ON A NEW CANKER-DISEASE OF PRUNUS YEDOENSIS, P. MUME AND OTHER SPECIES CAUSED BY VALSA JAPONICA MIYABE et HEMMI sp. n.

BY

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(With Plates VII–X)

1. Introduction.

The canker-disease of *Prunus yedoensis* MATSUM. *(Somei-yoshino)* was first noticed in 1913 by Prof. K. MIYABE in the Botanic Garden of our University, and was proved by him to be due to a species of Valsa. It was in the early spring of 1914, that my attention was drawn to the seriousness and widespread occurrence of the disease in the vicinity of Sapporo, when, at the suggestion of Prof. K. MIYABE I resolved to undertake the investigation of the disease. Since that time, many samples of the diseased branches and stems have been collected on different species and varieties of Prunus. Among those affected by the disease, *Prunus Mume* S. et Z. seems to be very susceptible to the disease, and its causal fungus was proved to be the same as that of *Prunus yedoensis* by my cultural and inoculation experiments. The peach tree (*Prunus Persica* S. et Z.) was also proved to be sometimes attacked by the same fungus, but the extent of its damage was not investigated in my study. According to my observations, *Prunus sachalinensis* KOIDZ. *(Ōyama-zakura* or *Yezo-no-yamazakura)* seems to have a strong resistance against the disease, though not immune. As to the other host plants my observations and studies are not yet complete, and I wish to defer their report to a future occasion. The fact that the canker disease has not yet been found on the European Cherry tree (*Prunus Cerasus* L.) in the vicinity of Sapporo, notwith-

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standing its wide cultivation, shows the plant is immune to the disease. As the result of my studies this destructive canker disease was proved to be caused by the attack of a new species of Valsa acting as a wound parasite.

I wish to express here my heartiest thanks to Prof. K. MIYABE for his constant and kind direction; and to Assistant Prof. S. ITŌ to whom I am also indebted for his many valuable suggestions. I wish also to express my sincere thanks to Prof. H. KŌRIBA, Messrs. Y. KUDŌ, S. NISHIDA and other gentlemen, who have kindly helped me in many ways.

2. Historical Review of Valsa-Diseases.

Valsa is a large Pyrenomycetous genus, having more than 400 species, most of which are generally described as saprophytes in mycological literature. Up to the present time only five species have been recognized as the cause of plant diseases. They are, namely,—*Valsa leucostoma* (PERS.) FR., *Valsa oxystoma* REHM, *Valsa ambiens* FR., *Valsa (Eutypella) Prunastri* (PERS.) FR. and *Valsa Mali* MIYABE et YAMADA.

Among them, *Valsa leucostoma* (PERS.) FR. is most widely known as the cause of a destructive disease of the drupaceous trees throughout Europe, Australia and N. America. This fungus attacks the cherry, peach and other drupaceous fruit-trees, causing the disease known as the "Die-back" of the twigs, and also the cankers upon the trunks and limbs. In the Rhine-district, from about the year 1899, the cherry trees have been so severely affected by the fungus as to arouse the attention of many phytopathologists. FRANK (1899)[9] studied the disease and ascribed it to *Cytospora rubescens* FR. On the other hand, GOETHE (1899)[11], ZAPFE (1899)[35], SORAUER (1900)[27] RASCHEN (1900)[21] and LABONTÉ (1900)[15] considered the disease to be due to some unfavorable climatic conditions or to other physiological derangements. It was by ADERHOLD (1903)[1] that this disease was most thoroughly investigated; and he proved it to be caused by *Valsa leucostoma*, the generally ascrived climatic causes to be merely its promoting agencies, and *Cytospora rubescens*, which was observed by FRANK (1899)[9], to be the pycnosporous stage of this fungus.

In Australia, McALPINE (1902)[17] described the same pycnosporous stage, by the name of *Cytospora leucostoma*, on almond, peach, plum and cherry twigs. In America it was first described by ROLFS (1907)[23], who found it upon peach and Japanese plum.

Valsa oxystoma REHM is known as the cause of a disease of the alder-tree in Europe. It was first described by REHM (1882)[22], who thought it to be a saprophyte. But in 1893, TUBEUF [30] ascribed the death of the twig of *Alnus viridis* to the attack of this fungus in Germany, and in 1899 the same fungus was observed by P. NYPELS[19] in Belgium to cause the disease of *Alnus glutinosa*.

Valsa ambiens FR. was shown by M. C. COOKE[7] to seriously affect the living bark of apple-trees in Europe, although the fungus is usually considered as a pure saprophyte. *Valsa Prunastri* (PERS.) FR. is known to be the cause of a serious disease of plum, apricot, peach and apple trees in Europe.

In our country there is a species of Valsa causing the destructive canker disease on apple trees. The disease was first discovered in the Aomori Prefecture, but it is now widely spread in Hokkaidō. Prof. K. MIYABE and Prof. G. YAMADA studied the fungus and named it *Valsa Mali* MIY. et YAM. It is an endemic species. Its description appeared for the first time in 1909 in IDETA's Hand book of the Plant-Diseases in Japan[12].

There are, besides, two more very destructive Valsa diseases in our country, which have not yet been reported. One is on Paulownia * and the other is on the Japanese flowering cherry-trees, Japanese apricot and peach-trees. The latter was taken up as the subject of my present investigations, as it is the more important from the economic standpoint.

3. Symptoms of the Disease.

a. General Appearance of the Diseased Trees.

It is easy enough to distinguish this disease on the bark of the branches

* From our studies, this fungus also seems to be new to science. We named it, therefore, *Valsa Paulowniae* MIYABE et HEMMI. Its diagnosis will be given in another paper.

or trunks throughout the year, if careful search is made. Although the effect of the disease on the general appearance of the tree is noticeable at any time during the growing season, it is most conspicuous during the spring months, at least in the vicinity of Sapporo, when the causal fungus is under most favorable conditions for its growth.

In the vicinity of Sapporo, where the disease is common, it is not rare to find the entire tree or some of its branches which are in blossom or in leaf suddenly wither and then dry up at the beginning of May. The brown, shriveled flowers and leaves are readily seen even at a distance, while the healthy part of the tree is still in blossom or in foliage. If one examines the base of such a withered branch, a comparatively large canker or a part entirely girdled by the disease will be found. Such symptoms are also noticed in summer, although not numerous. If the point of infection is near the base of the trunk, as is most frequently the case, one side of the tree will wilt first, then a month or so later the rest of the tree. From the point of entrance, the fungus hyphae pass rather more slowly toward the center of the tree than they do up and down through the water ducts, causing the formation of a gummy substance, which plugs up the lumina of the ducts and cuts off the water supply for the transpiration organ of the tree.

