On the Primary Occurence of Late Blight of Potatoes and the Change of Chemical Components of the Suscept during the Growing Season

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馬鈴薯疫病の第一次発生並びに馬鈴薯生育期間に於ける体内成分と疫病罹病度との関係について

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I Introduction

Late blight is, no doubt, one of the most serious diseases of potatoes, and elucidation of the problem of its primary occurence and the nature of the resistance of potatoes to *Phytophthora infestans* has great importance to the control of this disease and to the breeding of resistant varieties.

In this report, the possibilities of diseased plants and tubers as a source of the occurence were first examined, and then chemical analyses were carried out in order to get some suggestions regarding the susceptibility of potato plant to *Phytophthora infestans*.

II Primary Occurence of Late Blight

According to literature, overwintering of *Phytophthora infestans* is said to be possible mainly (a) as a saprophyte in the soil, (b) as a oospores, (c) as a mycelium in diseased tubers.

DEBRUYN[4], and MURPHY & ROBERT[8] recognized the occurence of the disease from oospores in the soil but HORI[6] denied this The occurence from diseased shoots was treated by ALCOCK & MCINTOSH[1] but MURPHY & ROBERT showed their opposite opinion. HORI[6] stated the possibility of the occurence from affected plants in northern districts of our country. ASUYAMA[2] & YAMAGUCHI[3], HORI[6], SALMON[7] & WARE[11] and ZAAG[20] described the occurence from affected tubers. YAMAMOTO & KIMURA[19] has already reported some informations on the primary occurence of late blight of potatoes.

1. Vitality of conidia

In order to ascertain a possible source of the primary occurence, the vitality of conidia was examined. Leaves and stems of diseased plants were collected and kept outdoors under natural conditions. These materials were put in Petri dishes with water. The liberation of swarmspores from conidia (sporangia) on plants was examined under the microscope at 12°C or at 20°C.

As shown in Table 1, the number of conidia decreased with the lapse of time, until at the end ol December, a few conidia were recognized in a vital condition.

Using the diseased plants grown in Fall, the same experiment was carried out. Shortly after the beginning of investigations, the number of conidia in vital state decreased suddenly and very few conidia were proved to be in a vital condition.

Table1. Vitality of conidia on the diseased plants which were kept outdoors under natural conditions

Date	June 22	July 14	August 10	September 3	October 11	November 10	December 10	December 30	January 10
Spring Crop	+	+	+	+	+	+	+	+	−
Fall Crop						+	+	+	−

N. B. + = Vital condition
− = Non-vital condition

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Table 2. Average temperature, humidity and precipitation under investigations.

Month	June	July	August	September	October	November	December	January
Temperature (C)	20.9	25.6	25.1	22.7	16.6	10.1	5.1	4.5
Humidity (%)	8.2	8.2	8.0	8.1	8.0	7.7	6.7	8.0
Precipitation (mm)	305.4	96.9	273.9	130.9	167.3	85.4	62.9	222.4

N. B. These figures were supplied from the Matsue Meteorological Station

As to the vitality of conidia on the diseased plant kept outdoors YAMAMOTO & KIMURA[19], ASUYAMA & YAMAGUCHI[2] stated that the conidia survived until the middle of December but not till the following year.

From these considerations, the possibility as a source of primary occurence of late blight for the Fall Crop might be supposed but its possibility for the Spring Crop remains as a question.

2. Translocation of mycelium of *Phytophthora infestans* within potato tubers.

i) The existence of pathogen within the plant bodies was proved under the microscope by examining the cross sections of a potato stem prepared at intervals of 2cm from the ground. Such plant (variety Warba) was obtained in the field in whose neighborhood there was no other cultivation of potatoes.

ii) A healthy potato plant (variety Irish Cobbler) was cut from the ground and was immersed in the conidial suspension of *Phytophthora infestans* (Concentration of conidial suspension was 5 conidia in one microscopic field under 150 magnifications) at 20°C. The conidial suspension was inoculated on the part of the potato plant 15 cm apart from the ground. The sections were examined under microscope after stained with Cotton blue solution.

iii) Tubers inoculated with *Phytophthora infestans* at the end of December were planted on the ground and undergound (5, 10, 15 cm below the surface of ground) at the end of January. Ten tubers which had been kept indoors were also planted in pots on May 10th.

