

Observations and Experiments on the Leafroll Disease of the Irish-potato in Japan.

(Preliminary report)

By

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Introduction.

It was in June 1919 that the writer's attention was first drawn to a serious potato disease prevalent in several parts of Okayama-prefecture, where it is locally known as "Shashaki"-disease. This colloquial term appears to have been derived from "Shashaki," the local name for *Eurya japonica* Th. var. *Thunbergii* Thw., a common shrub of the family Theaceae. It is because the general appearance of the affected potato plants, in the case of the variety "Nagasaki-aka," is closely similar to this kind of shrub in its rigidity and discoloration. In Okugun, a potato-growing section in this prefecture, it has been necessary to import every year the "seed" tubers from Nagasaki on account of this trouble. Closer observations on the symptoms and nature of this disease have led the writer to believe it to be identical with leafroll,* frequently reported in Europe and North America. In order to confirm this, some field trials were undertaken, and all available literature on this malady has been consulted, and it has been proved beyond doubt that our "Shashaki"-disease is nothing but the leafroll of the occidental countries.

The leafroll disease of the Irish-potato is a serious plague spread throughout the world wherever this crop is grown. The designation "leafroll" ("Blattrollkrankheit") has been differentiated from other similar types by APPEL (1906) and it is of the type that is transmissible but that has not been proved to be parasitic. It is considered by QUANJER (1916, 1920) to be an infectious disease caused by a virus or an ultramicroscopic parasite. In spite of prolonged inquiry, the actual cause of the disease is not as yet fully determined. But whatever the primary cause of the disturbance, the effect is sufficiently great, since tubers produced by affected plants give rise to diseased plants the following season.

* Throughout this paper the term "leafroll" will be used as a substitute for "apparently non-parasitic transmissible leafroll," "phloem-necrosis," or "Lepto-necrosis."

Since, so far as the writer is aware, few reports on this disease have ever appeared from this country, it seems worth while to submit a preliminary report for publication with the following purposes:—

- 1) To make it known that the disease has recently been recognized in Japan and that it is already fairly prevalent, and is increasing.
- 2) To describe the symptoms and nature of the disease.
- 3) To record the results obtained up to the present in our field and pot experiments.
- 4) To uphold the opinion of BOTJES and FOLSON that the disease is transmitted by insects.
- 5) To make it known that our "Shashaki"-disease and the leafroll of other countries are identical.

Here the writer desires to express his indebtedness to Prof. Dr. K. MIYABE, Dr. S. HORI, Dr. H. M. QUANJER, Dr. E. BLANCHARD, Dr. J. G. OORTWIJN BOTJES, Dr. D. FOLSOM and Dr. F. W. NEGER, for the information or reprints so kindly supplied by them.

Historical Sketch.

That there are some indications that leafroll is the same trouble, which was already causing damage to potato culture in western Europe in the second half of the eighteenth century, is found in the works of QUANJER (1916, p. 130), APPEL (1909) and others. However the term "leafroll" ("Blattrollkrankheit") is comparatively modern, and it is only in 1905 that APPEL (1906) distinguished this type of disease from other similar ones. It was first noticed in western Germany, Westphalia and the Rhine Provinces in 1905. At the beginning of 1908 it caused great alarm in Germany and the adjacent countries. This was emphasized by an alarming article, written by GRAF ARNIM SCHLAGENTHIN (1908), one of Germany's most prominent potato cultivators, and entitled "Europas Kartoffelbau in Gefahr." It expressed the fear that in all Germany—with very minor exceptions—there was not to be found one sound potato for "seed."

In the year 1909 this disease appeared in many places in Europe, especially in the south-eastern part of Germany and in Austria-Hungary, and caused greater devastation than in the previous year. For instance, in Bulgaria, it spread to such an extent that not a single district was free.

It is now known in most European and American countries. In France the disease was first noticed, according to BLANCHARD & PERRET (1918), in 1909. FOËX (1914, p. 44) writes in 1914 literally as follows:

"La maladie de l'Enroulement s'est montrée redoutable en Allemagne, Angleterre, Autriche, Hongrie, Hollande, etc. Bien qu'elle existe en France

depuis plusieurs années, elle y a passé presque inaperçue jusqu'à ce jour." In the U. S. A., after the writing of ORTON (1914), the first outbreak of this disease attended with some economic loss was noticed in Nebraska in 1911 and 1912, and the second attack was found by Melhus in two places; Onley, Va., and Northern Maine in 1913. In England it appears to have been making some headway during recent years. It is common in all parts of that country, but its attacks are more severe in the south (cf. Jour. Min. Agr. 1921, p. 956). It is considered by PETHYBRIDGE (1919), that, owing to the moist climate and cooler summer, the disease has not yet made its presence felt to any considerable degree in Ireland.

The existing literature on the leafroll disease of the Irish-potato is very numerous and confused. It is beyond the scope of this paper to touch upon it here, but the most important investigators of Europe and America, with the dates of the publication of their writings, are:—

Germany.—APPEL (1905—1918), SCHANDER (1910—1915), STÖRMER (1910—1911), SPIECKERMAN (1911—1913), DOBY (1910—1915), SCHLUMBERGER (1911—1920), VANHA (1910), HILTNER (1905—1919), NEGER (1918—1920), ESMARCH (1919—1920), etc.

Austria-Hungary.—KORNAUTH (1909—1914), REITMAIR (1910—1913), KÖCK (1909—1914), HIMMELBAUR (1912—1914), BOHUTINSKY-KRUIZEVCI (1909—1910), etc.

Sweden.—HEDLUND (1910).

Holland.—QUANJER (1913—1920), OORTWIJN BOTJES (1919—1920), etc.

France.—FÖEX (1914—1921), BLANCHARD (1917—1919), PERRET (1920), etc.

America.—ORTON (1913—1914), WORTLEY (1918), MURPHEY (1918—1920), ARTSCHWAGER (1918), SCHULTZ (1921), FOLSOM (1921), etc.

We have few reports on the study of this disease ever appeared in Japan. So far as the writer is aware the earliest statement on this disease is one issued by the Agricultural Experimental Station of Okayama Prefecture in 1916. More recently in 1920 Dr. S. HORI (1920) of the Central Agric. Exp. Sta. at Nishigahara, Tokyo, while describing other leaf-curling or dwarfing diseases of plants, made a passing mention of this disease, to which he proposed to give the name "Hamaki-byo" (i. e. "leafroll disease") as the technical Japanese name.

The writer's study on the disease starts in June 1919, and it is hoped the research will still be continued further.

Geographical distribution.

It may be inferred from the reports on the distribution of leafroll that it is as widely distributed over the world as are potatoes.

ORTON (1914) mentions its occurrence in Austria-Hungary, Denmark, Germany, Holland, Sweden, Switzerland, and the United States of America. He also states its probable existence in Norway, Russia, Roumania, and Bulgaria. It is also reported from France, England, Belgium, Canada, Bermuda, Java, Jamaica (ASHBY, 1919), and Dutch East Indies (VAN HALL, 1921).

The disease is fairly widely prevalent in Japan. The affected specimens examined by the writer have proved its existence in Okayama-, Nagasaki- and Shimane-prefecture. It seems widely spread also in Fukuoka, Saga, and Saitama.

Economic Importance

Leafroll causes considerable losses wherever an appreciable percentage of the plants is diseased, for affected plants produce the tubers which may be greatly reduced in size and number. Severely diseased plants will produce only culls, and these will result in an almost total loss to the grower. In some districts in Okugun section the affected hills have yielded only from 40 to 60 percent as much as the healthy distincts. This percentage decreases from year to year so that the danger and loss become steadily greater. It is because of this fact that, as previously stated, in the said section it has been necessary to import the "seed" tubers from Nagasaki every year. This reduced yield is caused by the dwarfing of the plants and by the injury to the food-transporting channels, that ordinarily serve for the passage of the materials needed to form starch and other substances in the tubers.