The wilted dead leaves or flowers remain clinging to the branches for a long time. If the plugging of the ducts by the gum or the girdling of a limb by the fungus takes place during the winter, the plant will appear in the spring as if it had been killed by a very low temperature.

b. Appearance of the Diseased Portions of the Bark.

On the smooth bark, by the death of the tissues of the cambium and inner bark, a canker formation takes place, accompanied generally by the exudation of gum around the affected portion. These cankered portions are slightly sunken, and distinguished from the healthy bark by a dark-brown or blackish-brown color, whereas the normal bark is more of a chestnut-brown or light-brown color. Often the bark on these cankered spots is more or less

cracked at the marginal portion. These cankers occur on branches of all sizes,—rarely, however, on young branches. The usual shape of the canker is elliptical, being longer in the direction of the long axis of the branch. The margin of the canker is usually fairly regular, but it may be irregular. According to my observations, the cankers formed on the large branch become deeper year by year as healthy wood is formed about them, thus causing a condition similar to the apple-tree canker or the American chestnut-tree canker. When the fungus affects the rough bark of the trunks, the cankers do not show very distinctly, since the change in color of the bark is not evident and the shape of the canker is mostly irregular. It is frequently distinguished from the healthy parts only by the lenticel-like openings of the stromata. But the cankers on the rapidly growing branches are usually outlined by a distinct ridge of slightly hypertrophied tissue. It seems to me that there are two different types of the diseased branches. The one is the cankered type (Pl. VII, Fig. 2–4) as already described, and the other is the girdled type (Pl. VIII, Fig. 1) which does not produce the canker. The latter takes place mostly in the case of the weakened trees or when the rather fine branches or twigs are infected in spring. The girdling is caused by the rapid growth of the mycelium without inducing the formation of a definite canker; and the fruiting pustules break out rather scatteringly all over their surface. Such a diseased portion extends sometimes to the greater part of the branch, which at once withers and dies. Although this girdling is seen more or less on all host plants, it is most common on *Prunus sachalinensis* KOIDZ. (Pl. VIII, Fig. 1), which is rarely attacked by this disease and on which I have not yet found the cankered type.

The fruiting pustules are at first covered by the periderm, which becomes lifted up and finally ruptured, exposing the brown or blackish brown stromata of the fungus. These slits appear at first glance like the lenticels, for they are usually elongated horizontally. During the growing season, when the weather is damp, from these pustules are pushed out very fine reddish threads or spore-horns which are composed of innumerable pycnospores. In the

early spring the red crusts composed of masses of ascospores are sometimes seen on the stromata.

c. Development of the Canker.

The canker appears first as a more or less discolored area about the point of infection, which takes place most frequently through cracks and wounds on the bark, or where a branch or twig has been pruned, or killed from some cause, as winter injury. It is also not seldom that the fungus gets a start from the wound which is given by an insect such as *Sesia hector* BUTL. or a crack in the crotch of the branches. Judging from my observations, the disease may also arise through the injured buds. The affected area of the branch soon becomes sunken, making the boundary between the dead and living tissues very marked. Gum pockets are often formed at this point. The gummy exudation pushing through the tissue of the bark appears on its surface as a copious gum flow. The bark of such a diseased portion gradually dries up and at last produces numerous pustules which are the beginning of the stromata. Callus soon pushes out from the edge of the canker, but it is very soon attacked by the fungus and produces a large new canker surrounding the previous one, requiring for its formation just one year. One can see, therefore, at times on a large tree cankers as large as five or six feet long, having three or four concentric ridges. Such injuries are gradually extended, often girdling the branches and even the trunks of the tree. The gummosis is also constantly associated with these cankers, being especially conspicuous on *Prunus Mume*.

d. Age and Parts of the Hosts affected.

According to my observations, the disease attacks the large trees more frequently than the smaller ones. This is evidently caused by the facts that the larger trees have a much greater chance of getting wounded by insects and other causes than the young trees, that the healing power of the former is not so strong as in the latter, and that moreover the younger it is, the

greater its resistibility to this disease.

Even on a single tree the disease is most frequently found on the thick branches or trunks, and the cases on the twiglets are very rare, if ever attacked. When the disease affects the young twig, the pustules of the stromata are very small, covered by the periderm for a long time without being ruptured; and the spores are in such a case very rarely produced, for the twigs die and dry up very rapidly. In the case of *Prunus Mume*, the canker is generally produced on comparatively small branches, having the diameter of one and a half centimeters or more.

4. Host Plants.

The disease in question was first found on *Prunus yedoensis*, which is most severely attacked. By extending our observations to other species of Prunus we found a similar disease on most of them. The trees affected by the disease are *Prunus sachalinensis* KOIDZ. (オホヤマザクラ), *P. Koidzumii* MATSUM. (ムシヤザクラ), *P. serrulata* LINDL. (ヤマザクラ), *P. serrulata* LINDL. var. *nobilis* (KOIDZ.) *f. Yōkihi* (八重櫻 楊貴妃), *P. kurilensis* MIYABE (チシマザクラ), *P. Mume* S. et Z. (ウメ), and *P. Persica* S. et Z. (モヽ).

The identity of the fungus which attacks *P. sachalinensis*, *P. Mume*, and *P. Persica* causing the canker-disease was proved by cultural and inoculation experiments.

On *Prunus serrulata*, the pycnidial stage only of the fungus has been observed. On *Prunus serrulata* LINDL. var. *nobilis*, the canker is most beautifully formed, showing apparently the weak power of its resistance to the disease. The specimen on *Prunus kurilensis* having both ascosporous and pycnidial stages was collected by Prof. S. ITŌ in May of 1915 on the dead twig. The specimen on *Prunus Koidzumii* was collected by myself on the dead twig, but the fungus is lacking in the ascosporous stage, and its parasitism is not clear. In regard to the causal fungus of the disease on these host plants, I have not been able to undertake the cultural and inoculation experiments.

In July of 1914, I collected a species of *Cytospora* on the dead twiglets of the European cherry, *Prunus Cerasus* L. The morphological characters of its spores are very much like those of the pycnospores of our fungus; but its cultural characters seem to be more or less different, and the inoculation experiments have also shown negative results.

5. Distribution of the Disease and Extent of the Damage.

This disease is common not only in the vicinity of Sapporo, but seems also to be widely distributed throughout Hokkaidō.

In September of 1914, I found diseased branches on *Prunus yedoensis* at Asahigawa, and an examination showed that it also was due to the same fungus. In February of 1915, Mr. S. NISHIDA collected the same fungus, also on *Prunus yedoensis*, at the Hakodate Park, and in May of the same year he again visited the same place for me and counted about thirty trees attacked by this disease.