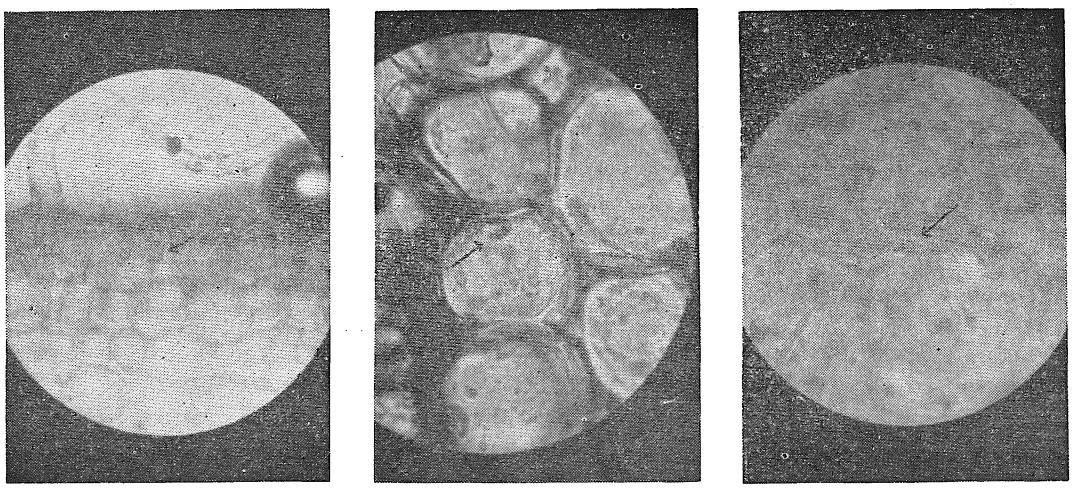
The results were as follows:

i) The existence of mycelium was proved within a potato plant subjected to the primary occurence without a break from the ground to the blighted part. Mycelium was found in epidermis, cortex, xylem, and pith, especially in cortex. From these observations, the diseased spots seemed to be caused by the mycelium which had moved from the diseased tubers.

ii) Within the plant on which *Phytophthora infestans* was inoculated at the part 15 cm apart from the ground, mycelia were found even in the under ground part. And the existence of mycelia was proved on the part 15 cm apart from the ground, when the conidial suspension was inoculated on the cut end of the stem. In this case, mycelia were found in the epidermis, cortex, xylem and pith, though the last case was very few.

These observations coincide with the results of YAMAMOTO & KIMURA[19]. ASUYAMA & YAMAGUCHI[2] desribed that the mycelia of *Phytophthora* grew upward within or around the vessel of stem, but did not kill the tissues. ZAAG[20] concluded that *Phytophthora infestans* could grow upward from the diseased tubers into a stem and that this was the normal way in which the above-ground parts of a plant, whose tuber was diseased, could become infected. Fig. 1 shows the existence of hyphae in a cross section of a potato stem infected with *P. infestans*.

Fig. 1. Mycelium of *Phytophthora infestans* in the cross section of potato stem.



(1) (2) (3)

N. B. (1) Epidermis (2) Cortex (3) Pith

iii) Potato tubers previously were inoculated with *P. infestans* were buried in soil. They were damaged by soft rot. ASUYAMA & YAMAGUCHI[3] described that tubers buried in soil rotted by the middle of February and late-blight fungus was not detected in the following Spring.

Potato tubers, inoculated with *P. infestans*, which had been planted in pots, sprouted on May 28th. In one of them, diseased spots appeared on the nearest leaf to the ground on June 14 and the plant was killed soon. The plant was 4 cm in height. According to ASUYAMA[2] & YAMAGUCHI[3], among 50 potato tubers infected with *P. infestans*, 23 were sprouted, and 6 became sick and had lesions on the top of buds which sprouted from tubers. YAMAMOTO & KIMURA[19] also described that diseased spots occured on the buds which sprang from potato tubers infected with *P. infestans*. ZAAG[20] stated that on the firstly diseased plants, there was always small diseased shoot, starting from infected tubers, which had turned brown. The fungus sporulated on this small brown shoot. These plants exhibited the same symptoms as the plants that had been found in the field. At first the fungus sporuleted only on the lower part of the diseased stem. From these considerations, the possibility of the primary occurence of late blight from the potato tuber in which *P. infestans* existed, was suggested. But severely damaged tubers might be destroyed by soft rot or become cavity for some secondary reasons. These tubers lose their germinating ability, and their possibility of the primary occurence might be diminished by elimination of these dirty tubers.

