A WILLOW-CANKER DISEASE CAUSED BY *PHY-*
SALOSPORA MIYABEANA AND ITS CONIDIAL
FORM *GLOEOSPORIUM*.

BY

TEIKICHI FUKUSHI.

(福士貞吉:—*Physalospora Miyabeana* 及其分生孢子 *Gloeosporium* に依りて生ずる杞柳の炭疽病に就きて)

In the early autumn of 1919, while visiting the College Nursery, Mr. K. Sakurai called the attention of the writer to a number of the Japanese osier, *Salix purpurea* L. var. *angustifolia* affected with a canker disease. It was apparently unlike the bacterial disease of this plant, and a preliminary examination of these specimens by Dr. T. Hemmi and the writer revealed the constant association of two forms of fungi, *Physalospora* and *Gloeosporium* with the lesions. Since some species of *Physalospora* are known to possess a *Gloeosporium* as their conidial stage, the writer was led to the idea that there would be a possible connection between these two fungus forms; and this assumption has been verified by a number of cultural experiments.

In the next season there appeared again the disease in the same nursery, and the writer was successful in isolating a *Gloeosporium* from the affected leaves and stems of the osier and also in inoculating the spores thus obtained on healthy plants with positive results.

Studying more carefully the symptoms of the disease as well as the nature of the causal fungus, the writer has come to the conclusion that the disease is due to a heretofore-undescribed species *Physalospora* and its conidial stage *Gloeosporium*.

It is highly probable that this disease has been prevailing for many years at several osier-holts in Hokkaido, since certain basket-makers inform us of the outbreak of this trouble at Kotoni, Barato, Urashinotsu, and Shinshinotsu in the province of Ishikari. According to a basket-maker this trouble has been prevalent at Kotoni since seven years and at some osier-holts, as at Motomura near Sapporo, the osier-

cultivation was abandoned owing to the severity of the trouble. He showed the writer a few osier-rods produced at Barato and Shinshinotsu, which were apparently affected with this disease. The affected rods are, as he said, apt to be broken at the lesions, producing the baskets of inferior quality on account of the brownish spots on the rod after the bark has been peeled off.

In the fall of 1920, another type of willow canker disease, which is probably due to a fungus parasite belonging to the genus *Marssonina*, made its appearance at several osier-holts in the vicinity of Sapporo. The willow plants in the College Nursery were also affected with the same disease. According to the basket-maker the disease is less injurious to the osier-cultivation in this district. The symptoms of the two maladies are entirely different from each other as will be shown later.

SYMPTOMS OF THE DISEASE.

The disease appears on the stems as well as on the leaves and most abundantly on the upper part of the plant. The lesions on the stem are whitish gray to gray in color and elliptical in shape, varying 5 to 30 mm. in length. Often the lesions become united involving a considerable portion of the stem or extending so widely as to completely girdle it. The stem is often attacked at the base of petioles, although the points of infection are by no means confined to such a portion only. The lesion is usually surrounded by a blackish border line with considerably sunken central area, where black minute pustules are found in cluster. These pustules are conspicuous on account of a contrast in color with the surrounding tissues. Later the lesion may become cracked and finally naked exposing the woody tissue by the peeling off of the dead bark tissues.

The indication of the disease on the leaves are dark brown spots on the upper surface. The spots are circular or irregular in outline, measuring 2 to 5 by 3 to 7 mm. and often showing concentric marking. When the lesion involves the midrib, a considerable portion of it may be lengthwise blackened.

The symptoms of the other disease, probably due to *Marssonina*, are entirely distinct from those just mentioned. The disease is characterized, by elliptical tar-black lesions on the stem and punctiform black spot on the leaf. The lesions on the stem are not sunken and there the pustules are rather scarce and indistinct.

CAUSE OF THE DISEASE.

Cross sections of the canker spot on the stem show the typical acervuli and the spores characteristic of the genus *Gloeosporium*. Under the epidermis is found a stromatic layer of mycelium which extends through the cortical tissue to the wood. The cortical tissue is discolored and disorganized and the woody portion is also discolored, showing the effects of the fungus. From the upper surface of the mycelial layer are developed short conidiophores which produce masses of conidia. With the accumulation of the conidia the epidermis is broken and the spores are exposed on the surface of the stem. On the leaf the acervuli are scattered in the spots and provided with brown setae at the peripheral portion.