Further, when net-necrosis appears as a symptom of leafroll, the tubers which otherwise could be used for culinary purposes become of less value, as they are often rejected. Furthermore inasmuch as the disease is inheritable, it decreases the value of the tubers for "seed" purposes.

Simptoms of the leafroll disease.

The following symptoms of the diseased plants are those observed in the case of the variety "Nagasaki-aka," which is preferably and widely grown in our districts.

While potato plants are still young and are feeding mainly on the reserve material of the tubers, no symptoms of the disease are to be seen. The symptoms do not appear until a large amount of the products of assimilation is beginning to be conveyed. After a while the lower leaves become discolored and then the marginal end of each leaflet begins to roll upward and

to colour. These phenomena appear at first in the side leaflets of the lower leaves, which appear more or less rigid. If one touch these leaflets they will rattle and feel brittle. Afterwards the whole plant becomes stunted and in poor condition. The stems, stolons, tubers, and roots remain far behind in their development, even though no diseased spots show themselves. In addition to these symptoms, which QUANJER designates the "secondary stage," still another stage, the "primary stage" exists. The primary stage begins with the rolling up and discoloration in the upper leaves. Rigidity may sometimes occur in the primary stage, but is never so conspicuous as in the secondary stage. The primary stage appears when a plant is newly infected and the secondary stage assumes itself in the plants grown from the diseased mother tuber used as "seed." It is thought that the plants infected relatively late in their development may show rolling in the upper leaves only, while those becoming infected from the tuber, or when very young, show it in the lower leaves first, usually followed by rolling of progressively higher leaves later in the same season until most or all are rolled.

The symptoms observed in the case of the variety "Nagasaki-aka" will be precisely described in a manner as follows:—

(I) External features of the diseased plants.

a) The vines of diseased plants grow but slowly, do not attain to the normal height, the internode of the stem being shorter than usual. The whole plant appears xerophytic, rigid, turgescient, brittle and flexible. This rigidity or stiffness is apparent to the eye, and when the leaves are handled it is also apparent to the touch by the leathery, brittle texture and to the ear by a peculiar rattling as the leaves strike each other.

b) The edges of the leaflets roll upward. It consists of an upward curving of the sides of each leaflet with the midrib at the bottom of the trough or spoon thus formed. Its extent depends largely upon the time when infection occurred and upon the age of the affected plants. The rolling may appear upon the upper leaves alone or only upon the lower leaves or, rarely, throughout the entire vine.

c) A pronounced pink-purplish discoloration of the rolled up leaves appears at first confined to the apical portion of the terminal leaflets, and afterwards it becomes manifest along the midribs and veins on the undersurface of the leaves, and in the petiole; but sometimes it spreads gradually over the whole. The deepest tinge, when observed from the upperside of the leaflets, approaches to rosolane purple (RIDGWAY), but from the under surface, along the mid-ribs and net-veins seems to be argyle purple or bishop's purple (RIDGWAY). The discoloration is more conspicuous in the dry seasons than in wet. Its intensity depends also upon the severity of the disease.

d) Some authors have asserted that the dark patches of dead tissue appear on the discolored portion of the leaves. However it is not really the case, as far as the variety "Nagasaki-aka" is concerned.

(II) Internal symptoms of the diseased plants and tubers.

a) A pronounced starch accumulation is found at first in the terminal leaflets of the lower leaves. This phenomenon reveals itself earlier than the external symptoms of the trouble.

b) When the disease is far advanced necrosed phloëm is found in the midribs, petiols and stems. It is most noticeable in the distal growing region of the stem. Since the necrosis of the phloëm causes a stoppage in the translocation of elaborated food, it must be very injurious to the whole life and development of the plant. The discoloration and rolling of the leaves seem to be caused by this disturbance. Further details of phloëm-necrosis will specially be touched upon in the next page.

c) The affected tubers after being stored for a while will show a dull magenta purple or magenta (RIDGWAY) discoloration in the flesh, in the vascular bundles, often originating at the rhizoma scar. The appearance of this discoloration differs in particular cases, and often it is more or less smaller spots, streaks, or map-like, and sometimes extending throughout the whole tuber. If exposed to the air the discolored cut surface of the flesh soon changes into brownish-black probably by oxidation. This in all probability is identical with the net-necrosis of the tubers described by SCHULTZ and FOLSOM (1921).

(III) Characteristics observable when the diseased plants are lifted up.

a) The tubers produced by plants in the secondary stage of the disease are found often attached directly on the underground part of the stem. They are reduced both in number and size, while there is a great decrease in total yield. The extent of this decrease depends largely upon the time when infection occurred and upon the age of the affected plants.

b) The tubers from the diseased plant, if used as "seed," are often found still firm, unexhausted and sound at the time of digging. They are even larger in size than when planted. This persistence of the mother tubers is a peculiarity of the leafroll disease in the secondary stage. In the primary stage of the disease this phenomenon is not necessarily the case.

c) The runners, or stolons, get so short that the tubers are close against the stalk, and none of them attain a normal length. It stands to reason, that the new tubers are clustered together, directly at the subterranean stem end. However the shortness of the stolons is by no means constant; for plants in the primary stage of the disease often show some elongated and rather well developed runners.

These symptoms as mentioned above, lead us to consider that our disease and the leafroll of the occidental lands must be the same.

Phloëm-necrosis.

QUANJER (1913) is convinced, as far as the variety Paul Krüger is concerned, that phloëm-necrosis is the only internal symptom which distinguishes true leafroll from a number of other pathological conditions which resemble it. However, there are some opinions contradictory to this. For instance KÖCK and KORNAUTH (1913) declared that necrosed phloëm was also to be found in the potato-plants attacked by other diseases, and that it was only a chance symptom of little diagnostic value. SCHANDER and TIESENHAUSEN (1914) hold similar views. They found that necrosis of the phloëm is to be found in plants suffering from other diseases and even in healthy ones. In plants suffering from "Kräuselkrankheit" and "Bukettkrankheit" it may be even more pronounced than in those suffering from leafroll. Most recently of all, ESMARCH (1919) also rejects the view of QUANJER and claims that the so-called necrosis may be a proper age phenomenon and a sign of early maturity. He writes (1920) literally:—".....Phloëmnekrose kein spezifisches Merkmal der Blattrollkrankheit ist und weder einen diagnostischen Wert besitzt noch zur Erklärung der Krankheit dienen kann.Demnächst dürfte die Phloëmnekrose als eine, der Kartoffel eigentümliche Alterserscheinung zu betrachten sein; ihre häufigeres und früheres Auftreten an kranken Pflanzen wird dann als Symptom einer Notreife ohne weiteres verständlich." ARTSCHWAGER (1918), although confirming the opinion that the phloëm cells are affected in the cases of leafroll, still suspects that the development of necrotic tissues is not confined to plants affected with leafroll, but it is common to the so-called degeneration troubles and perhaps to others also. FOËX (1920), however, accepting the existence of the necrosed phloëm in the leafrolled potato plants, claims that this process is of pectic degeneration tending toward a sort of gummosis; while it is believed to be a lignification by QUANJER and is thought to be a kind of cutinization by ARTSCHWAGER.

We find one more advocate of QUANJER's view in OORTWIJN BOTJES. BOTJES says (1920, p. 136), "Die Phloëmnekrose ist ein typisches Krankheitsmerkmal, das nur bei der Blattrollkrankheit auftritt und auch immer vorhanden ist, wenn die Krankheit schon weiter vorgeschritten ist. Sie ist aber erst wahrnehmbar, nachdem die Stärkeschoppung und die äussere Merkmale schon während kürzerer oder längerer Zeit beobachtet werden können. Sie kann denn auch nicht als die Ursache der Stärkeschoppung und des Auftretens der äusseren Symptome betrachtet werden." Also FOËX (1921, p. 27) writes; "Les résultats,tendent à démontrer l'existence d'une réelle concordance entre la leptonécrose et l'enroulement."