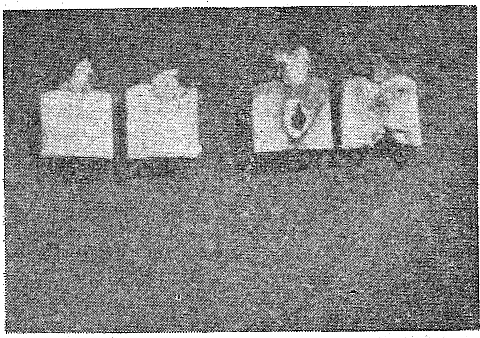
3. Movement of mycelium of *Phytophthora infestans* within potato tubers.

In order to prove the possibility of pathogen moving from tubers to the upper part of the plant, the followng experiments were carried out.

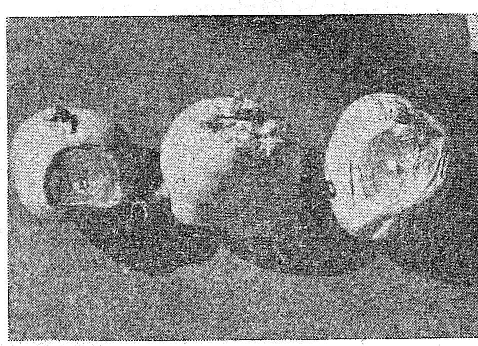
Potato tubers (variety Irish Cobbler) were inoculated with swarmspores of *P. infestans* (Race O) on holes (mm in dianeter, 10 mm in depth) apart 3, 6, 9, 12, 20, 25 and 30 mm from the eyes on which buds had sprouted about 1.0-1.5 cm. Longitudinal and cross sections were prepared from these samples and they were stained with Cotton Blue solution. Then the existence of pathogen in them was examined under microscope.

All of the sproutings infected with the pathogen in the neighboring place of the bud were killed a month later. If pathogen exists in a large amount within the tubers or spread more quickly, remarkable lesion will arise and the buds will be attacked or destroyed by soft rot pathogens and at last totally damaged.

Fig. 2 Potato tubers and buds infected with *Phytophthora infestans*



Healthy



Infected

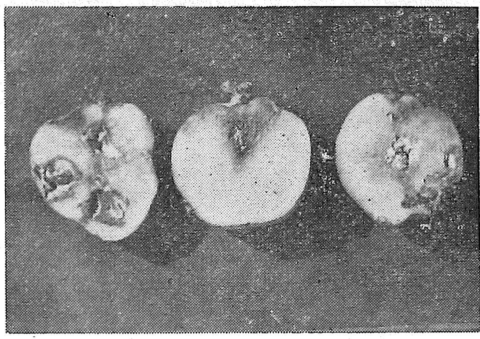
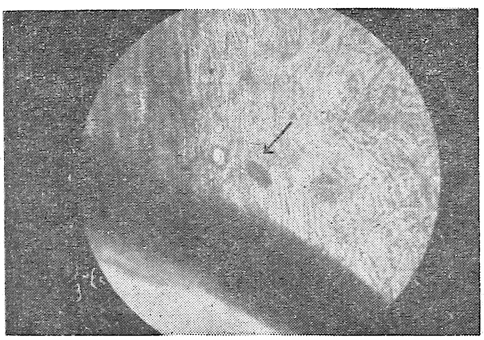


