ON A DISEASE OF SOME LEGUMINOUS PLANTS CAUSED BY *Ceratophorum setosum* KIRCHNER

By

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(With Plate II)

ケラトフォールム、セトースム菌の寄生に基因する二三荳科植物の病害に就きて

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1. Introduction

In Hokkaidō, *Lupinus polyphyllus*, known by the name of *Hauchiwa-mame* or *Tachifuji*, is commonly cultivated as an ornamental plant. A destructive disease of the leaves and sometimes petioles of this plant, due to a Hyphomycetous fungus, is common in the vicinity of Sapporo and very probably also in other districts, where the plant is cultivated. So far as I know, it has never been described, although the disease has been recognized by us and some studies have already been done in our laboratory since the summer of 1915. The investigation on this subject was at first undertaken by the writer, partly with the assistance of Mr. T. FUKUSHI in order to identify the specific name of the causal fungus and to make a close observation of it's pathogenetic characters. Our observations in the field, the experiments in the laboratory, as well as the studies of the literature relating to the subject have not only led us to recognize the fungus to be *Ceratophorum setosum* Kirchner of *Dematiaceae* which has yet been known as a parasite on the leaves of some *Cytisus*, but to prove at the same time that *Pestalozzia Lupini* Sorauer of *Melanconiaceae* is identical in every respect with the present fungus.

By a careful search in the Botanic Garden of our University for the disease of the *Cytisus*-leaves in the summer of 1917, we collected the fungus in question affecting the leaves of *Cytisus capitatus* and one other species of the same genus as well as those of *Lupinus polyphyllus*. The writer isolated the fungus from each

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of these host-plants, and then made their comparative cultural studies as well as some cross inoculations with expected success.

2. Historical Review

In 1892, KIRCHNER (2) reported the fact that the seedlings of *Cytisus capitatus* were attacked by a disease similar to the downy mildew caused by *Peronospora Cytisi* in its external appearance. He stated the fungus, for which he proposed the name of *Ceratophorum setosum*, to be the cause of this disease. The description of the fungus appeared in 1895 in SACCARDO's Sylloge Fungorum, Vol. XI. In 1905, ROSTRUP (8) reported that the same fungus affects the leaves of *Cytisus Laburnum*. He made the artificial cultures of the fungus and carried out the infection-experiments. In 1908, MALKOFF (5) reported this fungus to be parasitic also on *Cytisus Laburnum* in Bulgaria.

In 1910, LINDAU gave the description of this fungus in RABENHORST's Kryptogamen Flora, Aufl. II, Abt. IX and stated it to be parasitic on the leaves and stalks of the seedlings and also on the leaves of grown plants of *Cytisus capitatus* and *Cytisus Laburnum*. Further he remarked on the hyphae and chlamydospores of the fungus in the host-tissue and in the artificial media and also on the setae of the conidia accepting the results of the investigations of ROSTRUP and others. In 1913, LIND (4) revised the Danish fungi as represented in the Herbarium of E. ROSTRUP; and in his work he mentioned the fungus as parasitic on the leaves of *Cytisus Laburnum*, giving the ROSTRUP's figures of the hyphae and chlamydospores as well as the conidiospores. But up to the present time, no one has reported on the identity of *Pestalozzia Lupini* Sorauer to *Ceratophorum setosum* Kirchner. It was in 1898, that WAGNER and SORAUER (13) described *Pestalozzia Lupini* as the cause of a disease of *Lupinus Cruikshanksii*, *L. mutabilis* and others. Subsequently, in SACCARDO's Sylloge Fungorum, Vol. XVI (1902) and RABENHORST's Kryptogamen Flora, Aufl. II, Abt. VII (1903), the description of the fungus was reproduced without any comment. In STEVENS' "The Fungi" (12), which was published in 1913, both names were recorded separately in different families.

3. Symptoms of the Disease

KIRCHNER (2) described the symptoms of the one year seedlings of *Cytisus capitatus*. The important part of his description is as follows: "Die Krankheit äussert sich gleichfalls im Erscheinen brauner Flecke

auf den jungen Pflanzen; diese Flecke treten auf Blättern, Blattstielen und Stengeln auf, sind auf beiden Seiten der Blattspreite gleichmässig sichtbar, von dunkelbrauner Farbe, anfangs sehr klein, später über das ganze Blatt oder den grössten Teil desselben sich ausbreitend." In our cases of the disease, the general effect on the plant is more or less different according to the difference of its hosts. The *Cytisus*-leaves affected with the fungus generally fall down very quickly; but the affected leaves of *Lupinus polyphyllus* dry up gradually in summer and remain attached for a long time. Therefore, the brownish shriveled leaves are readily seen even at a distance.