From other parts of Japan we have not yet been informed of the outbreak of this disease. I received negative answers to all my inquiries to various places in Honshū. In spite of careful search for the disease made by my friends Mr. M. YATAGAI and Mr. Y. TAKENOUCHI and me during our journey through Honshū, we did not find it. Among the specimens collected by Mr. M. YATAGAI in Tōkyō, there is one on a cherry tree which resembles our disease in its external appearance, but whose microscopical characters are very different. Through the kindness of Prof. K. MIYABE, I was able to examine the diseased branches of the peach which were sent to him from Gifu. It is due to the attack of a certain species of Cytospora, the morphological characters of which are quite different from those of our fungus. Judging from these facts, it is safe to conclude for the present that the disease on the species of Prunus is limited to Hokkaidō.

In the vicinity of Sapporo the damage caused by this disease is by no means small. Almost all aged trees of *Prunus yedoensis* have their branches more or less attacked by this fungus. There are, indead, numerous branches

and even whole trees, that were cut off in consequence of the disease. On *Prunus sachalinensis*, however, we have very few cases of this malady. On *Prunus Mume* the disease seems to be not uncommon, but its damage is not so great as in the case of *Prunus yedoensis*. Judging from the extent of the damage, we may safely infer that the disease had existed in Hokkaidō for many years without drawing the attention of pathologists. I may furthermore infer that the causal fungus is indigenous to Hokkaidō growing on the wild *Prunus sachalinensis* or other species of *Prunus*, having a comparatively strong resistance, and that it has recently found more congenial hosts in the cultivated cherry and apricot trees introduced from other parts of Japan.

6. Morphology of the Causal Fungus.

a. Stromata.

The infected tissues do not show at first any external signs of the fungus itself; but in time, on the smooth bark, numerous fruiting pustules gradually protrude through the horizontal rent of the periderm, having generally a small lenticel-like appearance. In the young stage such pustules are covered by the cork layers of the bark and on young twigs, especially on *Prunus Mume*, they remain covered for a long time. On rough barks or in special cases, as in *Prunus Mume*, they break out from irregularly ruptured periderm.

In the young stage, a section through the pustule shows the compactly united mass of the hyphae under the periderm (Pl. IX, Fig. 8.). It is at first quite small and more or less flattened, but in time it becomes subspherical, ellipsoidal or irregularly oblong in shape. These young stromata increase their size little by little until they reach the matured conical or wart-like shape. The stromata are composed mostly of the fungus hyphae with some host tissues intermixed. From about the time when the stroma is exposed by the rupture of the periderm, a pycnidial cavity begins to be produced. It is at first composed of a small simple chamber, but gradually it increases its size, irregularly ramifying and filling up the stroma, having a single common exit. The exit or opening of the pycnidium appears to the naked eye as a

black point at the center of the surface of the stroma.

Such a stroma is said to be "ectostroma" by RUHLAND (1900)[21], separating it from "entostroma", in which perithecia are produced. The color varies with age, being white or light greenish black at first, later becoming grayish black or light blackish brown, finally greenish brown or black and sometimes lighter-colored near the center. The size of such "ectostroma" varies also with external conditions, and generally in moist situations they seem to develop much larger than in drier places. The stromata formed on the large branches are also much larger than those on the twigs. The matured ectostromata are usually elongated horizontally and their average size is about 2.2 mm. in breadth, 1.5 mm. in height and 1.0 mm. in depth.

In autumn the entostroma is produced under the ectostroma on the old canker, increases its size displacing the ectostroma, and at last takes possession of its site completely. Of course it is not infrequently happens that the entostroma is independently produced without any connection with the ectostroma.

In the formation of the entostroma, the initial stage of its development is exactly the same as in the case of the ectostroma. It is also elongated horizontally and has a conical or wart-like shape, having a round or elliptical base. Its average size is about 3–5 mm. in breadth, 2–4 mm. in height and 1–1.5 mm. in depth. The entostromata also vary widely in size with the environment and season; they become much larger in moist situations than in dry surroundings. The color also varies with age, being brownish gray at first, later becoming gray or light black on the surface, but always gray or yellowish gray near the center. The stromata are subcoriaceous and easily torn apart.

The matured entostroma has on the exposed surface numerous minute black papillae which project scarcely above its surface. On a single stroma from only a few to thirty or more of these papillae may be found. Each papilla is the opening of a long neck that forms a canal from the perithecium buried in the stroma.

In the systematic studies on the genus *Valsa*, a great importance is attached to the structure of the stroma especially at its basal portion. In the case of the present fungus there is no black boundary line, so called "Conceptaculum", between the stroma and the host-tissue. The presence of such a boundary line is the most essential character of the Subgenus *Leucostoma*, which was founded by NITSCHKE (1867) [15] and accepted by many subsequent authors. But in the case of our fungus, the stroma is composed mostly of the mycelial tissue in which disintegrated cells of the host are more or less intermixed; while stromata of many other species belonging to the Subgenus *Euvalsa* consist mostly of the host tissues, especially at their basal portion. Some sections of the stromata in our fungus have an appearance as if the whole body of the stroma is composed entirely of mycelial tissue, and that there is a distinct boundary between the hyphal portion and host tissue. A careful observation, however, showed at once that the above mentioned appearance is caused by the compact arrangement of the numerous perithecia in a single stroma. We do not find here a black compact stratum or boundary line, and moreover, the closer observations revealed the presence of the host tissue scattered deeply in the stroma, even in the portion above the bodies of the perithecia (Pl. IX, Fig. 3, 5.). We can more easily see the relation of such structures of the stroma by means of microtome sections of the material imbedded in celloidin.

b. Pycnidia and Pycnospores.

On the smooth bark of the diseased twigs or the young cankers, especially in summer, the cork layer is raised in numerous little pustules, from the apices of which slender, reddish, waxy curling threads may be frequently seen in moist weather. As already stated, such pustules are the ectostromata in which the pycnidia are produced. The pycnidium appears at first as only a small cavity in the young stroma. If a cross-section is made through the young stroma, a lighter colored portion, which is composed of a loose growth of white or slightly yellowish mycelium, is seen at first, which gradually

increases in size and at last produces a pycnidium. The pycnidia, even in the matured stage, are only cavities in the stromata, and their wall is not clearly distinguished from the surrounding tissue of the stroma. Their size and shape are also various and irregular. The pycnidial cavity is almost single in a cross-section of a stroma at first. In a single cross-section of a matured stroma, a few or several disconnected irregular shaped cavities are usually to be seen (Pl. IX, Fig. 1.). But if the entire stroma is cut into serial sections, it shows clearly that there is but a single pycnidium with a number of communicating chambers, having a single exit.