An Ascomycetous fungus was generally associated with the lesions similar in appearance to those caused by *Gloeosporium*. Perithecia are immersed in the host tissues, with the mouths breaking through the epidermis. The characters of the perithecia, the paraphysate asci, and the one-celled hyaline, ellipsoidal ascospores correspond well to those of the genus *Physalospora*.

Several attempts were made to prove the connection between these conidial and perithecial forms. The fungus could readily be cultured both from the conidial-masses and from small pieces of the diseased tissues, on any ordinary culture media, such as corn meal agar, oat meal agar, apricot extract agar, onion soy agar and willow-stem decoction agar, producing the spores of the *Gloeosporium*-type in 3 to 5 days. Since no perithecial stage was developed in the cultures just mentioned during a period from October to January (1920), the fungus was transferred to sterilized pieces of willow-stem in culture tubes. On the 23rd of February, 5 days after the transplanting, the fungus made its growth on the surface of the stem, forming a perfectly white mycelial growth and producing the conidia of the *Gloeosporium*-type. Twenty days later, small mycelial tufts of grayish color were developed here and there rupturing the epidermis.

Microscopical examination of the section of such a portion reveal the thick stromatic layer of the fungal hyphae below the epidermis, producing the conidiophores in large numbers and exposing the conidial mass through the ruptured epidermis. The hyphae were found profusely extending in all directions through the host tissues. On March 17, the perithecia were found, immersed in the stromatic layer of the fungal hyphae under the epidermis as well as on the surface of the substratum. The

perithecia arose singly or in little aggregate groups in the stroma and contained numerous asci and ascospores having the same characters as those produced in the lesions of the host plant in nature. Even at that date there was no indication of the formation of the perithecia on the agar media.

MORPHOLOGY OF THE CAUSAL FUNGUS.

1. ASCOGENOUS STAGE.

The perithecia are immersed in the host tissues with the mouths slightly protruded through the ruptured epidermis, giving rise to the minute pustules in the lesions. The perithecia are globose, dark brown, 100–170 μ in diameter, mostly solitary, or sometimes two or three of them aggregated in such a manner as if to present an appearance of being produced in a stroma. The asci are numerous, club-shaped, 55–75 μ long and 11 μ broad, with slender paraphyses measuring $66 \times 1.2 \mu$. The ascus contains 8 ascospores in an uniseriate or irregularly distichous arrangement. (Fig. 4). The ascospores are oblong-ellipsoidal, often slightly curved, unicellular, hyaline and granulated at both ends, and measure $15-17 \times 5.5-7 \mu$. (Fig. 5). The ascospores produced in the culture were somewhat smaller in size, being $14-16.5 \times 4.5-5.5 \mu$. The ascospores germinate readily in water, usually dividing by a median septum and then producing a germ-tube at the end of the spore. At the end of the germ-tube, there forms usually a light brown colored appressorium, 6.5–

Fig. 1. Conidia. X500. Fig. 2. Germinating conidia X 500. Fig. 3. Setae X 500. Fig. 4. Asci X 500. Fig. 5. Ascospores X 500. Fig. 6. Germinating ascospores X 500. Fig. 7. Conidia produced in the cultures X 500. Fig. 8. Chlamydospore-like bodies in the cultures X 500

$7.5 \times 7.5-9.5 \mu$. Sometimes the spore germinates at both ends and sometimes with a germ-tube which produces no appressorium. (Fig. 6). In one of the germination experiments on November 7, it took 5 hours for the ascospores to germinate and produce the appressoria in water at the temperature of 15°C .

2. CONIDIAL STAGE.

The acervuli are formed on the stromatic hyphal tissues under the epidermis of the willow-stem. From the outer part of the stromatic layer, closely arranged conidiophores arise, and these, by their growth raise the epidermis and ultimately rupture it, showing minute pustules on the surface. The conidiophores which bear the conidia singly at their tips, are long cylindrical, septate, and $30-45 \times 4-6 \mu$.

The conidia are one-celled, cylindrical or oblong-ellipsoid, with rounded ends, often slightly curved, hyaline or faintly tinged with an orange color and measure $13-23 \times 3.8-6.8 \mu$, mostly $17-19 \mu$ long and $4.5-6 \mu$ broad. (Fig. 1)

The spore-masses are light brick-red when produced on the lesions of the stem kept in a wet petri-dish.