Symtoms of *Cytisus sp.* The *Cytisus* plants affected by the disease and studied by us were all of grown plants. In the case of *Cytisus sp.*, whose name is not identified, the fungus produces characteristic spots on its leaves. The diseased areas show first as dark brown very minute spots. These rapidly enlarge, and soon form brown or blackish brown spots 4 to 8 mm. in diameter. The spots appear on the both sides of the leaves, but on the upper surface they are somewhat deeper in color. These spots are mostly circular or semi-circular or sometimes irregular in shape. The margin of the spot is not especially bordered with a deeper color, but on the larger spot the concentric dark-colored rings are formed generally. The diseased leaves fall down immediately. The symptoms in the case of the grown plant of *Cytisus capitatus* are, on the whole, similar to those of the above described *Cytisus sp.* But it seems to me that the spots are generally smaller in this case and rather irregular in shape. The semi-circular spots extended from the tip of the leaves are often found.

Symptoms of *Lupinus polyphyllus*. WAGNER and SORAUER (13) described the symptoms of the disease of *Lupinus Cruikshanksii*, *L. mutabilis* and others caused by the present fungus. They reported that the fungus attacks the cotyledons as well as the leaflets of these plants. In the vicinity of Sapporo, we have always noticed the disease affecting the leaves and rarely petioles, but we have not yet had an opportunity to find the cotyledons attacked. The fungus produces a characteristic spot on the leaflets of the affected plant. The diseased areas show first as small, brown or dark brown spots on the both sides of the leaves. They are irregular in shape in the early stage. But they rapidly enlarge, producing at last various sized brown spots, which are mostly circular or semi-circular in shape and 5–10 mm. in diameter, not seldom exceeding 14 mm. The margin of the spot is not especially bordered with a deeper color. The spots become confluent often forming large irregular brown or dark brown patches on the surface of the leaves. They extend also often from the margin to the center of the

leaflets. The old spots are brown or dark brown and sometimes chestnut-brown on the upper surface of the leaves, and very much lighter on the under side. Although the spots are not bordered with a special color, they are on the upper surface sharply separated from the green healthy part of the leaf, while on the under surface their margin is not distinct. The large circular spots show sometimes many concentric rings of the dark colored lines on the upper surface. The diseased leaflets die early and soon dry up in the summer or in the early autumn.

4. Morphology of the Causal Fungus

The mycelium of the fungus is composed of slender, hyaline, septate hyphae which grow mostly between the cells of the host. Some of the hyphae, appearing on the upper side of the leaves, creep on the surface of the diseased spots. A section through the diseased spot shows the hyphae to be ramifying in the tissue of the leaf, and the chloroplastids destroyed. The mycelium within the tissue or on the surface varies greatly in diameter. Many of the lateral branches are very slender, while the older hyphae may become greatly swollen. The hyphae found in the tissue are hyaline, many septated, 2-10 μ in diameter. The creeping hyphae on the surface are generally poorly developed and are also hyaline. In cultures, the mycelium is at first colorless, then gradually turning into light brown or brownish gray in color when seen under a microscope; and it develops largely in substratum, where it forms many intercalary knots of chlamydospores presenting an appearance of black dots in the media to the naked eye.

Conidiophores are short and straight and are formed here and there on the creeping hyphae on the surface of the leaf. They are generally simple, but rarely branched. They are not easily distinguished from the normal hyphae in their appearance. The spores are at first produced as the hyaline, club-shaped swellings at the tips of the conidiophores. Afterward a septum is formed at the base of each swelling. In the course of development, they produce 4 or 5 rarely 6 transverse septa, and 3 or 4 sometimes more setae from the uppermost cell. At the same time, the spores turn gradually into brown or dark brown color. The matured spores are very much like to those of *Pestalozzia* in their morphological characters. They are cylindro-fusiform in shape and mostly more or less curved. The middle one or two cells of the spore are darkest, while the basal and terminal end cells are generally very light in color or sometimes nearly hyaline. Accord-