The conidiophores form a dense hymenium on the inner wall of the pycnidium, extending directly out into the cavity from every point of the wall. They are of uneven lengths, the majority being 14–28 μ long, and are about 1.75–2.1 μ in diameter. They are much longer in the pycnidial cavity produced on pure cultures, reaching about 40–60 μ in length. They may be simple or branched (Pl. IX, Fig. 9.). Spores are cut off successively from the conidiophores and soon fill the cavity, but since the production of the spores does not cease when the cavity is filled, they are forced out through an ostiole at the top in a reddish spore horn. The spore horn is usually spirally twisted into coils or other various shapes. In cultures, the spores are exuded in drops instead of horns because of the too great moisture present.

The reddish tendrils are composed entirely of the small hyaline pycnospores held together by a binding substance. When placed in water, the tendril first swells and turns white, then the binding substance dissolves and the spores float away free from each other. They commonly measure 5.25–10.50 × 1.4–2.1 μ in size. But the sizes of the pycnospores are very variable in accordance with the grade of their maturation; and the difference between the maximum and minimum sizes is remarkably wide, *e.g.*, 3.5–15.75 × 1.0–2.63 μ. Such vacillation of the spore size is most commonly seen on pure cultures. They are cylindrical, allantoid with rounded ends, usually curved but sometimes straight. The spore-wall is smooth and colorless (Pl. IX. Fig. 2.).

From the characters of the pycnidia and the pycnospores as described

above, we easily recognize our fungus to be a species belonging to the genus *Cytospora* of *Sphaerioidaceae*.

c. Perithecia.

The matured stromata on the older cankers have numerous projecting papillae on the external surface, especially near the margin. A cross-section of a stroma shows the subglobose sac-like perithecia each with a long black neck (Pl. IX. Fig. 3, 5.). Generally, there are fifteen to forty perithecia in a stroma, but the number varies greatly. The bodies of the perithecia arrange themselves compactly forming the base of a stroma.

The perithecial wall is gray or black and sometimes light brownish black in color under a microscope. But when seen under a hand-lens or with the naked eye, the perithecial wall appears jet black. The mature perithecia measure about 350–580 μ in diameter and are mostly spherical, but the shape is often modified by the pressure of the adjoining perithecia.

Since the perithecia are always in the bottom of the stroma, or even partly in the host tissue, the length of the neck varies with the luxuriance of the stroma; but, in general, it is 1.5–3 times the diameter of the body. In a microtome section the wall of the perithecia on artificial cultures is seen to be composed of eight or ten layers of thick-walled cells. Inside these, there are a few layers of thin-walled cells, from the inner surface of the basal part of which the asci grow out into the cavity. But the wall of the neck is composed of densely interwoven, septate, thick-walled hyphae running longitudinally, parallel with the long axis of the neck.

The hyphal branches, or periphyses, project out free into the canal and they are especially prominent at the swallen tip of the neck where the canal is also enlarged (Pl. X, Fig. 9.).

d. Asci and Ascospores.

When mature the cavity of the perithecium is filled with asci, each containing eight allantoid spores. The asci are formed from the base of the

perithecial cavity. The cavity is finally filled with detached asci. The asci are cylindrical or rarely clavate and subsessile, measuring 60.0–96.0 × 8.8–16.0 μ. The wall of the ascus is hyaline and more or less thickened at the tip (Pl. IX, Fig. 7.). When mounted in water or potash, they swell irregularly at first, then dissolve gradually and for this reason it is hard to make out their structure, unless they are mounted in acetic acid or stained. In nature, the asci usually dissolve themselves while they are in the perithecia. And it often happens that the whole perithecial cavity is filled with the spores presenting an appearance of a pycnidium. Indeed, it is only when the perithecium is young or new that one sees perfect asci in it; and in the old perithecia one occationally finds even the germinating ascospores. (Pl. IX, Fig. 4.).

The ascospores are arranged in an ascus mostly biseriately, sometimes irregularly or rarely uniseriately. They are allantoid, with rounded ends and one-celled (Pl. IX, Fig. 6.). They measure about 10.0–28.0 × 3.2–7.2 μ (most commonly 18.0–22.0 × 4.0–4.8 μ); and their contents are dense and homogeneous, and occasionally guttate. These ascospores are hyaline when young, but in the most matured stage some of them become slightly darker.

e. Mycelium.

The individual hyphae are septate and branched, the branching being always monopodial. The hyphae are not uniform in diameter, but vary from 1 to 8 μ. They are ramifying in the tissue of the bark, destroying the parenchyma cells, and they also spread deeply into the woody tissue. The color of the mycelium is almost hyaline in the tissue of the host; but in most cultures the mycelium becomes yellow or brown after a few weaks, due to the production of pigments.

The relation between the mycelium and host cells I shall consider in another chapter.

7. Cultural Studies of the Causal Fungus.

I have grown the causal fungus on various cultural media under observation for a year, and for the sake of comparison I have now and then had cultures of *Valsa Mali* on the apple tree and *Valsa Paulowniae*, which had been isolated from *Paulownia tomentosa*. I have grown many hundreds of these cultures on a variety of media in test tubes, Petri dishes and Erlenmeyer's flasks, though for most purposes, tubes of apricot-juice or host-bark-decoction agar have proved the most satisfactory.

a. Isolation.

The fungus is most readily isolated by removing the pycnospores from the host plants to agar media, when the spore-formation is most vigorous. If a piece of such a diseased branch is kept in a moist condition, a red spore horn oozes out from each stroma. If the fungus is in the ascosporous stage, the spores may be permitted to fall on sterilized plates or on the stromata themselves to form reddish crusts after natural ejection. It can be done only in the case of fresh materials. Therefore either kind of the spores may be sown on the agar, or streaks may be made on agar slants with the spore horns. If the material is not fresh or the causal fungus is immature, the fungus is isolated by removing, after sterilization of the exposed surface, a small piece of the diseased tissue of the inner bark, especially in the youngest part of the canker, and transferring it to agar tubes.

b. General Cultural Characters.

