soluble albumin than the roots, flowers and leaves ; only with cabbage the stalk contained a little more than the root.

As to the influence of the flowering stage on the content of soluble albumin, a general conclusion can not yet be drawn.

Further investigations will determine the influence of different ages, different manuring and various other conditions upon the contents of soluble albumin in plants.

Note on the Enzymes of the Japanese Sake-yeast.

BY

T. Takahashi.

The occurrence, formation and action of enzymes form one of the most interesting questions in modern physiology and fermentation chemistry, especially since it has been shown that the agency of alcoholic fermentation is not the protoplasm of the living yeast cells, but an enzyme-like substance that can be pressed out from the contents of the cells. This justifies the hope that some day also other fermentations may be recognized as actions of enzyme-like compounds.

Among the enzymes found in various kinds of yeast the following are the most important.

1. Sucrase (Invertin).
2. Melibiase (Bau 1873; Dienert 1899).
3. Trehalase (A. Kalanthar 1898; Bau 1899).
4. Maltase (Fisher and Lindner 1895).
5. Trypsin (Hahn. 1898).
6. Zymase (E. Buchner 1896).
7. Catalase (O. Loew 1901).[1]

Since the occurrence of certain enzymes is characteristic of certain varieties and species of yeast, and since sake yeast[2] differs more or less in its physiological and morphological properties from common beer or wine yeast, I tested this yeast for various enzymes. I prepared from the so-called Moto-mash

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[1] No ordinary oxidase, but a trace of peroxidase occurs frequently in yeasts.

[2] This yeast is derived according to Ōmori from the conidia of a kind of *Ustilago*. Further researches on this subject are however required.

by means of the " Tröpfchen Kultur " of *Lindner* a considerable amount of that yeast in pure culture. From this I prepared an extract according to *Albert's* method.[1] The quantity of the yeast, which served for the preparation of the juice amounted to 52 grms. (in the air dry state), from which 35 c.c. of a yellow colored liquid were obtained. In testing for sucrase I mixed 5 c.c. of this juice with pure cane sugar at 49—54°C. After 37 minutes, inversion became evident by the action of the liquid upon *Fehling's* solution. After further standing at 12—18°C. the reducing power for *Fehlings* solution was very much increased.

I could not suceed in proving the presence of maltose in the above mentioned juice, but maltose is very well fermented by this yeast.

In testing for zymase *Einhorn's* fermenting tubes were employed. 10 c.c. of the pressed juice were mixed with 2 grms. of cane sugar, and the mixture kept partly at 30°C. and partly at 14—16°C. In the former case some few bubbles of CO_2 developed after thirty minutes, while in the latter case a still smaller quantity of gas developed within 2—3 hours. The yield of carbonic acid was smaller than was to be expected, but this was probably due to the fact that the juice applied was very dilute.

An experiment with the expressed juice of 250 g. of beer-yeast (Carlsberg No. II) yielded in the way mentioned a very satisfactory result.

A proteolytic ferment is also present in the juice of the sake yeast, though it is only a trace. Moreover the liquifying rate of the gelatin (prepared after the method of *Hahn*) of this juice is only about one half of that of the beer-yeast.

In testing for oxidase 20 grms. of air dry yeast were rubbed with glass powder and with the addition of some chloroform extracted with 50 c.c. of distilled water. In the filtrate a blue coloration of guaiac tincture was not obtained directly, but a faint one after the addition of some hydrogen peroxid.

In testing for catalase neutralized hydrogen peroxid (2%) was used and thus a lively development of oxygen was observed not only with the juice of the yeast, but also with the washed residue showing the presence of α- and β- catalase.

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[1] Ber. Deutsch. Chem. Ges. 1900.

The sake yeast contains therefore the following enzymes :

Sucrase.
Zymase.
Trypsin.
Catalase.
Peroxidase (trace).

On the Juice of the Pseudotrunk of *Musa Basjoo, Sieb.*, in Winter-time.