Cause of the disease.

Few diseases have ever aroused greater divergences of opinion as to their causes than leafroll. In the earlier opinion of APPEL (1905—1908), he has attributed the cause of leafroll to *Fusarium* or *Verticillium*. HIMMELBAUR (1912 & 1913) published two papers on the "Fusariumblattrollkrankheit der Kartoffel." There it is stated that in the cross-section of the stalk of a diseased potato-plant, the vessel-ring is occasionally yellow-tinted and a fungoid mycelium is found. The mycelium upon cultivation brings forth spores, which might be classified as belonging to *Fusarium*.

However, in many cases doubt has been expressed about the parasitical nature of the disease, as there is no mycelium whatever. It was supposed that the disease arises from abnormal and unfavorable conditions of the atmosphere and the soil, in connection with the use of immature "seed" tubers. As a result of these co-operating causes, there may have taken place inside the potato-tuber enzymotical disturbances, hence a disorder which possibly is nothing but a recurrence of the long-known "curl-disease."

The followings are some of the hypotheses as to the cause of leafroll :

- (I) Biological hypotheses.
 - (a) *Fusarium* (APPEL, ZEDTWITZ, REITMAIR, HIMMELBAUR, KÖCH & KORNAUTH).
 - (b) *Verticillium* (APPEL, KÖCK & KORNAUTH).
 - (c) *Phoma* (STÖRMER).
 - (d) *Solanella rosae* (VANHA).
 - (e) *Tylenchus*, a nematode (VANHA).
 - (f) *Helminthosporium* (BOHUTINSKY-KRIZEVCI).
 - (g) Bacteria (STÖRMER).
- (II) Physiological hypotheses.
 - (a) Severe drought (HAMANN, WODARG, GOLZ, SCHLEH).
 - (b) Excessive moisture (STÖRMER, VIBRANS, SORAUER).
 - (c) Storage at too high temperature (CAUSEMANN).
 - (d) Prematured "seed" tuber (HILTNER, STÖRMER, BÖHMER, ORTON, CAUSEMAN).
 - (e) Overmatured "seed" tuber (Jour. Min. Agr.)
 - (f) Soil character (STÖRMER).
 - (g) Lack of potash (FOFFIK, MAYER-WAGENINGEN).
 - (h) Excess of potash (HILTNER).
 - (i) Lack of fertilizers (OSTERSPEY).
 - (j) Excess of fertilizers (SORAUER, KRÜGER, WIMMER).
 - (k) Lack or over supply of mineral elements in the soil (ORTON).
 - (l) Nitrogen-hunger (BLANCHARD & PERRET).
 - (m) Too long asexual reproduction (BLANCHARD & PERRET).

- (n) Effeminacy ("Verweichlichung") (NEGER).
- (o) Hereditary, pathological mutation (HEDLUND).
- (p) Unknown changes of internal forces (HILTNER).
- (q) Physiological causes (NEGER, ESMARCH).
- (III) Virus, or ultramicroscopical germ hypothesis (QUANJER).

It will be seen, then, that though this disease has been thoroughly investigated, its real cause has not been ascertained. At present QUANJER's virus or ultramicroscopical germ hypothesis is accepted by many of the investigators. In the general nature, leafroll is like the mosaic disease of several plants. The exact cause of leafroll is known to be something in the juice of the plant. Therefore it will remain in the plants or their tubers as long as they contain juice or water, and it will spread from a diseased to a healthy plant only when some of the juice of the former in some way gets into the juice of the latter. Insects that extract their food from the channels of an affected plant and that pass from one plant to another are well fitted to spread the disease.

Explanations of the characteristic symptoms.

Explanations hitherto known of the symptomatic features of the affected plants are as following. All are caused by the check of the translocation of the material, which results probably from necrosis of the phloem.

- (1) Dwarfishness, rigidity and reduced yield.

Much of the food manufactured by the leaves does not pass down to the new tubers, as it normally should, but remains in the leaves in the form of starch. In consequence of this check there results an increase of the starch contents in the leaves of leafrolled plants. NEGER (1918, 1919) is of opinion that the starch accumulation stands in closest relation to the degree of leaf rolling. There cannot exist any doubt that starch accumulations and rolling of the leaves are coincident, yet this does not prove these phenomena to be causally related; both indeed may be symptoms caused by a third factor, as yet unknown. Nevertheless, excess of starch may be the primary, and the rolling of the leaf the secondary factor; though it is not clear how the former would cause the leaf to roll.

- (2) Discoloration.

A marked discoloration of the diseased leaves, in the opinion of QUANJER (1916, p. 99), is caused by anthocyan. He says the same phenomenon can be caused in many plants by cutting off the downward flow of sap. Thus it seems the discoloration is also of consequence of inhibition of translocation of the sap materials. The discoloration of the flesh of the tubers may be due to the same reason.

(3) Persistence of diseased "seed" tubers.

As to this, SPIEKERMANN (1910) is of the opinion that, in plants which produce limited quantities of dry substance, the salts and nitrogen compounds of the "seed" tubers can not be utilized, and, therefore, these remain in the stick-tubers, which thereby can survive longer.

Observed nature of the disease.

General nature of the leafroll disease may be stated as follows:

- (1) The disease is pseudohereditary. The diseased tubers always produce the affected plants. The secondary stage of the disease will only manifest itself, when the cause is present in the mother tuber. The primary stage starts not in the tubers but by infection.
- (2) The disease is communicable. The healthy plants near or somewhat apart from the diseased become infected.
- (3) In the newly infected, i. e. primary-diseased plants, some of the symptoms are not necessarily present. But in the next generation, i. e. secondary diseased plants, they are all exhibited.
- (4) The primary diseased plants may produce almost normal crop of seemingly sound tubers, while in the secondary the yield is much reduced.
- (5) When the mother plant is attacked so late that the symptoms are scarcely or not at all to be seen, only a part of its offspring will show the secondary disease and it may be that other offsprings derived from it have both the healthy and diseased stalks. When the mother plant is attacked early and severely the daughter plants will all be secondary-diseased.
- (6) The affected plants do not by any means die away earlier than the healthy ones; their leaves do not fall off.
- (7) Transmission experiments of the disease by tuber-grafting and juice injection have succeeded in our trials.
- (8) It is possible that insects seem to assist chiefly in the transmission of the disease.
- (9) Soil infection does not really take place.
- (10) Mosaic and leafroll are similar in their way of transmission.

Transmission of leafroll.

One strong proof that leafroll is a disease of the virus type is its transmission, through tubers that are usually without any very apparent sign of disease, through the grafting of tubers, and through the injection of the juice of a diseased plant.

(1) Transmission by the tubers.

Tubers of the leafrolled plants always produce similar diseased plants, except in very rare cases where they are too severely affected by the disease to produce more than spindling, sprawling vines. The disease is thus, in loose sense, of a pseudohereditary nature. Observations made by the writer upon the progenies of leafroll diseased stocks since 1919 show that these progenies consistently have developed the plants with the symptoms of the disease.

(2) Transmission by the grafting.

The first step to prove the suspected nature of the disease, as a virus type, is by means of grafts, where a diseased plant part and a healthy one can be joined into a single structure, with the best chance for the juice of the one to enter the other. This was proved with both tubers and shoots by QUANJER (1916) and SCHULTZ & FOLSOM (1921). The same result with tuber grafts was confirmed by the writer, as shown in Experiment VI and IX. Thus leafroll of the Irish potatoes is successfully transmitted by the grafting.