In the saprophytic condition the fungus in question seems to be almost omnivorous. But it likes on the whole such a comparatively high acidic medium containig sugar as the juice of fruits ; and on such media the fruiting pustules are not generally produced in spite of the vigorous growth of the mycelium. In the cases of fungi which form the stromata, it is reported by many authors that they have not succeeded in producing the ascospore stage in culture, even on sterilized twigs, but I have succeeded in producing such

ascospores of this causal fungus with cultures of the sterilized twigs. Speaking generally, the present fungus has the tendency to form the creeping mycelium rather than the aerial one. I can advocate, therefore, the cultural characters, such as the color of the mycelium and of the spore-masses, or the characteristic hyphal growth, as a means of distinguishing this species from the other very closely related species of *Valsa*. The chief cultural differences of our three species of *Valsa* are as follows:

(1) In general, the mycelial growth of *Valsa Mali* is comparatively poor in artificial cultures, while the growth of *Valsa Paulowniae* is most vigorous, and our present fungus shows an intermediate growth between the above two species.

(2) For most cases the growth of the aerial mycelium is most vigorous in cultures of *Valsa Paulowniae*, compared with cultures of the other two.

(3) The mycelium of the present fungus on cultures turns yellow, greenish yellow or light brown in color with age, especially on fruits-juice-agar or fruits-juice-gelatine. But the white color of mycelium is retained for a long time in *Valsa Mali*. In *Valsa Paulowniae* the mycelium shows, after a while, light flesh or light pink color on the same medium.

(4) The color of the masses of pycnospores is a most conspicuous characteristic to distinguish our species from the others. The color of the spore masses in the three fungi is as follows:—

Valsa Mali MIYABE et YAMADA.....Yellow.
Valsa Paulowniae MIYABE et HEMMI .....Greenish black.
Valsa sp. on Prunus .....Red or flesh color.

c. Cultural Characters on Different Media.

(1) Sterilized Twigs.

On all sterilized twigs of *Prunus yedoensis, Prunus Mume, Prunus Persica* and *Pirus Malus*, the causal fungus seems to grow. On the twigs of *Pirus Malus*, however, the growth of the hyphae was rather poor, and only a few stromata and spores had developed in most cases. On all of them, the fungus

produces at first a white, web-like growth over the surface of the twig as well as in the bark. In about two weeks after inoculation, thick globose masses of mycelium, where the pycnidia or the perithecia are to develop, are produced in large numbers all over the surface of the twigs. Here and there the creeping mycelium and the globose masses turn brownish-yellow, especially on the cut surface. Only under moist condition in the dishes do the pycnidia thus formed push out very long red threads, composed of innumerable pycnospores. If one observes closely the development of such globose masses of mycelium, it is seen that the stromata of the fungus are first produced under the periderm and then the excessive growth of the mycelium takes place, rupturing the periderm. The pycnidium is formed either in the globose mass of mycelium or in the stroma under the periderm; while only under the periderm of the sterilized twig can the perithecia develop, having long necks which form the canals through such a mycelial mass. The globose masses are composed of the entangled mycelium and are not so compact as the stromata in nature. Unless careful observations be made, the development of the perithecia may be overlooked, for they are produced in the bark. The sterilized twig was the only medium on which I have succeeded in producing ascospores in the artificial cultures.

(2) Bark-Decoction Agar.

When streak cultures are made on bark decoction agar slants with the spores from a spore-horn, the mycelium begins to spread along the streak as a white or light brown weft and spreads rapidly toward the edge. The mycelium is apt to creep on the surface, and the aerial mycelium is scant. But in a week, at ordinary room temperature, the brown color begins to appear along the streak, and it broadens until the whole surface of the slant is covered with such brown mycelium. After a while, the small bunches of the mycelium appear over the surface of the medium, and soon some of them turn into the compact stromata which produce numerous pycnospores, forming beautiful reddish masses. But I have not yet been able to get ascospores on

this medium.

When the spores are inoculated on the central portion of the same medium in Petri-dishes or Erlenmeyer's flasks, the creeping mycelium spreads radially toward the marginal portion of the medium, and the color of the mycelium is also brown. This character is one of the marks which can be used in distinguishing the present fungus from other species of *Valsa*. The fungus, isolated from *Prunus yedoensis*, grows on both bark-decoction agar of *Prunus yedoensis* and *Prunus Mume* with the undistinguishable characters; and the fungus, isolated from *Prunus Mume*, indicates the same characters. This fact may prove that those two fungi belong to one species. But I had no opportunity to make a culture of the fungi isolated from other host plants on the same medium.

(3) Apple-Fruit Slice.

On this medium, the white mycelial growth of the causal fungus is at first very active, but soon it turns grayish yellow or yellow. On the same medium *Valsa Mali* also grows actively, but the mycelium is white or gray in color and the substratum gradually turns black; while in the case of our fungus the color of the substratum remains unchanged.

Up to the present time, neither kind of spores and stromata of the fungus is produced on this medium, while *Valsa Mali* produced them on some of the same cultures.

(4) Fruit-Juice Agar.

I used apple, apricot or pear to make this culture; and the cultural characters are, on the whole, similar in all cases. The mycelial growth was most vigorous and rapid. The color of the mycelium showed also a common characteristic on these media. Cultures containing agar showed at first a white cottony mycelial growth, but it gradually turns yellow or greenish yellow, while in *Valsa Mali* the mycelium retains for a long time its white color, and in *Valsa Paulowniae* it gradually turns from snow white to light

flesh color. The mycelial growth is apt at first to be closely limited to a small area, whence it spreads toward the margin of the medium. Although such cultural media containing agar are prone to become darkened by the fungus growth, beginning from the margin and spreading toward the center, it is not so conspicuous as with *Valsa Mali* on the same medium. But *Valsa Paulowniae* never causes darkening of the same media and the mycelium is apt to extend loosely over its whole surface, without making any cottony growth on a small area. None of these three fungi seems able to dissolve the agar.

The present fungus and *Valsa Mali* do not produce any spores on those cultures, though only on pear juice agar and gelatine were produced the mycelial bunches which have somewhat the appearance of the stromata. But *Valsa Paulowniae* produced numerous stromata and greenish black masses of pycnospores throughout the surface of apricot-juice-agar cultures.

(5) Fruit-Juice Gelatine.

Cultures containing gelatine showed at first the same color and mode of the growth of the mycelium, but at last the color turns a deeper yellow than in the case of the agar. The gelatine is more or less dissolved and becomes darkened by the growth of any of these three fungi.

(6) Corn-Meal Agar.

This medium was used by the author as the standard medium of the tannic acid cultures, which will be described in the following chapter. Cultures containing no tannic acid showed a vigorous growth of the mycelium, which was, however, very loosely entangled, continuing as the aerial mycelium, and the growth of the fungus caused no darkening of the medium. Although the color of the mycelium is white for a long time on the plane cultures in the Erlenmeyer's flasks, the slant cultures of the same medium show a very light yellow color after three months and in a few tubes appear the round bunches of mycelium, turning into the compact stromata. But even after a period of

four months, the spores have not been observed in those stromata.