BY

S. Sawa.

The banana plants grown in central Japan lose their leaves during the cold months and there remains only the pseudotrunk,[1] the interior of which seems to be sufficiently protected against the freezing temperature of the cold nights by the dead part surrounding it. This inner portion is subjected to a period of rest for about four months. It seemed to me of some interest to ascertain whether during this time any noticeable decomposition of reserve protein matter took place, and for this purpose nearly 3 kilos served for the preparation of the juice. A portion of tissue was directly tested with a freshly prepared solution of ferrous sulphate, but only a weak tannin reaction was obtained and that only in the innermost parts. The expressed juice, however, did not yield any decisive reaction at all. Another portion of a fresh section was moistened with guaiacum tincture and a very marked blue reaction was observed, showing the presence of oxidase. A piece of the tissue was heated for a few minutes at 80°C. whereby the oxidase was killed, but the reaction of peroxidase was now obtained in a marked degree with a mixture of hydrogen peroxid and guaiacum tincture. As to catalase, a slow development of oxygen was observed when a piece of the tissue was placed in a diluted neutral solution of hydrogen peroxid, while the filtered juice gave no reaction; hence only *a*- catalase was present in a small degree.

In regard to the juice above mentioned I was surprised to find that it did not show any acid reaction at all, but that on the contrary the reaction was slightly alkaline. Phospho-

[1] This pseudotrunk consists mainly of a series of rolled up leaves.

tungstic acid in presence of nitric acid yielded only a moderate precipitate. On boiling a portion of the juice with nitric acid, some flocculent precipitate which became yellow, was obtained. Mercuric nitrate and basic lead acetate yielded moderate precipitates. On boiling with Fehling's solution, the presence of sugar was indicated.

50 cc. of the juice yielded 0.1355 gr. of ash equal to 0.27%. This ash showed an alkaline reaction, and consisted chiefly of carbonate and chloride of sodium.

10 cc. served for an estimation of the total dry matter which was found to be 0.927%.

The sugar determination gave 0.032% calculated as dextrose.

400 cc. of the filtered juice was mixed with basic acetate of lead until no further precipitate was obtained. This was filtered off and washed and decomposed with hydrogen sulphide. The filtrate of the lead sulphide yielded, on evaporation to a small volume, a crystalline mass; and after evaporation to dryness, the residue weighed 0.72 gr., or 0.18% of the original juice. As a portion of this mass developed, on heating slowly on a platinum foil, the suffocating fumes characteristic of succinic acid, the whole mass was now dissolved in a little water and shaken out several times with ether which left, on evaporation, crystals which agreed with the forms of succinic acid. Also the concentrated solution of the neutral sodium salt yielded with ferric chloride a brownish precipitate. Another portion served for a determination of the melting point which was found to be 179.5°C agreeing with that of succinic acid (180°C). However in the aqueous solution that had been shaken out with ether, there was still much organic matter present. On slow evaporation at the common temperature, prisms were obtained which, compared with crystals of succinic acid, were found to be identic. Hence most of the substance precipitated by basic acetate of lead consisted of succinic acid.

The filtrate from the lead precipitate above mentioned was precipitated with mercuric nitrate and the precipitate, after being washed, was decomposed with hydrogen sulphid. The filtrate from the mercuric sulphide was evaporated to a thin syrup with the addition of some ammonia to prevent any decomposition of asparagin supposed to be present, but further

careful examinations proved that neither asparagin, nor tyrosin, nor leucin, were present. *Hence in the period of rest, no decomposition of protein takes place in the juice of the plant.*

It is, however, of some interest that in the *absence of asparagin* the closely related succinic acid is present.

The juice of the pseudotrunk of Musa contained therefore :

Total dry matter —0.927%
Succinic acid———0.18 %
Sugar ——————0.03
Nitrogenous extract—0.44
Ash ——————0.27
Oxidase and Peroxidase.

On the Volatile Oil in the Wood of Cryptomeria Japonica.

BY

C. Kimoto.

One of the most common and most highly esteemed coniferous trees in Japan is Cryptomeria Japonica. The wood is durable and takes a good polish, and further it has an agreeable odor, somewhat suggesting that of peppermint, and on this account it is generally used as the material for sake casks. Thus the sake acquires a peculiarly agreeable although weak, aroma. In order to study the nature of this odoriferus principle I distilled about 2 kg of this wood in the form of small chips with water, and separated the oil rising to the surface of the distillate in the usual way by removing at first as much water as possible by means of a syphon, shaking with choroform and separating it again by distillation. Thus was obtained nearly 13 c.c. of an oily substance which had the characteristic smell of the original wood.