(3) Transmission by the juice.

The writer was able, as shown in Experiment X in the end of this paper, to produce diseased conditions in healthy plants through the injection of the juice of the leafrolled plant.

General observation indicates that leafroll may spread from one plant to another in the field. Healthy hills at different distances from leafroll hills became diseased often as they are closer to diseased hills. It was BOTJES (1919 and 1920, p. 123—136) that first proved plant-lice to be an agent in the spread of leafroll from plant to plant in the field. More recently SCHULTZ and FOLSOM (1921) conducted four types of experiment, each of which proved that plant-lice (*Macrosiphum solanifolii* Ashm), could transmit or carry leafroll from one plant to another.

That the potato-plants protected from insects, by means of the insect cages, remain free from leafroll has also been ascertained by the writer as is shown in Experiments IV, V and VII. This proof is made more convincing also by the fact that plant-lice transmit other virus diseases such as potato-mosaic and certain similar diseases of cucumber (DOOLITTLE, 1920), spinach (SMITH, 1919), sugar cane (BRANDES, 1920), sweet-pea (TAUBENHAUS, 1914) and tobacco (ALLARD, 1914), etc.

(4) Transmission by the soil.

Soil infection was once believed by QUANJER (1916). But afterward he has abandoned this view, for the following lines are found in his letter to the writer dated March 10, 1921. "In connection with the first paper (1916), it needs to be said that some of my later investigations showed that the supposition, that the disease is carried by the soil is not correct." As early as 1910 BERSCH reports that in some instances the disease appeared on land which had been for the first time planted in potatoes, thus excluding the

possibility of the infection coming from the soil. WORTLEY (1918) also reports negative results in Bermuda. BOTJES (1920, p. 129) says "Die negative Resultate.....bestätigen die Meinung, dass das Coftagium, welches von einer kranken Vorfrucht im Boden zurück gelassen werden könnte, keine Gefahr für gesunde Pflanzen bietet." From the results of our experiments it is believed by us also that soil transmission does not take place.

Biochemical aspects of leafroll.

The writer has conducted no inquiry as to the physiological or biochemical sides of the disease. The following is an outline of known results ascertained by different authors studying leafroll.

SORAUER (1913) first suggested that the diseased potatoes have more active oxidase reaction than the healthy ones. GRÜSS (1907) and afterward DOBY (1911, 1912) have proved that the diseased ones not only have higher reaction with regard to oxidase, peroxidase, and tyrosinase, but also they had a slightly higher ash content and less starch and protein. It would seem that katabolism is more rapid in the diseased plants. Extending his study to the amylase of tubers DOBY (1915) says that the amylase of potatoes is present partly as zymogen, which passes over continually into the active state. The activity in freshly expressed sap showed no relation to the variety or origin of the potatoes tested. In general, sound tubers possess more zymogen than diseased ones. This report of DOBY is not considered as having yet demonstrated a basal chemical criterion for the presence of leafroll, or as having decided whether the chemical or biochemical changes observed in diseased tubers are the cause or the effect of the disease, or how far the optimum and activities of amylase differ in sound and in diseased tubers.

In a paper 1913 QUANJER (1913) found that the chief cause of the disease shows itself in the phloëm strands. The sieve-tubes are shrunken so that the walls and lumina of the single tubes cannot be differentiated. This shrunken tissue is yellowed, and with acids and caustic potash gives the characteristic reactions of lignified cells. The physiological disturbances are traced back to the shrinking of the phloëm strands, which causes a checking of the sap flow, thereby indirectly producing the disease symptoms, roll-leaf, dwarfing etc. The occurrence of oxidizing enzymes in plants is considered to be a response of the plant to the stoppage of the sieve-tubes and not the cause of the disease.

SPIECKERMANN (1910) found that salts and nitrogenous substances are absorbed much more slowly by plants derived from diseased tubers than by healthy ones; on the other hand, at a later stage when the young tubers are being formed, the downward flow of salts and nitrogenous substances is

restricted. The salts referred to by SPIECKELMANN seem, in the main, to be potassium compounds. As for the nitrogenous compounds, these must be albuminous substances as it appears from the work of DOBY (1912) and we know that it is just the compounds of potassium and the albuminous substances, indispensable for the formation of new tubers, which move through the phloëm.

NEGER (1919) suggests that, coincident with the excess of starch in diseased leaves, there occurs an accumulation of diastase; and both factors would indicate serious disturbance of the enzymotic processes within the plant, but as to cause and origin of which no clear decision has yet been reached.

Again NEGER (1919) says that leafroll-diseased leaves contain much more diastase than sound leaves. That starch is not dissolved is apparently due to the accumulation of split products of starch as sugar, through the presence of which the amyolytic enzyme is rendered inactive. The same author points out that the "Bildungsstärke" is not or only very partially conveyed down from leafrolled leaves, hence the yield of tubers is reduced. Moreover sugars derived from hydrolysis of "Bildungsstärke" are found accumulated and they are stored in the leaves.

Furthermore NEGER in 1920 published a report in which the results of many experiments are shown. He was able to produce artificially the starch accumulation, which always accompanies and which he regards as the cause of leafroll. It was done either by more or less complete hindrance of the action of stomata or by removal of certain mineral nutrients such as calcium, sulphur, chlorine and nitrogen.

BOAS (1919) states that minute differences in the H-ion concentration may have marked effect upon the metabolic process of the plants. This caused him to inquire whether, in leafrolled potatoes, there could be determined any differences in the H-ion concentrations existing in sound and diseased plants. He ascertained from his experiments that, without exception, the cell sap of sound plants showed appreciably more acidity than that of diseased plants. The acid metabolism of diseased plants is plainly disturbed. In determining the albumen metabolism, that might be expected under the circumstances, he discovered that the diseased potato stems are flooded with amino acids, while the sound tissues are free, or only show traces of these acids. Examining then into the catalase contents of diseased and sound plants, he finds obvious differences, inasmuch as the diseased portions show an increase in catalase contents over the sound ones; but not all experiments gave identical results.

ESMARCH (1919) proved that there is a remarkable difference in the starch production of the diseased and sound potato-plants. While in the leaves of sound plants there remain no trace of starch after 19—68 hours, the old leaves of affected plants are found filled with starch even after 6—8 days' darkness. A microscopic examination of leafrolled leaves shows that mesophyll-cells are crammed with starch-grains, and this is the same also in the veins and

petioles. Thus, he says, rolling of leaves is a result of inner metabolic disturbances.

Experimental Data.

Since the autumn of 1919, seven field trials, as it is possible to grow the Irish potato twice a year in our district, were repeated and in addition to them three glass-house experiments were conducted. The data obtained from these tests will be stated here. The "Nagasaki-aka" was the only variety used for the tests throughout.

Experiment I. (Field trial—Sept. 4—Dec. 8, 1919)

This is a start experiment. In July 1919, seemingly sound and diseased plants were selected in a field which belonged to the department of agronomy of our Institute. The localities, from where the original stocks were brought, and the numbers, which were used in the line selection work conducted in the said department, are shown in Table I. The tubers of the selected plants were kept separately with care and were planted in Sept. 1919.

The selected diseased plants showed various disease symptoms; some of them very closely resembling to leafroll, others seemingly curly dwarf and the like. Were they independently different diseases or modified forms of the one disease, for instance, of leafroll? The writer determined to solve this question. Above all it was kept in mind to observe the behavior of suspected leafrolled plants.

The plot used for this experiment was obtained in a corner of the field where the Irish potatoes have been grown successively for a few years. The "seed" tubers were planted without previous boxing and without cutting.