(7) Bean Agar Slant.

On this medium the growth of the mycelium is very slow, and causes no darkening of the medium. But the mycelial growth is more or less thick, and after six months some of the tubes produced the stromata and pycnospores. The color of the mycelium is at first snow-white, but gradually turns to a very light yellow color.

(8) Soy Agar after Miyoshi.

The use of Japanese soy as a cultural medium of the fungi was at first proposed by Dr. M. MIYOSHI. The formula, which I used in this study, is as follows:—

Soy.....20 c. c.	Conc. boiled onion juice .....25 c. c.
Cane sugar..... 5 gr.	Dist. water .....50 c. c.
Agar ..... 1.5 %	

On this medium the growth of both aerial and creeping mycelium is very vigorous, covering the whole surface of the medium with a very thick hyphal layer, and its entanglement is more or less close. But both stromata and spores are not produced, and the color of the mycelium is at first white and then gradually turns into very light yellow or gray.

(9) Potato Agar.

This medium was also used as the standard medium of the tannic acid cultures. Cultures containing no tannic acid or citric acid showed a very poor growth of the fungus, lacking the aerial mycelium, and producing no stromata and spores. The growth of the fungus caused no darkening of the medium. Cultures containing low percentages of such acids showed more or less good results as the cultural media and about them I shall explain in detail in the following chapter. Although the color of the mycelium was white on slants in test tubes, containing no such acids, the plain cultures of

the same medium in Petri-dishes showed a special characteristic, namely,— that the creeping mycelium spreads toward the margin from the inoculated point, forming concentric rings of a dirty or purplish brown color.

(10) Oat Agar Slant.

On this medium the causal fungus grew very vigorously and also produced numerous pycnospores. At first the white aerial mycelium grew actively and after a while its color gradually turned more or less yellow. Then the fungus produced a cotton-like growth over the whole surface of the medium. From the first, the mycelial growth was very thick and in about eighty days the comparatively large stromata appeared and numerous pycnospores were produced from them as reddish masses.

On the same medium, *Valsa Mali* and *Valsa Paulowniae* also grew actively and produced pycnospores.

(11) Synthetic Solution.

The synthetic solution, which I used as the cultural medium, has the following formula:—

KH_2PO_4	0.50	$MgSO_4$	0.25
NO_3NH_4	1.00	$FeSO_4$	trace
Cane sugar	5.00	Water	100.00

The pycnospores, when sown in this cultural medium, germinated readily, and after a while, a poor thin growth of the mycelium was formed at the bottom of the flask. But the growth was very poor and it soon died without growing up to the surface of the liquid.

8. Effects of Tannic Acid on the Causal Fungus.

a. Tannin and its Relationship to the Fungi.

In nature, the causal fungus grows in the bark which contains tannin and in artificial cultures its spore production was most rapid and vigorous on twigs and bark decoction agar. From my histological studies of the diseased

branch, it was found that the amount of tannin is more or less increased in the diseased bark. It was thought desirable therefore to study the causal fungus in artificial cultures containing different percentages of tannin, to determine how it affected their vigor, growth and spore production. According to the previous researches, tannin is said to occur in practically all parts of the plant, but reaches its maximum in the bark of trees. It varies in amount in different parts of the plant, and at different seasons of the year. It also differs in different plants, but all tannins, from whatever source, have many properties in common. Tannin has generally been supposed to be largely a waste product, which serves more or less as a protective agent against animal and fungus attack. A few writers have raised the question whether or not it might serve some use in the physiological activities of the plant, possibly in the way of food. For instance, PFEFFER (1897)[20] and a few other authors are inclined to consider the tannin as something more than a by-product.

PFEFFER (1897)[20] says that fungi can assimilate many aromatic bodies such as tannin, resorcin, hydroquinone, phloroglucin, etc., but, except in the case of quinic acid, most of these afford very poor food materials; and again, he adds, tannins and glucosides are undoubtedly produced for definite purposes, and are not mere by-products produced under all circumstances.

COOK (1911)[6], who studied the effects of tannic acid on different species of fungi in artificial cultures, says that the results of his experiments in some respects confirm the investigations of MASSEE, WARD and others concerning the positive and negative chemotactic action on the germ tubes of the fungi. By his report, it appears that tannin is an important factor and that its importance varies according to the other substances with which it is associated in the cells of the host plant; and while tannin no doubt serves as a protective agent, its efficiency in this direction will vary somewhat with the character of the other substances within the cell. He thinks this may account for the variation in power of resistance between species, varieties, and individual plants. He further says: "The fact that plants which produce large quantities

of tannin are subject to disease is no argument against the preceding. The organism may live in tissues which bear little or no tannin, or which contain other substances that in a measure counteract the influence of the tannin. Furthermore, some species of fungi are much more resistant to tannin than others, and the species which attack these high tannin-bearing plants no doubt possess this quality."

CLINTON (1913)[5], who studied the effect of tannic acid on the chestnut blight fungus, *Endothia parasitica*, and saprophytic *Endothia gyrosa* in artificial cultures, asserts in his summary that both fungi can use tannic acid, at least in small amounts, as food. He also says: "To the writer it has occurred that possibly tannin may serve as an unusual source of food for certain trees rich in this product under unfavorable conditions for active formation of their normal food supply, such as drought years, and that such a use would lessen the supply of tannin laid down in the annual growth of wood formed in these years. Or possibly if not used for food, these unusual conditions do not favor its normal production. In any case, if tannin content bears a relation to the blight disease, it is not the tannin of the whole tree that counts so much as the tannin of the bark and wood of that year's growth. If it bears any relation to the chemical activity of the tree, we can readily see that it could easily vary from year to year according to external conditions more or less favorable for its production."

b. Tannic Acid Cultures.

To prove the above statements by COOK and CLINTON, I made three series of cultures; one was prepared after Clinton's method which was used in his study of the chestnut blight fungus, and in the two others I used the corn-meal-agar after COOK's formula. I have always used for these cultural experiments the pycnospores produced on the fresh cultures of the causal fungus isolated from *Prunus yedoensis*.

EXPERIMENT I.

In this case, I used potato-juice-agar as the standard medium. At first

500 grams of peeled potatoes were sliced as thin as possible and cooked in 500 c. c. of water for one hour, then strained through cloth. 15 grams of agar were melted separately in 500 c. c. of water. The two were mixed and enough water was added to make a total of 1000 c. c. It was boiled sufficiently in Koch's steam sterilizer and then filtered through cotton. Even if prepared by the same method, when the standard media had been made in many different flasks, they were thoroughly mixed just before they were divided into small flasks, in order to add the tannic acid.