Since no trace of crystallization had manifested itself after several weeks I subjected the oil to a fractional distillation with the following result :

Fractions	boiling point	reaction	quantity
A.	150—180°c		very little
B.	180—185°c	acid	„
C.	185—200°c	„	„
D.	200—230°c	„	„
E.	230—235°c	„	„
F.	235—250°c	slightly acid	„
G.	250—260°c	acid	„
H.	260—270°c	neutral	4 g.
I.	270—275°c	„	3 g.
J.	275—280°c	„	3 g.
K.	280—300°c	———	very little
L.	Residue.	———	2.4 g.

Since the fraction H, which distilled between 260-270°c, and and most of it more exactly at 264,° as a second trial showed, exceeded all the other fractions in quantity and showed the very agreable odour of the wood in a high degree, I subjected it to an elementary analysis with the following result :

I. 0.1740 g. yielded 0.1940 g. H_2O, and 0.5376 g. CO_2 corresponding to 12.38% H, and 84.43 of C.

II. 0.2414 g. yielded 0.2453 g. H_2O, and 0.7542 CO_2 corresponding 11.3 % H, and 85.2 % C.

This would correspond tolerably well to the formula $C_{30}H_{48}O$.

	Theory	Experiment	
		I.	II.
C.....	84.9	84.43	85.2
H.	11.3	12.38	11.3
O..	3.7	4.0	3.5

The supposition might be entertained that the new compound is a camphor-like substance related to triterpene, or

to the cholesterins. Indeed the solution in chloroform produces on the addition of some concentrated sulphuric acid a dark red color.

I propose to call it *Sugiol*, from sugi, the Japanese name for *Cryptomeria Japonica*.

Sugiol is an oil of neutral reaction, almost completely insoluble in water, but easily soluble in alcohol, ether and chloroform. Its boiling point is 264°, spec. gravity 0.935. It yields no crystalline acetyl and hydrazon compound, but reduces alkaline silver solution very slowly in darkness.

On the Poisonous Action of Quinone.

BY

T. Furuta.

Numerous phenol derivatives have been tested for poisonous properties, but the ordinary quinone has been almost wholly neglected in this regard, although it is of some physiological interest. *Beijerinck* has recently shown that *Streptothrix chromogena*, a soil fungus, has the remarkable property of producing quinone from proteids.[1] This author is even inclined to ascribe the process of the formation of humus in the soil largely to the quinone produced by this fungus.

Quite recently *Phisalix* has observed a peculiar poison in the secretion of a certain myriapod which he and *Bèhal* afterwards identified as quinone.

Since quinone is a labile di-ketone it appeared to me of particular interest to ascertain whether it is a general poison, and to compare it with related compounds. As subjects, served shoots, twigs, isolated leaves, algae, mould fungi, bacteria, insects, tadpoles and mice.

Experiments with Shoots.

1. Young plants of the soy bean 12—14 cm. high were placed in 1% solutions of quinone, hydroquinone, resorcin, pyrogallol and phloroglucin. The roots in quinone showed first, after a few hours, a decided injury and discoloration and it took only four days to kill the entire shoots, while it took eight days with hydroquinone, seventeen with pyrogallol, and twenty with phloroglucin. The control plants were still alive. Phloroglucin is trioxybenzene, but it is considered by some authors as a tri-ketone. Nevertheless one would expect that such a tri-ketone would prove about as strong a poison as quinone.

[1] Central-Blatt f. Bakt. (II. Abt.) VI. 1900.

2. Wheat. Young wheat plants of 15 cm. length kept in the same solutions were first injured by quinone, then followed hydroquinone and the other compounds in the same order as observed with the soy shoots. A second experiment with 0.5 per mille solutions yielded essentially the same results.

Experiments with Twigs.

Twigs of plum trees of about 20 cm. length and bearing 20—22 flower buds were placed in the same solutions. In quinone the buds withered and gradually dropped off; not one had opened. In hydroquinone, only four buds opened within twenty-one days; the others had withered. In resorcin, two buds opened within eleven days; later on all the buds developed into flowers. In pyrogallol seventeen buds opened within twenty-one days. A second test with bud bearing peach twigs 15 cm. long yielded similar results.—Vapours of quinone seem to be much more poisonous than the aqueous solution.