The acervuli on the leaf-blade are provided at the circumference with scattering setae, which are dark brown in color, lighter at the tip, with one or two septa, attaining the length of 55μ . (Fig. 3)

On germinations, a germ-tube is produced from the end of the spore, usually dividing previously into two cells by a median septum. Light brown appressoria are usually formed at the tips of the germ-tubes, when the conidia are sown in water. (Fig. 2)

TAXONOMY OF THE CAUSAL FUNGUS.

T. Johnson has described a canker on the European osiers (*Salix triandra* L.) in Ireland, caused by *Physalospora gregaria* Sacc. According to him the conidial stages of the fungus are *Tetradia salicicola* Johnson and *Macro dendrophoma salicicola* Johnson. Although the symptoms of the disease described by him closely resemble those of our willow canker, the causal fungus is entirely different, the ascospores of *Physalospora gregaria* being twice as large as those of the fungus in question, and also the conidial stages of the former belonging to the genera entirely distinct from *Gloeosporium*.

Besides the fungus just mentioned, several species of *Physalospora*, viz., *Ph.*

Salicis (Fuck.) Sacc., *Ph. Salicis* var. *gregariella* Sacc., *Ph. hyalospora* (Ges.) Sacc., and *Ph. apiculata* (Kalch.) Sacc. have been reported to occur on *Salix*. No mention, however, can be found which indicates the occurrence of any species of *Physalospora* on *Salix purpurea*, except *Ph. Salicis* which infects according to Lind⁽²⁾ a hybrid plant between *Salix dasyclados* and *Salix purpurea* and also one between *Salix purpurea* and *Salix viminalis*. The ascospores of *Physalospora Salicis* are somewhat smaller than those of the fungus under consideration, measuring $12-4 \times 5 \mu$ (according to Winter) or $14 \times 2 \mu$. (according to Saccardo).⁽⁴⁾ Furthermore the conidial stage of the former is of the *Phoma*-type with oblong-oval spore $8 \times 4 \mu$, according to the original description of Fuckel⁽³⁾ who referred the perfect stage of the fungus to the genus *Sphaeria*. *Physalospora Salicis* var. *gregariella* was described by Saccardo⁽⁶⁾ on the dead stem of *Salix vitellina* and seems to be different from the typical *Physalospora Salicis* only by its gregarious appearance of the perithecia. Saccardo makes no mention regarding the conidial stage of the fungus. *Physalospora hyalospora* differs from the fungus in question to the broad shape of the ascospore, while *Physalospora apiculata* cannot be compared owing to its imperfect description.

As to the conidial stage, according to the present taxonomical usage, the fungus under consideration is to be referred to *Myxosporium* when it occurs on the stem and to *Colletotrichum* when it appears on the leaf, as the acervuli are provided with the setae. Recent investigations, however, have shown that the species of *Gloeosporium* cannot be kept distinct from *Colletotrichum*, since the hairs, which alone separate the two genera are not constant character, but very variable, their formation being dependent often on external conditions. It seems also reasonable to the writer to consider that there are no intrinsic characters sufficient to separate *Gloeosporium* from *Myxosporium*. The following species of *Myxosporium* and *Gloeosporium* are according to European authors, found on various species of *Salix*, viz., *Myxosporium Mitharditium* Sacc. et Roum., *M. salicellum* Sacc. et Roum., *M. salicinum* Sacc. et Roum., *M. incarnatum* (Desm.) Bon., *M. melanotrichum* (Cast.) Sacc., and *M. rimosum* Fautr. and *Gloeosporium boreale* Ell et Ev., *G. Salicis* West., *G. Beckianum* Bäuml., *G. Capreae* Allesch., *G. amentorum* (Delacr.) Lind, *G. deformans* (Schroet.) Lind, *G. Oelandicum* Lind, and *G. lapponum* Lind. Of all these species *Myxosporium salicinum* has been referred by Lind to be found on *Salix purpurea*. None of these species, however, are identical with the conidial form of the fungus under consideration. And there seems to be no records in mycological literature of any *Colletotrichum* growing on *Salix*, as far as the writer is aware of. The fungus which the writer is describing seems to

differ from all the described species of *Physalospora*, *Myxosporium* and *Gloeosporium* on *Salix*; and therefore the writer wishes to treat it here as a new species, proposing for it the name, *Physalospora Miyabeana*.

Physalospora Miyabeana sp. nov.