I grew the causal fungus and the other two fungi mostly on slant test tubes and partly on the plain standard medium in Petri-dishes or Erlenmeyer's flasks, as checks for comparison, and also on the same medium to which had been added the following percentages of tannic acid: 0.1, 0.2, 0.4, 0.8, 1.2, 2.0, 5.0, 8.0, 11.0, 14.0. These cultures were made in November, 1914 and the observations were continued to April, 1915. From the results of these investigations I obtained the following informations:

(1) The growth of any of these fungi causes no darkening of the standard medium, when tannic acid is not added; while if it is added, even as little as 0.1 %, a darkening of the medium takes place. Such a fact was observed by CLINTON[5], in the case of cultures of the chestnut-blight-fungus, and he proposed that this darkening indicates an oxidation of the tannic acid by the fungus, since those tubes without the introduction of the fungus remain undarkened except with the higher percentages, when they color as soon as made, upon cooling.

(2) The medium in the tannic acid tubes remains liquefied when 0.8% or more of tannic acid is added.

(3) Cultures of this causal fungus in media containing 0.1, 0.2, 0.4 % of tannic acid show a more vigorous growth than the check cultures of potato-juice-agar without tannic acid. In tubes, which contain 0.8 % of tannic acid, the fungus also grows vigorously, and tends to form a more or less firm coating over the surface, after the manner of growth on the solid medium; but it is only after a long time that it begins to show the ordinary mode of

growth on the liquefied medium.

(4) On the medium containing 0.2 % of tannic acid, the pycnospores are produced at about the ninetieth day after inoculation, and the spore development is not vigorous.

(5) In the liquefied tubes, from 0.8 % to 2 % tannic acid, the growth of the causal fungus becomes less evident, generally appearing in floating patches, embedded masses, or lateral growth along the side of the tube. But it never makes a continuous coating over the surface of the medium. It generally fails entirely to make any growth at above 5 %, and only in a few tubes of 5 % did a very poor growth take place. Finally, at the highest percentages, 8 to 14, growth or even germination entirely ceases.

(6) *Valsa Mali* and *Valsa Paulowniae* generally show a poor growth on these cultures, and spores have not been produced. In the high percentages of tannic acid, *Valsa Mali* shows an enfeebled growth sooner than does the causal fungus of this disease, since at 0.8 % it makes comparatively little growth, which corresponds to the condition shown by the causal fungus at about 1.2 to 2 %. *Valsa Mali* generally fails to make any growth at above 1.2 %, or only a very poor growth is made in a few tubes up to 2 %. At above 5 % the growth or even the germination of *Valsa Mali* entirely ceases.

Generally, the toxicity of tannic acid against *Valsa Paulowniae* seems to be more conspicuous than against *Valsa Mali*. The culture solution containing tannic acid in which the Paulownia fungus distinctly shows its growth are only up to 0.2 %, although up to 1.2 % in some tubes a very poor growth could be noticed. In the Petri-dish or Erlenmeyer's flask cultures with the same media, I obtained almost the same results. The results of this experiment are given in Table I.

Table I.

Table showing the different degrees of growth of the causal fungus and two other species of Valsa on the standard medium containing different percentages of the tannic acid. The + sign indicates the growth of the fungus and its number

means the grades of hyphal growth. The — sign indicates the negative results.

Per cent. of tannic acid	The causal fungus		Valsa Mali		Valsa Paulowniae	
	Growth	Remarks	Growth	Remarks	Growth	Remarks
No tannin	++		++		++	
0.1	+++		+++	Hyphal growth most vigorous	++	
0.2	+++	Pycnospores produced	+++	Hyphal growth most vigorous	+	
0.4	++++	Hyphal growth most vigorous	++		+(—)	
0.8	+++		+(—)		—(+)	Fungus growth in a few tubes
1.2	+		—(+)	Fungus growth in a few tubes	—(+)	Fungus growth in a few tubes
2.0	+(—)		—(+)	Fungus growth in a few tubes	—	
5.0	—(+)	Fungus growth in a few tubes	—		—	
8.0	—		—		—	
11.0	—		—		—	
14.0	—		—		—	

As already stated, the stromata and the pycnospores of the causal fungus were formed not only on the bark-decoction-agar, but also on the oat agar. It is, therefore, not by the tannic acid alone that the spore production is induced. On the fruit decoction, the hyphal growth is very vigorous, but the spores are not developed. *Valsa Paulowniae*, however, readily produces numerous pycnospores on the apricot-juice-agar. From these facts, one may see the importance of the knowledge of the effects of a certain acid on the fungi under consideration. For the purpose of determining this question, I added the 0.2, 0.4, 0.8, 1.2, 2.0 % of citric acid, to the same standard medium employed in the previous experiments, and on which I have cultured the three species of *Valsa* using the same methods and placed under the same conditions. In this series of experiments I got comparatively good results relating to both the spore production and the hyphal growth. According to my test by titrating with a certain solution of NaOH, using phenolphthalein as an indicator, the acidities of solutions of citric acid are very much higher than those of the same per cent solution of tannic acid.

From these experiments I obtained the following results:

(1) All the media remain liquefied when citric acid is added, but some of them were at last solidified in consequence of the vigorous growth of the hyphae.

(2) The growth of any of these fungi causes for the most cases no darkening of the media even when citric acid is added.

(3) The causal fungus grows in all the cultures containing citric acid except 2 %, showing a more vigorous growth than in the check cultures. The cultures containing 2.0 % of citric acid show a comparatively poor growth of the causal fungus and a darkening of the medium takes place in some of them.

(4) Some of the cultures of this fungus containing 0.4 % of citric acid produce the pycnospores at about the 120th day after inoculation, and the spore development is conspicuously vigorous all over the surface of the medium.

(5) The color of the mycelium of this fungus gradually turns from white to light yellow on the cultures containing citric acid, while on the cultures containing tannic acid it turns to dirty brown.

(6) Although *Valsa Mali* grows in all percentages, its growth is generally poorer than in the case of the causal fungus; and the spores are not produced.

Although *Valsa Paulowniae* grows vigorously in all percentages, yet in the tubes containing 0.2 and 0.4 % of citric acid its growth is most vigorous. The cultures of the fungus containing 0.8, 1.2 and 2.0 % of citric acid produce numerous pycnospores at about the 120th day after inoculation, and spore development is also conspicuously vigorous, with the exception of 2 % culture, in which the development is rather poor.

The results of this series of experiments are given in Table II, with the same signs as in the preceding Table.

Table II.