The results of the experiment are shown in a tabulated scheme in Table I in the next page.

Remarks:—This preliminary test suggests that:—

The sound tubers produced healthy plants in one instance. However in other case some of the plants derived from the sound tubers became diseased. This may be because of infection. The majority of the diseased tubers produced affected plants in their progeny. This indicates that the disease is of inheritable nature. A mother plant which shows leafroll and tubers of which are found closely attached will almost always produce leafrolled plants. Dwarf mother plants often produce also leafrolled plants. By this trial a closer observation on leafroll was made.

After all, all tubers newly yielded by the forty-one diseased plants in Table I were separately collected in accordance with four types as are classified below and were used as the "seed"-tubers in Experiment II.

Table I.
Showing the result of Experiment I.

State of the plants selected in July, 1919.	Provenance & pedigree number.	No. of tubers planted.	State of the plants under observation on Nov. 25, 1919.	No. of plants diseased.
Sound.	Shikii 216	4	All healthy, neighbor infection did not take place.	0
"	" 221	20	Almost healthy, but 3 were found leafrolled.	3
Leaves rolled, tubers small.	Nagasaki 203	8	Seemingly sound 1. Leafrolled 7.	7
Stunted growth.	" 176	4	All 4 with flaccid appearance in the leaves.	4
Dwarf, smaller tubers closely attached.	Shikii 109	13	Apparently sound 6, leafrolled 4, dwarf 1, and dwarf & enrolled 2.	7
Leaves rolled, but growth vigorous.	" 232	12	Apparently sound 2, conspicuously leafrolled 10.	10
Smaller tubers closely attached.	— 8	4	Dwarf 2, normal 2.	2
Stunted growth.	— 9	5	Normal 2, dwarf & enrolled 3.	3
Poor growth, smaller tubers closely attached.	— 10	9	Apparently leafrolled 3, dwarf & enrolled 2, sound 4.	5
Total,		79		41

N. B.—The diseased plants in the table were classified into 4 types; they are:—

- (a) Dwarf:—with dwarfed nature in general, leaves not enrolled, but flaccid as if in the case of mosaic.
- (b) Dwarf & enrolled:—leaves flaccid and enrolled, plants in a dwarfed development.
- (c) Seeming leaf-roll:—with apparent leafroll symptoms.
- (d) Leafroll:—conspicuously leafrolled, with discoloration in the leaves.

The provenance and pedigree numbers were left out of consideration after this experiment.

Experiment II. (Field trial—Apr. 14—July 6, 1920)

In April 1920, all progeny tubers yielded by the forty-one diseased plants and also the sound tubers obtained in Experiment I were planted. In this experiment it was hoped to determine whether the four types of the diseased features, when planted separately, would manifest themselves again exactly as before; and if they undergo any change how they behave. The plots used for this test were selected in a corner of the field where for successive years preceding no potatoes have been grown.

Results of the experiment will be shown in the following Table II.

Table II.
Showing the results of Experiment II.

Healthy or diseased.	No. of plants.	Behaviour of the plants observed on June 26, 1920.	
Healthy.	12	Neither enrollment nor discoloration appeared in the leaves, but the plants appeared feeble, leaflets being small, haulm slender.	
Diseased. {	Dwarf & enrolled.	3	Leaflets smaller, with evidence of dwarf and enrollment.
	Leafroll.	41	All either apt to leafroll or actually conspicuous leafroll, plant growth poor, leaves smaller.
	Nearly leafroll.	33	All evidently leafrolled. Plant-growth not vigorous, leaves smaller.
	Dwarf.	25	All in dwarf state, leaflets small and flaccid, no enrollment of the leaves appeared.

Remarks:—In the plants derived from the sound tubers, both enrollment and discoloration were not noticed. The reason why the number of plants of the dwarf and enrolled type used in this experiment is few, being only 3 in all, is because the produced medium sized tubers in Experiment I were scant, most of them being culls. Thus the results of this type in Table II are not reliable. The nearly leafrolled type was exactly the same as and nothing but leafroll. The dwarf and enrolled type as well as dwarf-type have acted just in the similar manner as they were. The majority of the mother-tubers of the leafroll type or nearly leafrolled type were found unexhausted. By this experiment true symptoms of leafroll were more closely and clearly observed. Rolling, rigidity, brittleness, leathery texture and reddish-purplish discoloration of the affected leaves; dwarfing of the vines; shortness of stolons; reduction in the number and size of tubers have all manifested themselves.

Experiment III. (Field trial—Sept. 17—Dec. 14, 1920)

The "seed" tubers used for this trial were those obtained from Experiment II and they were planted on Sept. 17, 1920. The number of "seed" tubers of the nearly leafroll type and the dwarf and enrolled type was only six each. This is because of the much reduced yield in these types in the foregoing experiment. The main object of Experiment III was to observe more minutely the characteristics and symptoms of the leafroll disease. And in this case infection of the disease to the healthy plants was to be watched with special care. The plots used were the same as in the preceding experiment.

Results:—The following Table III will show the results of the test.

Table III.
Showing the results of Experiment III.

Sound or diseased.	No. of "seed" tubers.	Behaviour of the plants on Dec. 1, 1920.	Observations when lifted on Dec. 14, 1920.
Healthy.	66	l.r.*=46, dwarf=6, healthy=6, not germinated=9, thus seriously leafrolled.	In l.r., stolon short, tubers directly attached to the underground stem. In the healthy, stolon long.
Diseased. { Dwarf-enrolled. Leafroll. Nearly leafroll. Dwarf.	6	l.r.=4, dwarf=1, not germinated=1.	Mother tubers remain unexhausted, new smaller tubers closely attached to the underground stem.
	24	l.r.=13, dwarf=8, not germinated=3.	Stolon short, small tubers attached, the mother tuber remain unexhausted.
	6	l.r.=3, dwarf=2, not germinated=1.	Mother tubers unexhausted, no or very short stolon, tubers small.
	30	l.r.=15, apparently sound=10, not germinated=5.	Majority of the mother tubers unexhausted, stolon short or absent, small tubers directly on the stem.

* l.r.=leafroll, with conspicuous symptoms of the disease.

Remarks:—Some 70 percent of the sound ones became leafrolled. This must be the result of infection. A few of the dwarf type appeared out of the types of leafroll and nearly leafroll, and apparently sound ones came out of the dwarf type. By this experiment also the most typical features of leafroll were observed, and the four types divided hitherto became almost all mixed into one type leafroll. Whether this is the effect of overcoming of leafroll over dwarf-enrollment and dwarf, or these latter two forms were of particular types of leafroll, it could not be certainly defined. However, it is probably due to the former reason, that is contamination. Potatoes planted near our experimental plots became extensively infected.

Experiment IV. (Field trial—April 1—July 6, 1921)

Is soil infection possible? If some plants derived from the diseased tubers were planted, being inserted among or along side of healthy plants, in a plot which had not been used for potato culture for several years, how does the disease spread? Does the infection more easily spread among the plants planted nearest to the diseased ones than among those which are apart from the latter? If insects are kept away, by means of insect cages, from the healthy plants, will the protected plants remain sound? The "seed" tubers used for this experiment were of following four origins.

- a) Healthy tubers newly brought from Nagasaki.
- b) Sound tubers newly bought in the market.
- c) Leafroll-diseased tubers newly brought from Ushimado.

d) Leafroll-diseased progeny tubers from Experiment III.