ing to my own measurement the matured spores are 56–72 × 15–20 μ in size, and at each septum they are more or less constricted. From the terminal light colored cell, three or four and sometimes more wart-like swellings appear in the early stage of their development; and the swellings grow little by little into the threads or hairs resembling to the setae of *Pestalozzia*-spores. One of these hairs is always first produced from the tip of the uppermost cell and the others then succeeding from the side more often near the septum of the same cell. They are at last very long, becoming often longer than the length of the spore, and always hyaline. These hairs or setae are sometimes branched near the base and as a consequence they present an appearance of the presence of many hairs. The setae are 2.5–3 μ in width at the basal portion.

The morphological characters above delineated are entirely similar to the descriptions and remarks of *Ceratophorum setosum* and *Pestalozzia Lupini* given by various authors. The latter name ought to be treated as a synonym of the former. Although the measurement and the number of the cells of the spores are more or less different according to the authors as shown in the following table, such a small difference has little value in distinguishing the species, when other important characters are in perfect accord.

Author	Size of the spore	Number of the cells of a spore	Given name of the fungus
KIRCHNER	40–80 × 15–19 μ	3–8 (mostly 6)	*Ceratophorum setosum*
WAGNER & SORAUER	54–60 × 16 μ	5–6	*Pestalozzia Lupini*
The writer	56–72 × 15–20 μ	5–6 (rarely 7)	*Ceratophorum setosum*

The spores germinate usually first from the basal cell, though they may also germinate from an upper dark colored cell—the next cell from the setae-bearing terminal cell, or from both of them at the same time. Any other cells have also the power to germinate under suitable conditions. These germ-tubes are relatively wide in diameter, branching at once and forming transparent septate mycelium. For germination tests, distilled water and host-plant decoction were used in our experiments.

5. Name of the Causal Fungus

From the morphological characters, we may easily recognize the present fungus to be a member of the family *Dematiaceae* of the *Hyphomycetes*. A careful consideration of the fungus, both in the natural condition and in the artificial culture, has led the writer to believe it to be *Ceratophorum setosum* Kirchner, as already stated. The genus *Ceratophorum* was first described by SACCARDO (9) in 1880; and in 1895 he founded the subgenus *Pleiochaeta* in his Sylloge Fungorum,

Vol. XI. But previous to his announcement, in 1892 KIRCHNER (2) already remarked in his paper about the subgenus as follows: "Meine Vermutung, dass er zu der Gattung *Ceratophorum* Sacc. gehöre, wurde auf meine Anfrage in liebenswürdigster Weise von den Herren Dr. DETONI und Prof. SACCARDO in Padua bestätigt, mit dem Bemerken, dass der Pilz als eine neue Art in die von SACCARDO noch nicht veröffentlichte Sektion *Pleiochaeta* (mit 2 oder mehr Borsten an der Spitze der Conidien) zu stellen sei."

The synonym, literatures and hosts of the fungus under consideration are as follows:

Ceratophorum setosum Kirchner

KIRCHNER: Zeitschr. f. Pflanzenkr. Bd. II, S. 324, 1892.
SACCARDO: Sylloge Fungorum. Vol. XI, p. 622, 1895.
ROSTRUP: Botanisk Tidsskrift. Bd. XXVI, p. 312, 1905.
MALKOFF: Annales Mycologici. Vol. VI, p. 36, 1908.
LINDAU: Rabenhorst's Kryptg. Fl. Aufl. II, Bd. I, Abt. IX, S. 24. 1910.
STEVENS: The Fungi which cause Plant Disease. p. 610, 1913.
LIND: Danish Fungi. p. 526, 1913.

Syn. *Pestalozzia Lupini* Sorauer

WAGNER & SORAUER: Zeitschr. f. Pflanzenkr. Bd. VIII, S. 266, 1898.
SACCARDO & SYDOW: Sylloge Fungornm. Vol. XVI, p. 1014, 1902.
ALLESCHER: Rabenhorst's Kryptg. Fl. Aufl. II. Bd. 1, Abt. VII, S. 694, 1903.
STEVENS: The Fungi which cause Plant Disease. p. 560, 1913.