Fig. 3 Mycelia within potato bud infected with *Phytophthora infestans*

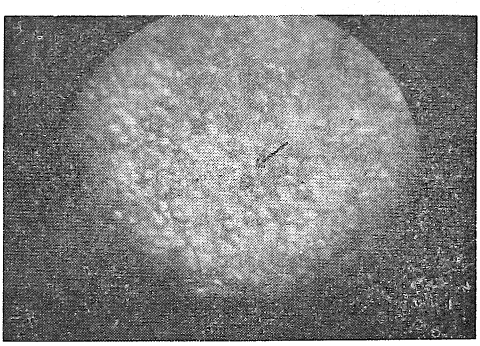
Longitudinal sections at (1) the boundary part of tuber and bud and at (2) the cortex



(1)

(2)

Fig. 4 Mycelia of *Phytophthora infestans*



Such kind of rotted tubers or the tubers with remarkable lesion in them should be eliminated from the seed tubers for actual use but slightly infected tubers might have an opportunity to be as a seed tubers and to become a source of primary occurence of the disease.

Within the tubers, the pathogen, first of all, might have a chance to spread into the vascular bundle and the cortex, then advance to the pith. cavity caused by the attack of the pathogen seems to be that which started from the pith. The movement of pathogens from tubers to buds seems to be caused through vascular bundles and cortices.

TANAKA & AKAI[17] stated that the pathogen invaded the stem through the affected tubers and the diseased part above the ground became a source of the primary occurence and that if the plants were young, all tissues must be invaded and the top killed, but if the plants grew 20 cm in height, the mycelia would move along one side of stem and into leaves. In spite of those circumstances, the top continued their normal growth, except the diseased part.

Ⅲ The change of the constituents of potato plants during the growing season and the susceptility of the suscept to late blight.

The susceptibility of potatoes to the late blight varies with their variety, growing stage, and portion of plant. To account for this variation the morphological characters, and chemical constituents of the suscept, and difference of the reaction of suscept cells to the invasion of pathogen should be put into consideration.

The writers carried out chemical analyses of potato plants in order to get some suggestions to this point.

1. Varietal difference in the susceptibility to late blight among suscepts.

Irish Cobbler and Warba (susceptible), Interspecific Hybrid Kennebec and 48005-68 (resistant) were planted on April 2nd and harvested on June 20th (late stage of flowering period) for chemical analysis. Third compound leaves from the top downward were collected for analitical use. As a inoculum, swarmspores were liberated from conidia on the naturally infected potato leaves at 2°C in 2 hour (concentration of conidial suspension was 5 conidia in one microscopic field under 150 magnifications). A definite amount (0.04cc) of these swarmspores were dropped on the terminal leaflet in the upper or and the lower parts. Inoculation was done at 20°C in a moist chamber in 36 hours and the inoculated were kept in room temperature of about 20°C for 5 days. The shape of lesions was copied on cellophane paper and their area were measured by a planimeter. Ten plants were used for this measurement. These leaves were dried at 60°C. The total nitrogen, total sugar and reducing sugar were analysed with the use of these materials. For quantitative analyses of the total nitrogen, the Kjehldahl method was adopted, i. e. lg of the sample was decomposed with 5g of oxidiser (K_2SO_4 10: $CuSO_4$ 10: $MgSO_4$ 1) and 10cc of H_2SO_4. For the analysis of the total sugar, 0.1lg of the samples was put in Erlenmeyer's flask with a glasstube lm in length, After hydrolysing with 20cc of 25% HCl in water bath in 2 hours, it was neutralysed with HaOH and was added with distilled water until the whole amounts to 50cc. For the determination of the reducing sugar, 20cc of distilled water was added to lg of the sample and an extraction was made in 2.5 hours at 60°C. The extraction was filtered and added with distilled water, thus 50cc of sample being obtained. From this sample, reducing sugars were measured as glucose, using Micro-Bertrand method. Non-reducing sugar was shown as of the total sugars minus the reducing sugar.

As shown in Table 3, the area of lesion is larger in lower leaves than in upper ones. Susceptible varieties have larger lesion area than resistant ones.

Table 3. Varietal difference of diseased spot

Variety	Irish Cobber		Warba		Kennebec		48005-68	
Portion of leaf	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
Area of disease spot (cm^2)	2.92	3.22	2.81	3.06	0.64	2.00	Slight lesion	
S (cm^2)	4.7012		4.2993		0.6400			

N. B. S=Area of diseased spot of upper leaf × area of diseased spot of lower leaf × ½
Figures were shown in the average of 10 plants.