Five long parallel plots were prepared in a field where during some years preceding no potato crops have been grown. On the middle plot some cruciferous vegetables were planted to serve as a separating space of 3 meters. On the two plots in one side of the vegetable plot the sound tubers of "a" (Plot A) and "b" origin (Plot B) were planted in each of them separately. In Plot A and Plot B some diseased tubers of "d" origin were planted lineally acrossed at an interval of 4 meters. On the remaining two plots in other side the diseased tubers of "c" origin (Plot C) and "d" origin (Plot D) were planted. The total number of the plants for this field trial was 324 in all. Large cheese cloth insect cages were used to protect plants from insect, two each for Plot A and Plot B, thus protecting sixteen healthy plants, and also one each for Plot C and Plot D as control. The insect cages were applied on April 29. At this time the sprouting of the tubers had scarcely begun and the majority of the sprouts were still underground.

Results of the experiment will be shown in the following Table IV.

The data of observations, made during the growth of the plants, and when at the time of digging on July 1, 1921, will be written in the following way.

Plot A.

- (1) A comparatively satisfactory yield of tubers has resulted. The tubers were of medium size in general.
- (2) Enrollment and discoloration appeared to the full extent chiefly on the upper leaves.
- (3) Stolons shorter in general. Longer ones, however, were also often noticed.
- (4) The mother tubers were all well exhausted.
- (5) It seems all plants in this plot have been "primary" diseased. This must have been the result of new infection from the neighbouring diseased plants. For a smaller number of the tubers of the same origin, used in an experiment conducted under a glass-house as is stated in Experiment VI, has behaved all sound.
- (6) The plants under the insect cages had well developed haulms. Their leaves, though mottled, being normal, and had no indication of leaf-roll. Their production of new tubers, however, was not satisfactory. Thus it seems that to protect the plants from insects it has an appreciable effect on them.
- (7) The mother tubers of the diseased strain inserted in the plot were found consumed or remaining unexhausted. The stolons were very short in common. The yield very much reduced.

Plot B.

- (1) All plants, even though sound in origin, were affected, showing nearly

Table IV.
Showing the results of Experiment IV.

Plots and preparations.	No. of plants.	Observed results on (figures show the number of plants).		
		May 21.	June 13.	June 23.
A { Healthy Nagasaki (a) { exposed. under cage. Diseased tubers (d) inserted.	55	*m.l.r.=33. m=7, l.r.=12, n=3.	l.r.=55, i.e. all at last leafrolled.	entirely all distinctly l.r.
	8	m=8 (pale spots on leaves).	m=8, mottled appearance.	not leafrolled, though mottled.
	18	l.r.=13, n=5.	l.r.=18, i.e. all leafrolled.	all typically leafrolled.
B { Healthy Market (b) { exposed. under cage. Diseased tubers (d) inserted.	55	l.r.=21, m=15, n=19.	l.r.=55, all leafrolled.	all typically leafrolled.
	8	m=8 (pale spots on leaves).	m=8.	not leafroll, though mottled.
	18	l.r.=15, m=2, n=1.	l.r.=18, i.e. all leafrolled.	all typically leafrolled.
C Diseased Ushimado { exposed. under cage.	77	l.r.=70, m=3, n=4.	l.r.=77, all leafrolled.	all entirely leafrolled.
	4	m=4 (pale spots).	l.r.=4, leafrolled.	l.r. but not rigid as others. discoloration not appeared.
D Diseased progeny { exposed. under cage.	77	l.r.=64, m=8, n=5.	l.r.=77, all leafrolled.	all leafrolled.
	4	m=4 (pale spots).	l.r.=4, leafrolled.	l.r. but no discoloration appeared.

* m.l.r.=mottled, flaccid, and leafrolled.
l.r.=leafroll, m=mottled, n=normal.

typical leafroll. Indications of the "primary" diseased stage were evident.

- (2) The size and number of the new tubers were not necessarily smaller or scanty. In one case some fourteen of them were found attached to one plant.
- (3) Stolons generally shorter, but not always so.
- (4) All plants under the insect cages were free from leafroll. *Phytophthora infestans*, however, was found on their leaves late in season.

Plot C.

- (1) Stolons always shorter.
- (2) The production of new tubers were not necessarily scarce in this case. The size also was of medium.
- (3) The plants under the insect-cages became all diseased as has been expected, and their new tubers were only culls or sterile. *Phytophthora* was also noticed late in season.

Plot D.

- (1) Stolons short in every case.
- (2) Very few tubers were produced.
- (3) The mother tubers, in an unexhausted condition, were comparatively few. In the case of every plant to which comparatively larger new daughter tubers are attached, its mother tuber was always well consumed. On the contrary whenever it was not consumed, the produced tubers are always small in size.
- (4) The plants under the insect cages, being also affected, produced culls only.

Remarks :—Answers to the objects aimed at this experiment will be as follows:—

Soil infection is not the case. For the healthy tubers planted in an uninfested soil became severely diseased. The plants nearest to the affected plants, as well as those which are somewhat apart, are equally found to be diseased. Hence neighbourhood infection through the soil seems not to take place. The healthy plants protected from insects with the insect cage did not indicate any sign of leafroll. General contamination, occurring in the exposed plants, must be due to insect-transmission of the disease. The plant lice were extraordinarily numerous in the experiment plots and found aggregated in all tested plants except those under the cages.

Experiment V. (Field trial—April 1—July 6, 1921)

How do the healthy tubers behave when planted in an infested soil? What effect has the protection from insects upon the healthy plants planted on the same soil? Experiment IV and this experiment were conducted in a parallel way at the same time in different fields. The plots used in Ex-

periment III were divided into four long plots, the two on the right being planted with the tubers of healthy origin "a" (Plot A) and "b" (Plot B) and the two on the left with the diseased "c" (Plot C) and "d" (Plot D). The materials for this experiment were just the same as in the case of Experiment IV. One insect cage covered with cheese cloth was applied on April 29 in Plot A and Plot B each. At this time sproutings were as yet not observable above the ground. In Plot D 44 of the mother tubers remained unexhausted in Experiment III were replanted. Thus total number of the tubers was 480 in all.

Results:—The results of the experiment will be shown in a tabulated scheme in the next page as Table V.

Observed data obtained while digging up the crop were as follows:—

Plot A.

- (1) The mother tubers were found almost all consumed.
- (2) Many stolons were not long, though very short ones were absent.
- (3) Newly formed tubers were all of medium size, 4 or 5 being attached to every plant. Often, however, numerous smaller nuts-sized ones were attached, clustered together.
- (4) All plants "primary" diseased.
- (5) The total yield was less than that of Plot A in the preceding experiment.
- (6) The daughter tubers of the plants under the insect cage were few and of medium size.

Plot B.

- (1) The mother tubers were almost all consumed.
- (2) Stolons shorter in general.
- (3) New tubers were of medium size, most of them smaller but occasionally a few larger ones were found among them.

Plot C.

- (1) The mother tubers were exhausted in general.
- (2) New tubers were very few and much smaller.
- (3) Stolons commonly short.

Plot D.

- (1) Old unexhausted mother tubers of Experiment III, when once more planted, sprouted and developed a feeble shoot, but after a meanwhile withered away.
- (2) Majority of mother tubers were found consumed.
- (3) Production of new tubers was very scanty.
- (4) Stolons were very short.

Remarks:—The healthy strain of tubers grown in an infested soil became affected without any apparent signs of difference from that from the healthy soil in Experiment IV. The protected plants, though grown in the infested soil, did not show any trace of leafroll. The transmission of disease to the healthy ones in Plot A and Plot B must be due to infection though insect-transmission of the disease.

Table V.
Showing the results of Experiment V.