Hab. On the leaves of *Lupinus polyphyllus* (new host), *Cytisus capitatus* and *Cytisus sp.* (recieved under the name of *Cytisus uralensis*)—Botanic Garden, College of Agriculture, Sapporo, Hokkaidō.

6. Cultural Characters of the Causal Fungus

The fungus is easily isolated by transferring small pieces of the diseased tissue on the poured plates of the host-leaf decoction agar. I have kept growing the causal fungus thus isolated from three different hosts for the sake of comparison. But those three strains showed the same features in their cultural characters. The cultural media which we have used are the slants in test tubes containing corn-meal agar, apricot-decoction agar and host-leaf-decoction agar, and also the soy-agar-plate in the Erlenmeyer's flasks.

Although I have kept the fungus under observation for a year, I have not succeeded in producing the ascospore stage and even the conidia are rarely pro-

duced in the culture-media I have used, while the chlamydospore production is generally conspicuous. On the soy agar and apricot-decoction agar media, the aerial growth of the mycelium is comparatively vigorous, and there is at the same time some growth in the substrata. The characters on different media are as follows:

a. *Cultures on the leaf-decoction agar of Lupinus polyphyllus.* The mycelium begins to spread from the infected portion at first as a white or light brown weft and spreads rapidly toward the edge. The mycelium is apt to creep on the surface of the medium, and the aerial mycelium is very scanty. The mycelium turns gradually brown in color. After a while, numerous small black dots are seen in the peripheral portion of the medium, where the creeping mycelium is thinly formed. Under a microscope, I have proved those dots to be the knots of chlamydospores. The conidiospores are rarely produced in old cultures.

b. *Cultures on the corn-meal agar.* The mycelial growth on the surface of this medium is sometimes entirely lacking or very scant. The mycelium grows entirely in the medium and it presents macroscopically as dense radiating groups of many grayish-black fine stripes or lines. On these stripes or lines are found numerous black spots, which are the knots of the chlamydospores. The conidial production is, however, very scant.

c. *Cultures on the apricot-decoction agar.* On this medium, the fungus grows vigorously and caused it to become more or less darkened. At first a white aerial mycelium grows actively and after a while its color gradually turns gray and sometimes blackish gray. The knots of the chlamydospores are also more or less produced on the hyphae growing in and on the medium.

d. *Cultures on the soy agar.* The use of the Japanese soy or *shōyu* as a cultural medium for fungi was at first proposed by Prof. M. MIYOSHI (6-7) in 1895. On this medium, a velvet-like brownish-gray mycelial layer is produced within a week after inoculation. The chlamydospores are produced abundantly. The conidiospore is not produced even on the medium of two months old.

7. Chlamydospores of the Causal Fungus

The chlamydospores are most abundantly produced in the corn-meal agar cultures, although in other cultures they are also formed to more or less extent. The hyphae, from which the chlamydospores are to be formed, turn gradually brown or light brownish gray in color, accompanied by the process of the division into a series of numerous short ellipsoidal cells. These cells transform at once into the chlamydospores first by their swelling and then by the thickening of their cell-walls. Consequently the chlamydospores thus formed are usually arranged in a chain, although a single chlamydospore is rarely produced intercalarely in the hyphae. Our fungus is, however, characterized by the formation of the knots of the chlamydospores, having remarkable forms. A knot of the chlamydospores consists of a mass composed of from a few to thirty cells having a thick and deep brownish-black cell-wall and granular contents. The knots

seem to be originated by the more or less dense formation of short lateral irregular branches composed generally of a few chlamydospores which adhere firmly to each other forming irregular dark masses. The single chlamydospores are globose, subglobose or ellipsoidal in shape, or they become often more or less angular in shape by the mutual pressure of the adjoining cells in a knot. The chlamydospore-formation of the fungus was first noticed by ROSTRUP (8) in 1905, and LIND (4) reproduced two of the ROSTRUP's figures in his Danish Fungi in 1913.

8. Inoculation Experiments

The inoculation experiments, from which the conclusions of the parasitism of the fungus and also the identity of the fungus which attacks *Lupinus polyphyllus*, *Cytisus capitatus* and one other species of the same genus have been drawn, were made in the laboratory of our institution in the month of July of this year.

Experiment I.