A young cabbage branch placed in a flask covered with a glass plate, was exposed to the vapours which one gram of quinone developed from the bottom of the vessel at the ordinary temperature. This branch died within twenty-two hours; the turgor was lost and the normal color was changed. Young leaves of *Trifolium*, *Photinia*, and *Rhododendron* died under these conditions within twenty-five hours. Leaves with a thick cuticle, as those of *Camellia*, succumbed more slowly. Isolated leaves placed on a 1 per mille solution of quinone died within two days; while it took 4—5 days with hydroquinone, and ten days with resorcin and pyrogallol.

Experiments with radish seeds left no doubt, that the germinating power was greatly injured by three days soaking in a 1 per mille quinone solution. Of 50 seeds only 8 germinated, as compared with 25 in the control case.

Experiments with Algae.

Diatoms and filaments of *Mesocarpus* and *Spirogyra* were placed in 1 per mille solutions of quinone, and observed every thirty minutes under the microscope to ascertain whether the protoplasm was contracted and the contents of the cells would

stain quickly by highly diluted methylene blue. It was thus found that diatoms and the cells of *Mesocarpus* were killed by quinone in three hours and forty minutes; soon afterwards also *Spirogyra*. Towards algae hydroquinone also proved very poisonous; indeed with diatoms hydroquinone acted nearly as powerfully as quinone. With resorcin, pyrogallol, and phloroglucin, death was observed more than a day later.

Experiments with Mould Fungi and Bacteria.

Penicillium glaucum developed on beerwort to which 1 per mille quinone had been added, while *Aspergillus oryzae* did not.

Bacteria were inoculated in bouillon to which after sterilization 1 per mille quinone had been added. The bacillus of typhoid fever of mice could not develop in this solution, while *Bac. pyocyaneus* gave a meager growth on the surface.

Experiment with a Mouse :—

The vapour which quinone emits at the ordinary temperature exerts a highly poisonous action on warm blooded animals.

About $\frac{1}{2}$ gram of quinone was placed in a deep beaker glass ($1\frac{1}{2}$ liter capacity) and upon this at a height of 3 cm. above the quinone a wire gauze was placed. A small white mouse was then put in the beaker glass and this covered with a glass plate, which was often lifted to admit fresh air. The animal became at once very much agitated, tried to escape and soon gave evidence of irritation of the eyes and nose. After one hour all the motions became sluggish, and after two hours more the animal was dead.

Under the same conditions horse-flies died within three hours.

Experiments with Tadpoles.

Tadpoles were placed in the 0.1 per mille solutions of quinone, and the time required to kill the animals was :

(1) with quinone : 33 minutes.
(2) ,, hydroquinone : 58 ,,
(3) ,, pyrocatechin : 22 hours and 23 minutes.
(4) ,, pyrogallol : 31 ,, ,, 13 ,,
(5) ,, resorcin : 54 ,, ,, 53 ,,
(6) ,, phloroglucin and control (plain water) : they were still actively moving after six days.

The highly poisonous character of quinone became still more evident by the observation that even in 0.005 per mille solution it killed tadpoles within one hour and thirty-five minutes.

Other aquatic animals (*Asellus*, *Copepoda*) were killed by this highly diluted solution within eight hours.

Conclusion: We can infer from all these results that quinone is a very strong poison,—a much stronger poison indeed than the other closely related benzene derivatives. In the face of these facts does there exist any probable basis for the hypothesis of *Beijerinck*, that the relatively large amount of humus in the soil is due to the action of quinone? It seems to me that another hypothesis would be more natural than this namely, that the quinone produced by *Streptothrix chromogena* must be changed almost as soon as formed, for otherwise animal and vegetable life in the soil becomes impossible!

Are Coffeine and Antipyrin in High Dilutions Poisonous to Plants?

BY

S. Sawa.

Many poisons show a stimulating action of some kind or other when they are so highly diluted that any further injurious action can not take place. *Gamaleia* [1] has observed that coffeine exerts a stimulating action on the growth of yeast and certain bacteria. Since coffeine is trimethylxanthine and xanthine is a constituent of the nucleoproteids of the protoplasm, I thought it of interest to observe the action of coffeine in high dilution on phaenogams.