Perithecia solitary or in small cluster, abundant, immersed in the host tissues, with the mouths slightly protruding, dark brown, globose, 100–170 μ in diameter. Asci numerous, clavate, 55–75 $\times$ 11 μ . Spores uniseriate or sub-distichous, one-celled, oblong-ellipsoidal, often slightly curved, granular at the ends, hyaline, 15–17 $\times$ 5.5–7 μ . Paraphyses slender, 66 $\times$ 12 μ .

Acervuli gregarious, erumpent. Conidiophores crowded 30–45 $\times$ 4–6 μ , bearing spores at the tips. Conidia unicellular, ellipsoidal or oblong-ellipsoidal, sometimes slightly curved, with rounded ends, hyaline, 13–23 $\times$ 3.8–6.8 μ , mostly 17–19 $\times$ 4.5–6 μ . Setae scattered around the acervuli on the leaf, straight, dark brown, septate, 40–55 $\times$ 3.7 μ . Conidial masses light brick-red.

Forming cankers on the stem, or round spots on the leaf of *Salix purpurea* var. *angustifolia* Sapporo, Japan.

The type specimens have been deposited in the Herbarium of the Botanical Institute, Hokkaido Imperial University, Sapporo.

THE CULTURAL CHARACTERS OF THE FUNGUS.

The fungus is readily isolated by means of poured agar plates both from the conidial masses and from small pieces of the diseased tissues of the willow leaves and stems. It grows on the ordinary culture media, such as corn agar, oat agar, apricot agar, onion-soy agar, and host-decoction agar, forming a white mycelial growth which later turns to gray. On corn agar and oat agar the vegetative growth is very meagre. The spores of various sizes and shapes may be produced in the cultures in three days or more (Fig. 7), though no production of the spore masses can be found. The formation of chlamydospores is also abundant. (Fig. 8). They are entirely terminal or intercalary, brown in color and 10–30 μ in diameter. An abundant development of very small sclerotia-like black hyphal masses may be found around the edge of the agar surface and also on the sides of the tubes.

With the mycelial growth, the willow decoction agar changes from reddish brown to deep chestnut brown, and the apricot agar assumes a beautiful red color on the

agar surface. On the corn agar the pink color which is frequently found in the cultures of *Gloeosporium* may appear or not in our fungus according to its strains even under the same cultural conditions.

According to Dr. Hemmi the fungus in question sometimes affects apple fruits when inoculated at the cut wounds, but the rotting is very slow and entirely different from that caused by the inoculation with the apple anthracnose fungus.

The development of the acervuli and perithecia was secured only on the sterilized pieces of willow stems as already mentioned.

THE PATHOGENECITY OF THE FUNGUS.

The inoculation experiments with the fungus were carried out in the autumn of 1920. On Sept. 19, two willow plants, with or without incisions made on the stem, were inoculated by spraying them with the water suspension of the conidia. The fungus strain isolated from a lesion on the stem was used for the inoculation. After 4 days, the sign of the disease was evident on one of the inoculated plants, showing dark brown spots of 1–3 mm. in diameter on the surface of several leaves, while the control plant remained perfectly sound. Microscopical examinations revealed the spores of a *Gloeosporium* ($13-17 \times 4 \mu$) on the spotted areas. On Oct. 28, three willow plants were inoculated with the same fungus strain as above. Unfortunately these plants were defoliated owing to the plants being kept too long under bell glass. On some of the remaining leaves, however, there appeared small brown spots which proved afterwards to be the lesions of *Gloeosporium*. The control plant showed no signs of the disease. No evidence of the canker formation on the stem was to be found in these experiments.

GLOEOSPORIUM RELATED TO PHYSALOSPORA.

There are a few species of physalospora which are known to have *Gloeosporium* for their conidial stage. *Gloeosporium Physalosporae* Cavara⁽⁷⁾⁽⁸⁾ is considered to be the conidial form of *Physalospora baccarum* of the same author found on the Concord grape. Maublanc and Lasnier state that the conidial form of *Physalospora Cattleyae* M. et L.⁽⁹⁾⁽¹⁰⁾⁽¹¹⁾ is *Gloeosporium macropus* Sacc.