On July 4, the first inoculation test was made on the healthy leaves of the seedlings of *Cytisus capitatus*, which were growing in a pot and kept on a laboratory table. Small bits of the mycelium and chlamydospores from corn-meal agar cultures isolated from *Lupinus polyphyllus* were placed by a sterile needle in drops of water on both sides of the uninjured leaves, which were previously sprayed with sterilized water. To keep it moist, we placed the pot under a bell-glass covered inside by the moistened filter-paper for two days.

All of the leaves which had been inoculated showed signs of infection at about the fourth or fifth day. The disease did progress rather rapidly, forming blackish brown spots on the leaves which gradually shrunk, dried up and at last dropped to the ground. But all uninoculated leaves treated in the same way as controls showed no changes for a long time.

Experiment II.

On July 6, the second inoculation test was made on the healthy young leaves of *Lupinus polyphyllus*. When the plant was transplanted into a pot, all the old leaves were cut off and the pot was kept in the laboratory in order to avoid the natural infection.

Small bits of the mycelium and chlamydospores from corn-meal agar cul-

tures isolated from a species of *Cytisus* were placed by a sterile needle in the drops of water on both sides of the uninjured leaves, which were previously sprayed with sterilized water. To keep it moist, we treated in the same way as in the case of the first experiment. The inoculated leaves showed the signs of infection at about the third or fourth day as small patches of brown spots. The diseased leaflets wilted then gradually from the ends. In the case of this experiment, we inoculated the fungus also on some healthy petioles in the same way. It showed also the positive result at about the fifth day and at last the petioles were broken at the infected points. Although the symptoms of the disease are rather different from the naturally infected leaves, such a difference is undoubtedly due to the wideness of the inoculated area and youngness of the leaf. The leaves treated in the same way as controls showed no changes for a long time.

Experiment III & IV.

On July 10, the third and fourth inoculation tests were made on the healthy leaves of *Lupinus polyphyllus* grown in pots. In those cases, we used also bits of the mycelium and chlamydospores from corn-meal agar cultures as the inoculum. But in the case of the third test, the fungus isolated from *Cytisus capitatus* was used, and in the case of the fourth test, that isolated from *Lupinus polyphyllus* was used as the inoculum. The methods of these inoculation experiments were quite the same as those above described. The results of these two cases were nearly the same. The signs of the disease could be detected on the leaf as brown patches at about the fourth or fifth day and then the infected area shrunk gradually and at last the leaflets wilted from the end. But the control-leaves treated in the same way were all healthy for a long time.

Judging from the results of the above experiments, we may safely infer that the causal fungus isolated from each of the three different hosts belongs all to one species, and that it is a virulent parasite, easily infecting the uninjured leaves. It is not always easy to explain how a parasite gains entrance into its host. It enters always from the inoculated portion on the both sides of the uninjured leaves and also on the healthy petioles. Although we have not been able to demonstrate clearly whether the fungus makes the stomatal infection or the cuticular, we have a conviction from what we have observed that the cuticular infection is taking

place when a bit of the mycelium and chlamydospores was used as the inoculum. Unfortunately we have not been able to make infection experiments with the conidiospores, as they are very rarely produced in the artificial cultures we have used.

9. Summary

(1). A serious leaf-spot disease of *Lupinus polyphyllus* is prevalent almost every year in the vicinity of Sapporo and probably also in other districts, where this plant is cultivated.

(2). This disease is caused by *Ceratophorum setosum* Kirchner, which has been known up to the present time only as the parasite of certain species of *Cytisus*.

(3). According to the description and figures, *Pestalozzia Lupini* Sorauer described by WAGNER and SORAUER as the parasite of *Lupinus Cruikshanksii*, *L. mutabilis* and others does not belong to the *Melanconiaceae*, but is identical to *Ceratophorum setosum* and should be treated as its synonym.

(4). By a careful search we have collected the same fungus severely affecting the leaves of *Cytisus capitatus* and one other species of the same genus in the Botanic Garden of our University. The identity of the fungi which attack the two species of *Cytisus* and *Lupinus polyphyllus* was proved by cultural and inoculation experiments.

(5). Pure cultures of the causal fungus were isolated from the diseased areas on the leaves of each of these three host-plants and its parasitism has been demonstrated by successful cross inoculations on the healthy plants.