These experiments were done on the leaves previously cut. TAKASE[14] recognized the availability of cut leaves for assaying resistance. TAKASE[15] and those in Dept. Crop, Hokkaido Agr. Exp. Sta.(5) desribed that the number of diseased spot was greater in the upper leaves than in the lower ones and the infection percentage and the conidial formation were also more abundant in the upper leaves. However, in the case of cut leaves grown under the same conditions, the area of diseased spots, was larger in lower leaves than in upper leaves. These findings were supported by YAMAMOTO, KIMURA & KUDO,[18] and TAKASE.[13] As to the varietal difference of the invading and infection percentage, SUZUKI, SUGAYA & HASHIMOTO[2] stated that the percentage in largest in the lower leaves of susceptible varieties. The results of analyses were shown in Table 4.

Every constituent was greater in the upper leaves than in the lower leaves in all varieties used. As for the total nitrogen, resistant varieties contain greater quantity of nitrogen than susceptible varieties, though lower leaves of Kennebec are an exception.

Total sugar was more in both upper and lower leaves of susceptible varieties than in resistaut ones. Although the reducing sugar was more in suceptible varieties, the non-reducing sugars did not show a definite tendency. It might be suggested that there was no definite relation between the area of diseased spot and the contents of total nitrogen.

Generally speaking, the area of diseaed spots is smaller in the upper leaves and in the resistant varieties, and the total nitrogen content is larger in the resistant varieties and in the upper leaves.

Table 4. Varietal difference of total nitrogen and sugar content

Variety	Portion of leaf	Ratio of dry matter to crude matter	Total nirogen: mg per lg of dry matter	Total nirogen: Ratio to orude matter	Total sugar: mg per lg of dry matter	Total sugar: Ratio to crude matter	Reducing sugar: mg per lg of dry matter	Reducing sugar: Ratio to crude matter	Non-reducing sugar: mg per lg of dry matter	Non-reducing sugar: Ratio to dry matter
Irish Cobbler	Upper	14.30	41.69	0.599	225.1	3.218	47.50	0.637	177.5	2.581
	Lower	39.49	32.26	0.299	116.7	1.080	36.33	0.336	80.3	0.744
Warba	Upper	16.75	43.34	0.748	166.7	2.654	58.33	0.954	108.3	1.700
	Lower	9.85	25.06	0.247	108.3	1.066	33.67	0.332	74.7	0.734
Kennebec	Upper	16.72	50.15	0.839	127.1	2.124	27.50	0.460	99.5	1.684
	Lower	7.95	29.01	0.229	106.7	0.862	27.08	0.214	79.6	0.628
48005-68	Upper	16.73	47.10	0.787	134.2	2.241	20.83	0.349	113.3	1.872
	Lower	10.11	34.67	0.308	99.9	1.001	15.00	0.152	85.0	0.849

As to the nitrogen content, YAMAMOTO, KIMURA & KUDO[18] stated that in the upper leaves, the total nitrogen contained proteid nitrogen is smaller than in the lower leaves. SUZUKI, SUGAYA & HASHIMOTO[2] described that the reducing sugar content is larger in the upper leaves than in the lower ones, and until the flowering stage, the leaflet of the resistant varieties had larger quantities of reducing sugar than in the resistant varieties.

2. Suscepts in different growing stages.

Potato plants (variety Irish Cobbler) cultivated in an underground room under artificial light at 20°C. Three days after the inoculation (Race O), concentration of conidial suspension was 5 conidia in one microscopic field under 150 magnifications, the area of diseased spots was calculated by a planimeter. The average figures of the results were shown in Table 5. In all cases the lower leaves showed a greater value than in the upper leaves and the plants in the advanced stage of growth showed larger area.