The first experiment was carried out with onion plants which were placed in a solution of 1 p. mille of coffeine and antipyrine with and without the addition of nutrients. It was observed that the plants remained alive for nearly four weeks. In both solutions some new branches started but these developed much better with antipyrine than with coffeine. Coffeine proved gradually to be a much more noxious compound than antipyrine, and the primary leaves withered much sooner in the former than in the latter case.

The development after twenty-three days in the presence of all mineral nutrients is seen in the following table giving the measurements of the still living parts.

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[1] Jahresbericht für Thierchemie, 1896 p. 923.

		Length of principal shoot at the beginning. cm.	Increase in length of all shoots combined.	
			absolute cm.	relative %
Antipyrin	A	26.0	3.0	11.5
	B	21.5	3.5	16.3
Coffeine	A	10.0	0	0
	B	21.5	0.2	0.9
Control	A	38.8	26.7	68 8
	B	40.5	26.5	65.4

In the second experiment the amount of these bases was reduced to 0.1% and 0.25%. This time young celery plants of about 15 cm. height served for the test. After about two weeks some injurious action was observed, commencing with the wilting of the rim of the leaves.

The injurious effect of coffeine in this high dilution appears very remarkable since the epidermis of the tea leaves is comparatively rich in it and nevertheless remains uninjured. The coffeine is here probably shut up in the vacuole surrounded by a tonoplast of such density that the cytoplasm and nuclei are sufficiently protected against the intrusion of the coffeine. A stimulating effect of some kind or other can only be expected in still higher dilutions of coffeine and antipyrine.

Has Urea any Poisonous Action on Phaenogams?

BY

S. Sawa.

Sometimes urine is applied as manure before its urea is completely split up by bacterial action into ammonia and carbonic acid. But while the ammonia can be absorbed in the soil and thus becomes only gradually accessible to the roots, a poisonous action by a too high concentration of ammonia being thus avoided,[1] the urea is not absorbed in the soil as *Kellner* has shown. It might be supposed, however, that urea could act injuriously and I have therefore made an experiment with young onion plants in order to test this point. Thus far it has been generally assumed that urea would be a good source of nitrogen for the phaenogams, but some objections may be raised against the tests thus far made.[2]

On April 22nd two plants were placed in *Knop's* solution with an addition of 0.5 p.m. urea and also two control plants without this addition. These plants were kept over five weeks, in a room near the window at 15—18°C. The solutions were twice renewed. A start towards the formation of flowers was made by each plant, but only in the one to which urea had been applied was a full flower developed, in the other three plants there being no further development.

Gradually new branches developed with the four plants, but much better with the control plants than with those in the urea solution; soon afterwards, however, the old leaves commenced to die off from the tip downward. Finally on the 30th of May, the parts still living were measured. The results may be seen in the following table:

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[1] On the poisonous action of ammonium carbonate in water culture, see Bul. of the Coll. of Agr., Tokyo, Vol. III., No. 3.

[2] Comp. *Hampe*, Landw. Versuchs. St. Vol. 10. Also *Thompson*, Centr. f. Agr. Chem. Aug. 1901; the urea solution being permitted to act for only 3—5 hours each day.

		On April 22. cm.	On May 30. cm.
Urea.	A	85.0	67.0 (decrease 18)
	B	81.0	52.0 („ 29)
Control.	A	68.0	127.0 (increase 59)
	B	69.0	127.0 („ 58)

The injurious action of urea even in the high dilution of 0.5 per mille is therefore quite evident. This recalls another observation of *Loew* and *Bokorny* on algae.[1] *Spirogyra* was much injured within five days in a solution of 0.2 per cent. urea, and microscopic observation showed that it was especially the chlorophyll bodies that were attacked.[2] Probably in these organoids, the urea is too readily split up into ammonia and carbonic acid, and the nascent ammonia killed the chlorophyll bodies. The fact that urea in such dilutions does not act poisonously on fungi and bacteria, and is even a good source of nitrogen for them, would be in accord with this explanation.

[1] Journ. f. prakt. Chemie, Vol. 36 (1887) p. 379.

[2] Thio-urea acted still more poisonously.

On the Poisonous Action of Potassium Persulphate on Plants.