Plots and preparation.	No. of plants.	Observed results on (figures denote the number of plants).			
		May 21.	June 13.	June 23.	
A—Nagasaki	{ exposed	116	l.r.=81, m.l.r.=5, m=23, n=7.	l.r.=109, m=7.	l.r.=114, m=2, i.e. all diseased.
	{ under cage	4	mottled.	no indication of l.r.	no leafroll.
B—Market	{ exposed	116	l.r.=33, m=44, n=39.	l.r.=97, n=19.	l.r.=111, n=5, i.e. all diseased practically.
	{ under cage	4	mottled.	no indication of l.r.	no leafroll.
C—Ushimado		120	nearly all diseased.	evidently all diseased.	completely diseased.
D—Diseased progeny	{ new	76	nearly all diseased.	ditto	ditto.
	{ mother	44	Sprouts were coming forth, shoots very feeble, slender and scarcely having any smaller leaves. After a mean-while all withered away.		

Experiment VI. (Tuber-grafting trial—April 1—July 6, 1921)

It was to test in this experiment the possibility of contamination with the disease by tuber-grafting. Twelve tubers of approximately the same size and shape were selected from the healthy stock newly brought from Nagasaki, that is, one part of the tubers used in Experiments IV and V. Twelve tubers also were selected from the leafroll-diseased stock produced by our previous experiment.

Each healthy and diseased tuber was cut in half, and the fresh surfaces of each of both kinds of tuber were intimately bound with a thread to form a combination set. When cut into halves discoloration of the flesh was observed in every case of the diseased twelve tubers. Such discoloration was, of course, not noticed in the case of the healthy stock from Nagasaki. The diseased halves in the 12 combined sets were marked with white paint. And the combined 12 sets were immediately planted in 12 pots which were kept in a glass-house. When the sprouts came out of the soil those from diseased halves were completely removed, leaving only those originating from the healthy halves. Then five insect cages were applied to the five pots of the combined sets.

The other halves of each of the diseased and sound tubers, 12 in each, were planted in other pots separately as the control. Thus total number of pots makes 36 in all. Watering was duly applied.

Results:—The following three tables will show the results of the experiment.

Table VI.

Showing results of twelve control halves of sound tubers.

Pot-mark	Observed results on	
	May 21, 1921.	July 1, 1921.
A	Normal development, mottled spots in the leaves.	Mottled spots disappeared, free from leafroll.
B	Development somehow retarded.	Sound, free from leafroll.
C	Very good development, mottled spots conspicuous.	Mottled spots disappeared, sound, free from leafroll.
D	Well developed, leafroll pronounced?	Some indication of leafroll in the lower leaves? Doubt remains.
E	Well developed, flaccid and mottled spots in the leaflets.	Sound, normal development of plants.
F	Very good development, only mottled.	Sound, free from leafroll.
G	Development medium, mottled spots in the leaflets.	Sound, no indication of leafroll.
H	Good development, normal leaflets with a few mottled spots.	Healthy, no trace of disease symptoms.

Pot-mark	Observed results on	
	May 21, 1921.	July 1, 1921.
I	Development good, it is suspected a trace of leafroll in the lower leaflets.	The said suspicion disappeared, plant was sound.
J	Luxuriant growth, leaflets normal.	Sound, no indication of leafroll.
K	Very good development, mottled spots rather conspicuous.	Mottled spots disappeared, sound, no indication of the disease.
L	Very good development, conspicuous mottled spots.	Spots disappeared, free from leafroll.

As above table shows leafroll did not occur in any of the twelve plants that were derived from control halves of sound origin.

Table VII.
Showing results of twelve combined sets.

Pot-mark	Observed results on	
	May 21, 1921.	July 1, 1921.
I*	Good development, no leafroll, but mottled.	Sound, free from leafroll.
II	Good development, suspected leafroll, but very inconspicuous, mottling abundant.	ditto
III*	Good development, no leafroll, mottlings conspicuous.	ditto
IV	Good development, seemingly leafroll? mottlings conspicuous.	ditto
V	Flaccid, undulate and mottled leaves, development good.	ditto
VI*	Normal growth, mottlings conspicuous.	ditto
VII	Normal development, leafroll in the lower leaves, discoloration appeared, mottle inconspicuous.	Typical leafroll with conspicuous discoloration.
VIII	Good development, no leafroll and mottling, sound.	Sound, free from leafroll.
IX	Rather poor growth, leaflets few but all normal.	ditto
X*	Good development, mottling is absent, no leafroll.	ditto
XI	Vigorous development, conspicuous mottling, no leafroll.	ditto
XII*	Superior development, conspicuous mottlings, no leafroll.	ditto

N. B.—Pots with an asterisk are those with the insect cages.

This experiment lends support to the possibility of contamination of disease by tuber-grafting. It was proved in one case (Pots VII) only out of 12 pots. Upon examination it was found that this one was the only combination which had formed organic union with the corresponding diseased half. In

this case only, both halves of healthy and diseased were observed unexhausted, while in other sets healthy halves were all exhausted and diseased halves were on the contrary all remaining unexhausted.

Table VIII.
Showing results of twelve diseased halves.

Pot-mark	Observed results on	
	May 21, 1921.	July 1, 1921.
1	Dwarf, margins of leaflets rolled up, reddish-purplish discoloration appeared.	Characteristic rigidity, discoloration, in the lower leaflets, evidently leafroll.
2	Development pretty good, indication of leafroll observable, no discoloration.	ditto.
3	Dwarf, conspicuous leafroll and discoloration.	ditto (even in the upper leaflets).
4	Somewhat mottled, no leafroll symptoms as yet appear.	ditto (even in midribs and veins).
5	Failed to germinate.	—
6	Tips of the lower leaves rolled, no discoloration as yet, conspicuous mottlings.	Rigid, stunted, discoloration conspicuous, evidently leafroll.
7	Dwarfed, leaves flaccid and stunted.	ditto (in lower half leaves).
8	Development pretty good, no leafroll and no discoloration, conspicuous mottlings.	ditto (very typically).
9	Well developed, inclined toward leafroll, mottlings.	ditto (conspicuous in lower half leaves).
10	Poor development, rigid, conspicuously leaf-rolled with discoloration.	ditto (discoloration even in upper leaves).
11	Normal development, inclined to become leafroll.	ditto (very typically).
12	Superior development, plant and leaflets seem normal.	ditto (especially lower leaves, also in upper).

Thus all diseased half-tubers produced affected progeny as the table indicates.

Remarks:—At the end of the growing season all the control healthy plants were free from leafroll, and on the contrary all the control diseased plants became conspicuously leafrolled. The mother half tubers of from Pot A to Pot L were found all totally consumed. Some of them of from Pot 1 to Pot 12 were found exhausted or remained not digested. In the glass-house insects, if ever seen, were very few. Consequently the protection for them had less meaning, except that the caged plants were entirely free from insects. Possibility of communication of the disease through tuber-grafting was demonstrated in this experiment.

Experiment VII. (Field trial—Sept. 20—Dec. 22, 1921)

In September 1921, sound tubers obtained from Experiment VI, and also diseased progeny tubers from Experiment IV, were planted in the same plots used in the case of Experiment IV. In this experiment it was hoped to see repeatedly the behaviour of the sound plants under the insect cages.

Sixteen sound plants, four plants being under each cage, were protected under the cages. Twelve of them remained healthy while four manifested diseased features in earlier period of the season. Since these four diseased were all those protected under the one and same cage, it is considered, that insects might have get entrance into the cage by chance, though their presence was not noticed.

The plants from the diseased progeny tubers have seriously diseased. The new tubers yielded were practically nothing but culls. Thus the progeny descendants from Experiment I became totally into culls at last.

Remarks:—The majority of healthy plants protected from the attack of insects remained sound.

Experiment VIII. (Field trial—Sept. 20—Dec. 22, 1921)

This is a repeated trial of Experiment V. Diseased tubers from that experiment were planted in the same plots and in just the same manner in accordance with the Plots A, B, C and D. The only difference was that the insect cages were not applied in this case. Every one of the plants in this trial manifested diseased conditions in the earlier period of the growth or at the later end of the season. It was only poor culls practically that were yielded from them.