Table 5. The area of blighted spots of potatoes in different stages of growth

Average height of plant (cm)	20		55	
Position of leaf	Upper	Lower	Upper	Lower
Area of diseased spot (cm^2)	0.130	0.710	0.533	1.180
S (cm^2)	0.046		0.315	

N. B. S = Area of diseased spot of upper leaf × Area of diseased spot of lower leaf × ½

TAKASE[13] stated as a result of in test that a young plant showed a lower susceptibility and Irish Cobbler also indicated a lower susceptibility in the upper and young leaves. As sown in Table 6, the content of total nitrogen is larger in the upper part of plant and, the contents decreased with the lapse of growth. Reducing sugar was fonnd adundantly in the lower leaves, and decreased gradually with the advance of stages.

Table 6. Total nitrogen and sugar contained in potato plants in differeht stages of growth

Average height of plant (cm)	Portion of leaf	Ratio of dry matter to crude matter	Total nitrogen mg per 1g of dry matter	Ratio to crude matter	Total sugsr mg per lg of dry matter	Ratio to crude matter	Reducing sugar		Non-reducing sugar	
7	…	0.795	5.613	0.455	161.8	1.288	50.63	0.402	11.2	0.883
20	Upper	0.829	61.76	0.508	186.8	1.556	51.34	0.425	135.5	0.813
	Lower	0.660	44.29	0.293	150.8	0.997	69.60	0.459	81.2	0.536
55	Upper	0.618	34.41	0.216	11.2	0.686	36.82	0.227	74.4	0.462
	Lower	0.685	18.14	0.125	154.2	1.055	30.08	0.343	104.3	0.712

OTANI[9] stated that a rice plant of high susceptibility to the blast disease had a greater quantity of organic nitrogen compounds, e. g. soluble protein, amino acid, and basic amino acid.

IV Summary

This paper deals with the results of experiments and investigations made on late blight of potatoes to decide its primary occurence and to make clear the relation between the susceptibility of potato plant to late blight and the change of their chemical components in the growing season.

1) On the primary occurence of the late blight of potatoes

(1) Very few conidia of *Phytophthora infestans* on the diseased plants were observed by December.

2) When shoot cuttings of potato plants were dipped in the conidial suspension of late-blight fungus, the mycelia were observed in the epidermis and pith of the stem 15 cm up from the cutting place. In the eld, the writers recognized the existence of mycelia within the plants affected with late blight in their whole bodies down to the ground. After sprouting from the infected tubers, the plants which sprouted from the infected tubers showed the symptoms of late blight disease.

3) The translocation of the mycelia from tubers to shoots was investigated. It seems likely that mycelia invade shoots from tubers through cortices and vascular bundles.

(2) On the relation between the change of chemical components in the growing potato plants and the occurence of late blight.

The writers measured the area of late-blight lesion caused by inoculation of *Phytophthora infestans* and analysed the total nitrogen, and the reducing and non-reducing sugar in leaves.

The area of lesions were larger in the lower leaves, and in the later growing stages, and in the susceptible varieties. Negative correlation was shown between the total nitrogen content and the area of late-blight lesion in leaves.

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摘　　　要

(1) 馬鈴薯疫病の第一次発生

i) 罹病茎葉上の分生胞子の生存期間をしらべたところ，12月中は極くわずかではあるが生存を認めた。

ii) 幼葉を地際から切断し，断面を分生胞子懸濁液に浸けたところ，上方15cm位の所迄，表皮,皮層，木部，髄に菌糸を認めた。又地上15cmの所に接種したものは地際迄菌糸を認めた。又圃場に於て初発した茎葉中の菌を観察したが，地際迄菌糸の存在を認めた。病薯を鉢植にし，発芽したものの中で幼茎に発病を見たものがあつた。

iii) 塊茎中の菌糸の芽への移行をしらべた。感染初期には芽の皮層，維管束を通つて芽へ侵入するようである。

(2) 馬鈴薯の生育期間中に於ける体内成分の変化と疫病罹病との関係

生育時期別に罹病性品種及び抵抗性品種を用いて，接種による病斑面積，全窒素，全糖，還元糖，非還元糖含量を夫々しらべた。

接種試験の結果，病斑面積は上葉より下葉の方が大きく，又生育段階の進んだものが大で，品種間では抵抗性品種より罹病性品種が大きかつた。全窒素に於ては以上の結果とある程度の相関を示し，病斑が大きい場合に全窒素量は少なかつた。