BY

S. Sawa.

The salts of persulphuric acid exert a moderate oxidizing action on certain compounds. Thus *Morrell* and *Crofts* observed a slow action on glucose in the presence of ferrous sulphate whereby glucoson is produced.[1] *Hugounenq* observed an oxidation of uric acid, haematin, and bilirubin by these salts;[2] uric acid in alkaline solution yields thus allanturic acid. *Pröscher* found that while methyl green is quickly oxidized, other color bases are not attacked as e.g. methylen blue. He further observed that apomorphine is oxidized by it. The oxidizing character of these salts makes it very probable that they may act poisonously also on living cells in a way similar to the action of hydrogen peroxid. Indeed it was observed by *Wacker*,[3] and further by *Bérard* and *Nicolas*,[4] and finally by *Friedländer*,[5] that these salts easily kill bacteria. The last named author states that $\frac{1}{2}\%$ sodium persulphate in aqueous solution prevents the growth of pathogenic bacteria, and a 5% solution kills them; and further that one gram of sodium persulphate suffices to kill a rabbit of 2 kilo weight. *Nicolas*[6] observed that an intraveinous injection of 0.04 gram of sodium persulphate per kilo body weight kills a rabbit; and per os 0.3 gram per kilo, a guinea pig. *Loew* observed that infusoria died in a 0.5% solution of the potassium persulphate in about thirty minutes. I thought it of some interest to extend such observations also to chlorophyll-bearing plants.

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[1] Journ. Chem. Soc. 77, 1219. (1900).

[2] Chem. News, 1901.

[3] *Merck's* Report 1894, 35.

[4] Semaine Medicale 1899, No. 43, p. 342.

[5] Therep. Monatschefte 1899, Feb.

[6] La Semaine Médicale, Vol. 20 (1900).

For the experiment with algae, as *Spirogyra*, *Mesocarpus* and diatoms, a 0.5% solution of potassium persulphate was applied.[1] All these organisms died within one hour. Branches of the rape plant about 17 cm. long with numerous buds showed in the 0.5% solution of the same salt an injurious effect after twenty-four hours: the leaves became curled, the tips of the branches withered after three days and the lower part of the branches bleached out. One day later the plants had entirely died off. In the control cases with potassium sulphate and with distilled water, the branches had remained healthy and many of their buds had developed into flowers. The poisonous action of the potassium persulphate on chlorophyl-bearing plants is therefore plainly seen, but it is surprising to see that the poisonous action becomes very weak on a further dilution of the 0.5% solution to 0.1%. In such a solution plum branches as well as onion plants remained alive for a considerable time, although some injurious action could be noticed. Thus only a few buds of the plum branches developed within the next five days, while in the control case with distilled water all the buds had developed into flowers. After about ten days the branches exposed to the persulphate seemed to be entirely killed.

The onion plants remained alive considerably longer in the 0.1% solution; even after ten days only the tips of the leaves showed signs of withering. Further growth seemed to stop; while in the control cases with potassium sulphate and with distilled water alone it was not inconsiderable, as may be seen from the following table.

After the ten days of observation, all the necessary mineral nutrients were added in the form of *Knop's* solution, and while now the growth in the control cases was very marked, the plants in the persulphate were very backward. One of these plants showed a further drying up from the tip downward, while with the other only a very insignificant increase was noticed (see Table).

[1] In this case common well water was applied in preparing these solutions, to avoid the poisonous action of the very minute trace of copper sometimes contained in distilled water.

		Length of Plants.		Increase.		Length of Plants.	Increase.	
		At the beginning (March 16) individual leaves cm.	After 10 days (March 26) Summed up	Absolute cm.	Relative %	23 days after full nourishment (April 18) individual leaves cm.	Absolute cm.	Relative %
Potassium persulphate	A	o 20.5 o′ 16.9 (1) } 37.4	38.0	0.6	1.6	o 10.0 o′ dead } (4) n 23.6 } 41.6 n′ 8.0	3.6	9.5
	B	o 19.1 o′ 14.2 o″ 9.3 (2) } 42.6	42.0	−0.6	−1.4	o dead o′ 23.0 o″ dead } 36.5 n 13.5	−5.5	−13.1
Control — Potassium sulphate ..	A	o 24.4 o′ 10.9 } 35.3	45.4	10.1	28.6	o 17.0 o′ 34.2 n 26.5 } 87.2 n′ 9.5 (5)	41.8	92.1
	B	o 20.1 o′ 17.5 } 37.6	48.0	10.4	27.7	o dead o′ 30.6 n 41.5 } 80.6 n′ 8.5 (6)	32.6	67.1
Control — Water	A	o 14.9 (3) o′ 13.5 } 28.4	38.8	10.4	36.6	o dead o′ 30.0 } 65.5 n 35.5	26.7	68.8
	B	o 20.5 o′ 11.2 } 31.7	40.5	8.8	27.8	o dead o′ 30.0 } 67.0 n 37.0	26.5	65.4