Experiment IX. (Tuber-grafting trial, Sept. 8—Dec. 15, 1921)

This is a repetition work of Experiment VI, being followed in just the same way, the pots, the soil exactly corresponding to that used in the case of that experiment.

The following three tables will show the results of the trial:

Table IX.

Showing results of control twelve halves of sound tubers.

Pot-mark	Tuber name planted.*	Observed results at the end of the season.
A	A $\frac{1}{2}$	With a sign of leafroll, scarce discoloration in leaflets, mother tuber unexhausted, stolons short, 2 new tubers attached.
B	K $\frac{1}{2}$	Healthy, mother tuber well consumed, stolon longer, with 3 new tubers.
C	C $\frac{1}{2}$	Healthy, mother tuber consumed, stolon longer, with 2 tubers.
D	D $\frac{1}{2}$	ditto, with 4 medium sized tubers.

Pot-mark	Tuber name planted.*	Observed results at the end of the season.
E	E $\frac{1}{2}$	Healthy, with 3 smaller tubers.
F	F $\frac{1}{2}$	Germination failed.
G	G $\frac{1}{2}$	ditto.
H	H $\frac{1}{2}$	Normal, healthy, mother tuber consumed, stolon longer, 3 medium sized tubers attached.
I	I $\frac{1}{2}$	Healthy, mother tuber well exhausted, stolon longer, 3 smaller tubers attached.
J	J $\frac{1}{2}$	Germination failed.
K	K $\frac{1}{2}$	ditto.
L	L $\frac{1}{2}$	ditto.

* A $\frac{1}{2}$ denotes a half piece of the tuber produced in the Pot A in Experiment VI, and so on.

As this table shows all, except that the plant in Pot A was with a sign of the disease, were in normal condition.

Table X.
Showing results of twelve combined sets.

Pot-mark	Tuber name planted.	Observed results at the end of the season.
I	A $\frac{1}{2}$ + I $\frac{1}{2}$	Typically leafrolled, both halves of tubers solid and unexhausted, stolon rudimental, only one small tuber attached.
II	K $\frac{1}{2}$ + 2 $\frac{1}{2}$	Not diseased, sound half of tuber well consumed while diseased half solid and not consumed, stolon longer, 2 new tubers.
III	C $\frac{1}{2}$ + 3 $\frac{1}{2}$	Leafrolled, both halves of tuber solid, stolon short, 3 smaller new tubers.
IV	D $\frac{1}{2}$ + 8 $\frac{1}{2}$	ditto, stolon shorter, 6 cull-tubers gregariously attached.
V	E $\frac{1}{2}$ + I $\frac{1}{2}$	ditto, 3 smaller tubers attached.
VI	F $\frac{1}{2}$ + 6 $\frac{1}{2}$	ditto, only one medium sized new tuber is produced.
VII	G $\frac{1}{2}$ + 7 $\frac{1}{2}$	Not diseased, just alike with the case of II, 2 new tubers produced.
VIII	H $\frac{1}{2}$ + 4 $\frac{1}{2}$	ditto, with 3 new smaller tubers.
IX	J $\frac{1}{2}$ + 9 $\frac{1}{2}$	Germination failed.
X	J $\frac{1}{2}$ + I $\frac{1}{2}$	Typical leafroll, both halves of mother tubers not consumed, stolon short, with 3 smaller tubers.
XI	K $\frac{1}{2}$ + I $\frac{1}{2}$	Germination failed.
XII	L $\frac{1}{2}$ + I $\frac{1}{2}$	ditto.

Among 9 which were germinated and developed, 6 of them became diseased with the decided symptoms of leafroll, and 3 were free from it. Thus the possibility of disease-transmission through tuber-grafting was proved. It is interest to observe that whenever typical leafroll manifest itself in the combined sets it is always found that the two halve-tubers are in a close union and they both do not exhaust. On the contrary when only the sound half

is consumed, that is there the union does not take place, diseased symptoms do not appear.

Table XI.
Showing results of twelve diseased tubers.

Pot-mark	Tuber name planted.	Observed results at the end of the season.
1	12 ½	Typical leaf-roll, rolling and discoloration in the leaves, mother half tuber not consumed, stolon short, 2 smaller tubers attached.
2	2 ½	ditto, with 2 smaller tubers.
3	3 ½	ditto, with 2 tubers.
4	8 ½	ditto, with 3 small tubers.
5	11 ½	ditto, with 3 small tubers.
6	6 ½	ditto.
7	7 ½	ditto, no tubers.
8	4 ½	ditto, with 2 small tubers.
9	9 ½	ditto, with 2 small tubers.
10	10 ½	Germination failed.
11	11 ½	ditto.
12	12 ½	ditto.

Thus every nine that has germinated all manifested typical diseased conditions.

Experiment X. (Juice injection trial—Oct. 28, 1921)

On October 28, 1921, leaflets from a typical leafroll plant were taken and macerated in a mortar adding small quantity of water. The juice thus prepared was imbibed in a small bit of cotton and the imbibed cotton was put upon a wound artificially made on the petiole or on the stem of the healthy plants with the sharpe edge of a knife. A larger piece of cotton was used to overlap the wet bit of cotton in order to protect drying and the whole was bound with a thread. This trial was conducted in a glass-house. Number of the plants used for this trial was only four, the writer being unable to get more of them in this case.

After a week, on Nov. 4, expected interesting results were observed in two of the plants used as follows.

Plant A. Injection was made in the middle height of the stem. There were eight larger or smaller leaves on the stem part upper than the infection point. Of these eight leaves, lower four which were nearer to the injection point indicated a shade of characteristic discoloration and tendency of upward rolling in tip end of their terminal leaflets. These symptoms were

later observed in the leaf just below to the injection point. When this plant was dug out it was found that the mother tuber was well exhausted, and stolons being comparatively longer, the new tuber production was satisfactory. Then it is considered the disease symptom must have appeared as the result of the injection of the juice, the plant being in the primary stage of the disease.

Plant B. Injection was made in the middle height of the stem. No trace of infection was observable in this plant. The juice injection ended in a failure in this case.

Plant C. Injection was made on the petiole of a leaf. In the tip of the terminal leaflet there appeared decided discoloration and upward rolling. Careful examination was made of other leaves, but no symptom of the disease was noticed. Mother tuber of this plant was completely exhausted. Tuber production was satisfactory, many stolons being found long. Thus the said symptom of the disease must be due to the artificial injection.

Plant D. Injection was made on the petiole of a leaf. The result was somewhat obscure, it is safe to say that the infection did not take place in this case.

Remarks:—It seems possible to cause a healthy potato plant to a diseased condition by an artificial injection of the juice of affected plant. It is considered a virus is contained in the juice of diseased plants.

Summary.

- 1) A disease of the Irish-potatoes, which is known among practical growers under a local name "Shashaki"-disease, has been identified with the leaf-roll of the occidental lands. This disease was recently recognized in this country and is already fairly prevalent and is increasing in many places.
- 2) Certain symptomatic changes caused by it in the potato plants, namely, rolling, rigidity, and discoloration of the leaves, dwarfing of the plant, discoloration of the tuber flesh, shortness of stolons, persistence of diseased mother tubers, and marked reduction in the yield, are described as observed in the variety "Nagasaki-aka."
- 3) The pseudohereditary nature, through the tubers, of the disease has been proved by the repeated field tests.
- 4) It was proved that transmission of the disease through the soil is not really the case.
- 5) That the disease is transmissible through tuber-grafting and through juice-injection was demonstrated.
- 6) That the disease can be transmitted from one plant to another by insects seems to be really the case. For it was proved that the healthy offspring of the potato-plant, if protected from the attack of insects, remains free from the disease.

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