Sheldon⁽¹²⁾ obtained the perithecia in the cultures of a *Gloeosporium* parasitic on *Dracaena fragrans* and afterwards also its dead leaves, which he described under

the name of *Physalospora Dracaenae*. Shear and Wood¹²⁾ consider that *Physalospora citricola* Penzig on *Citrus nobilis*, *Ph. Phormi* Schroeter on *Phormium tenax*, *Ph. Vanilla* A. Zimmermann, on *Vanilla Planifolia* and *Ph. baccae* Cavares on *Vitis labrusca* are the perithecial forms of the *Gloeosporium* found on the corresponding host plants and that furthermore these fungi are identical with *Glomerella cingulata* which has been connected with the anthracnose fungi on a great variety of cultivated plants.

In the present paper, the writer has been able to add a new instance of the relationship between *Physalospora* and *Gloeosporium* in the canker disease of the Japanese osier.

SUMMARY.

In the autumn of 1919 as well as of 1920, a canker disease injurious to the Japanese osier, *Salix purpurea* var. *angustifolia* appeared in the vicinity of Sapporo. The disease seems to be prevalent at several osier-holts in Hokkaido.

The disease is due to a Pyrenomycetous fungus, *Physalospora Miyabeana* n. sp. and its conidial form *Gloeosporium*.

The relationship between the perithecial and conidial stages was established by cultural experiments.

The writer wishes here to express his heartiest thanks to Prof. K. Miyabe and Prof. S. Ito for their kind suggestions and criticisms and also to Dr. T. Hemmi, who has kindly helped him in the cultural study of the fungus.

LITERATURE CITED.

1. Johnson, T. Willow canker: *Physalospora* (*Botryosphaeria*) *gregaria* Sacc. Sci. Proc. Roy. Dublin Soc. X. 153-166. 1904.
2. Lind, J. Danish fungi as represented in the herbarium of E. Rostrup, pp. 211, 480. 1913.
3. Winter, G. in Rabenhorst's Kryptogamen-Flora, Die Pilze II. 411. 1887.
4. Saccardo, P. A. Sylloge fungorum I. 439. 1882.
5. Fuckel, L. Symbolae mycologicae, 115. 1869.
6. Saccardo, P. A. Notae mycologicae. Ann. Myc. VII. 432-437. 1909.
7. Saccardo, P. A. P. Syll. fung. X. 488. 1892.
8. Allescher, A. in Rabenhorst's Krypt., Die Pilze VII. 510. 1903.

9. Holtrung, Jahresbericht. VII. 273-274. 1904.
 10. Lindau, G. in Sorauer, Handbuch der Pflanzenkrankheiten II. 248. 1908.
 11. Stevens, F. L. The fungi which cause plant disease, 253. 1913.
 12. Sheldon, J. L. A study of the leaf tip blight of *Dracaena fragrans*. Journ. Myc. XIII. 138-140. 1907.
 13. Shear, C. L. and Wood, A. K. Studies of fungous parasites belonging to the genus *Glomerella*. U. S. Dept. Agr. Bur. Pl. Ind. Bull. No. 252. 1913.
-

摘 要

大正八年及九年秋、北海道帝國大學附屬苗圃の杞柳に一種の新病害發生し被害大なりき、柳行李製造者の談に徴し其齎らせる標本を検するに此病害は數年前より北海道諸所の杞柳栽培地に發生しつつあるものの如し、被害の杞柳は莖上に汚點を印して品質著しく劣るのみならず折れ易く此病害は一種の *Physalospora* 菌及 *Gloeosporium* 菌に依りて生ずるものにして予は培養試験に依りて一が他の分生孢子時代なることを確めたり。

Physalospora 菌にして *Gloeosporium* 菌をその分生孢子時代とすと報告せられしもの數種あれども、培養試験、接種試験に依りてその關係を明かにせられしは唯一種あるにすぎず、予は培養試験の結果茲に新しき一種を加ふるを得たり。

予は此 *Physalospora* 菌を新種と認め *Physalospora Miyabeana* なる種名を與へたり。

(附記) 大正九年秋、同大學苗圃並に札幌附近の杞柳に類似の病害發生したりこは一種の *Marssonina* 菌に依るものらしく、且曩に福井武治氏が病蟲害雜誌第五卷(大正七年)にて報告せられし杞柳の病害(氏は病原菌を *Marssonina Kriegeriana* Bres. とせり)に似たれども、予は研究の歩を進めざりしが故に、此點につきては明言し能はず、唯其病徴が *Physalospora* 菌に依るものと全く異なる(詳細は本文中にあり)ことを一言附記す。