Foot notes :—

(1) 1.6 cm. on the tip had died off.
(2) 3.3 cm. „ „ „ „ „ „
(3) 3.3 cm. „ „ „ „ „ „

(4) O = old leaves ; N = new leaves.
(5) A bud was formed on April 18.
(6) „ „ „ „ „ „ „ „

It seemed to me of interest to test the effect of a still higher dilution. Accordingly young *Cucurbita* plants of about 6 cm. length with 3 leaves were placed April 22nd in a 0.1 per mille solution of potassium persulphate containing all the mineral nutrients in the form of *Knop's* solution; but even in this case after two weeks some leaves had become curled and yellowish.[1]

We must infer therefore that potassium persulphate shows considerable poisonous effects upon phaenogams.

[1] Compounds which may not be stimulants in a certain dilution for phaenogams may however be stimulants for fungi. Thus *H. Hattori* (Journ. College of Science, Tokyo, 1901) observed that copper sulphate injures the growth of roots of the pea and maize even at 0.000001% while at 0.004% it stimulates the growth of fungi.

Note on Hamananatto, a Kind of Vegetable Cheese.

BY

S. Sawa.

This peculiar product is prepared from soy-beans, as are also two other kinds of vegetable cheese manufactured in Japan, the *Miso* and the *Natto*;[1] but it has a different flavour and taste, and lacks the slimy character of the common *Natto*. It is manufactured only in the central provinces of Japan—especially in those of Mikawa and Totomi, from which it finds its way all over the country. It has an agreeable salty taste and a peculiar odor somewhat resembling that of the fresh crust of brown bread. There is not any mycelium discernible with the naked eye. The soy-beans composing it form no compact mass, and are of a brown colour with a thin layer of a salty taste and a somewhat sticky consistency.

In preparing this product, the soy-beans are well washed, boiled to softness, spread on straw mats, and mixed with wheat flour (6 liters flour to 10 liters soy-beans). Moldfungi will now develop, but soon afterwards this mixture is exposed to the direct sunlight for three days, probably to kill the fungi, and is then put into flat tubs. After 12—13 days some common salt and ginger are added. The entire mass is then kept in tubs under pressure for about thirty days.

A portion, carefully freed from the pieces of ginger and particles of straw mats used in its manufacture, was dried, pulverized and sifted through a 0.5 mm. sieve. I found the chemical composition of the dry matter to be as follows :—

Albuminoid nitrogen	3.57%
Crude fat	3.44%
Crude fibre	6.87%
Total carbohydrate excluding cellulose	8.40%
Total ash including the salt added	18.54%

[1] The composition of *Miso* was studied by *O. Kellner* and *M. Nagaoka*. Bul. Col. of Agr. Tokyo, Vol. I., No. 6 ; and that of *Natto* by *K. Yabe*, ibid. Vol. II , No. 2.

The fresh sample contained 44.73% water and 55.23% dry matter.

There exist at least three different kinds of bacteria in this product. The most numerous colonies on agar are of two kinds.

I. The largest colonies on bouillon agar show under the microscope very large chain-like forms. It hardly liquifies gelatin. Surface culture on agar: white, without lustre. Stab culture in agar: much development on the surface, but very little along the needle track; no gas bubbles visible. On potato: light brown, with minute foldings. In bouillon: kahmhaut white and lustreless, breaking easily on shaking

II. The second microbe forms small colonies on bouillon agar. It readily liquifies gelatin producing thereby an acid reaction. Stab culture in agar: developing along the track, with chalk-like white colour and showing formations like the Bac. mycoides. On potato: greyish, with irregular foldings. In bouillon: developing on surface white and lustreless. Solution remains clear. On shaking, the skin does not break and it sinks.