These investigations were carried out in the laboratory of plant pathology of the Hokkaidō Imperial University. The writer wishes to express here his heartiest thanks to Prof. Dr. KINGO MIYABE for his kind suggestions and criticisms. He wishes also to express his thanks to Mr. T. FUKUSHI and other gentlemen who have kindly helped him in various ways.

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Explanation af Plate

1—3. Immature conidiospores. × 438.

4—6. Mature conidiospores. × 438.

7. Hyphae and a knot of the chlamydospores. × 438.

8—9. Hyphae and knots of the chlamydospores.

10. Diseased leaf of *Lupinus polyphyllus*.

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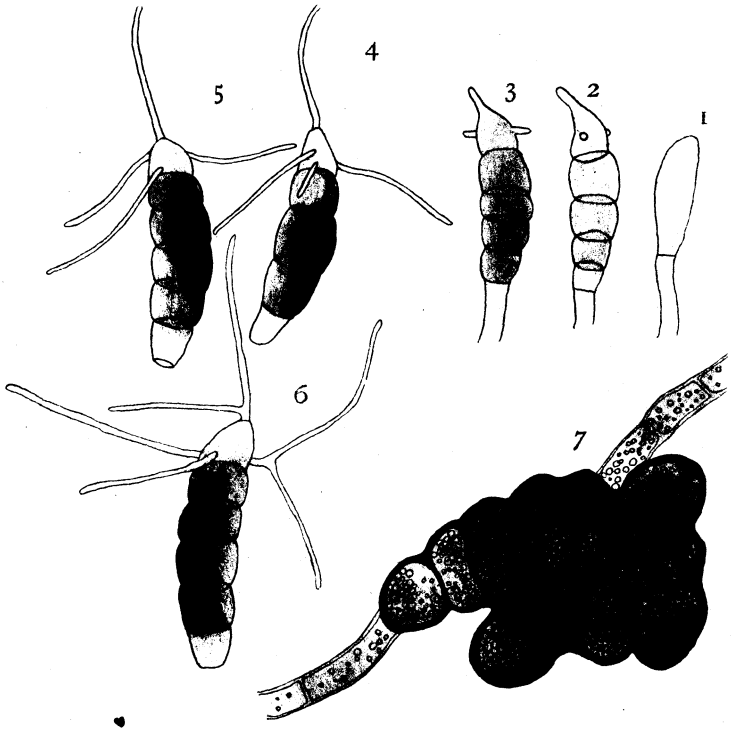

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摘　　要

廣く各所の庭園に培養せらるゝ觀賞植物**ハウチハマメ**の葉に線菌族に隷入すべき菌の寄生に基因する一病害發生し、札幌地方に於ては年々歲々被害を見ざる事なく、殊に病原菌の侵襲力極めて强烈なるが爲め晩夏若くは初秋の頃全株既に萎凋乾枯の慘狀を呈する事稀ならず。然も此病害猖獗を極むるは多くの場合開花期を過ぐるを以て世人の注意を喚起する事少なし、從て本邦に於ては未だ之に關する記錄の徵すべきものなしと雖、庭園の美觀を失するが故に園藝的見地より寔に觀過すべからざる病害なり。

予は大正四年八月初めて該病害を發見し、爾來幾年常に文獻に注意し、機會ある毎に之れが研究と觀察とを忘れず、今夏漸く報告すべき結果を得たり。即ち予は該病々原菌檢索の結果從來單に *Cytisus* 屬植物の寄生菌として廣く知られたる *Ceratophorum setosum* Kirchner 菌に外ならざるを認めたるのみならず、又從來 *Lupinus mutabilis, Lupinus Cruikshanksii* 等の寄生菌として知られたる *Pestalozzia Lupini* Sorauer 菌は全然本 *Ceratophorum setosum* 菌を誤りて命名記載したるものなる事を明かにせり。予は特に本菌を *Cytisus* 屬の植物より採集せんと欲し、搜索の結果遂に昨大正六年七月札幌農科大學植物園內に培養せる *Cytisus capitatus* 外一種の *Cytisus* 屬植物の葉が甚だしく之に侵蝕せられ居るを發見し、是等二種の *Cytisus* 屬植物並に**ハウチハマメ**より別々に病原菌を分離し、比較培養を爲し、更に交互接種試驗を施行し、終に豫期の結果を獲得せり。