Table showing the effect of the different percentages of citric acid in the standard medium on the development of the causal fungus and two other species of Valsa.

Per cent. of citric acid	The causal fungus		Valsa Mali		Valsa Paulowniae	
	Growth	Remarks	Growth	Remarks	Growth	Remarks
No acid	+		+		+	
0.2	++		+		++++	Hyphal growth most vigorous
0.4	++	Pycnospores produced	+		++++	Hyphal growth most vigorous
0.8	++++	Hyphal growth most vigorous	+++	Hyphal growth most vigorous	+++	Pycnospores produced
1.2	++		++		++	Pycnospores produced
2.0	+(—)	Some of cultures turn to black	+(—)		+	Pycnospores produced

Experiment II.

The next experiment was made with only the cultures of the causal fungus, using the standard medium of corn-meal-agar to which had been added the following percentages of tannic acid: 0.1, 0.4, 0.7, 1.0. To make the standard medium I used the following method:—

15 grams agar melted in 500 c. c. water.

15 grams corn meal cooked in 500 c. c. water for one hour.

Strain through cloth.

Mix the two and filter through cotton.

In this experiment, I used Erlenmeyer's flasks containing 25 c. c. of the media in each. The cultures were started on the 23rd of December, 1914. The observations were continued to April, 1915, and the following results were obtained.

(1) The growth of the fungus causes no darkening of the check-medium containing no tannic acid; but when tannic acid is added, even as low as 0.1 %, the growth of the fungus causes a darkening of the medium, as already stated in the case of the first experiment.

(2) The medium containing tannic acid remains liquefied when 0.7 or 1.0 % is added.

(3) On all cultures containing tannic acid, the mycelium turns to a dirty brown color, while on checks it has remained colorless.

(4) Check cultures containing no tannic acid show a vigorous growth of mycelium, which is, however, very loose, sending up the aerial mycelium. Spores have not been produced in these check cultures.

(5) Cultures containing 0.1 % of tannic acid also show a vigorous growth of mycelium, but its entanglement is a little closer, and some of them produced numerous red masses of pycnospores at about the 50th day after inoculation. In this case, the spore production is more vigorous than in the cultures containing 0.4 % of tannic acid.

(6) Cultures containing 0.4 % of tannic acid show the most vigorous growth of mycelium in this series of experiments, forming a close coating over the surface of the media. Some of them produced also numerous red masses of pycnospores at about the 50th day after inoculation.

(7) The growth of hyphae on the cultures containing tannic acid seems to be slow at first, but later becomes more luxuriant, compared with the cultures without it.

(8) Some of the cultures containing 0.7 % of tannic acid also show a vigorous growth of mycelium, but it takes a long time for the mycelium to appear and grow on the surface, due to the liquid of the medium; and at last the growth of the fungus tends to form a more or less close coating over the surface. But in some of these cultures the fungus entirely failed to grow.

(9) All cultures containing 1.0 % of tannic acid fail to allow the growth of the fungus.

The results of the second series of experiments are given in Table III.

Table III.

Showing the effect of the different percentages of tannic acid in the standard medium on the growth of the causal fungus.

Per cent. of tannic acid	Growth of Mycelium		Production of pycnospores
	Grade	Remarks	
No tannin	+ +	loose	—
0.1	+ +	close	+ +
0.4	+ + +	closest	+
0.7	+ or —	close	—
1.0	—		—

Experiment III.

In this experiment the same media and methods were used as in the case of the second experiment. It was started at the beginning of May, 1915. In this case, *Valsa Mali* and *Valsa Paulowniae* were taken up in addition to the causal fungus, for the sake of comparison. The results are given in the following table.

Table IV.

Showing the effect of the different percentages of tannic acid in the standard medium on the growth of the causal fungus and two other species of Valsa.

Per cent. of tannic acid	The causal fungus		Valsa Mali		Valsa Paulowniae	
	Growth	Production of pycnospores	Growth	Production of pycnospores	Growth	Production of pycnospores
No tannin	++	—	++	—	+++	—
0.1	++	+	++	—	+	—
0.4	++	—	+	—	+ or —	—
0.7	+ or —	—	—	—	—	—
1.0	—	—	—	—	—	—

c. Conclusion.

From the foregoing experiments on the effect of tannic acid on the development of the causal fungus and other species of *Valsa*, the following conclusions may be drawn:—

(1) The fungi can use tannic acid, at least in a small amount, as food,—shown by the blackening of the media through oxidation, and by a more luxuriant growth, with a low percentage of the tannic acid added, than in the case without it.

(2) Higher percentages of tannic acid (1.2 % and above) are detrimental to a vigorous growth of the causal fungus, and finally (8 to 14 %) entirely inhibit its growth.

(3) Different species of the same genus may vary in power of resistance. The resistant power of the causal fungus against tannic acid is the highest among our three species of *Valsa*. I agree, therefore, with COOK's opinion [6] that some species of fungi are much more resistant to tannin than others, and the species which attack the high tannin-bearing plants no doubt possess this quality.

(4) High percentages of tannin have the tendency to retard or inhibit the growth of fungi. But the growth of the fungi is frequently increased by the use of low percentages of tannin, and in this case the hyphal growth is closer than without it.

(5) In some cases the growth was at first retarded by certain percentages of tannin, but later became as good or better than on the medium without it.

(6) The formation of spores was stimulated, at least in the case of the causal fungus, by low percentages of tannin.

(7) Citric acid also stimulates the spore formation of this fungus, probably rather more than tannic acid. But on the cultures containing tannic acid, the time required for fruiting was shorter than on the cultures containing citric acid.

(8) The fruiting of this fungus is not determined by the acidity of the medium alone, but it seems to me to be due to a combination of various factors that constitute the real cause of the stimulation.

9. Confirmation of the Genetic Relation between Pycno- and Ascosporous Stages.

On the infected area of the branches and trunks, two spore forms, pycno- and ascospores, usually develop in different stages, as already described. It has been demonstrated by various authors that some species of *Cytospora* belong to *Valsa* as its pycnidial stages. In my case also, the pycnidial stage of the causal fungus is a species of *Cytospora*.

Inoculations made with pure cultures of our *Valsa* and *Cytospora* produced the same results. The characters of pure cultures of these two types of the spores are quite identical. As a direct proof, the ascospores of *Valsa* were inoculated on the sterilized twigs of *Prunus yedoensis*. As I had anticipated, a *Cytospora* was at first produced which is identical in every respect with one on the natural host, and finally the *Valsa* stage was reproduced. Moreover, when the *Cytospora* spores were inoculated on the sterilized twigs of the same plant, the same *Cytospora* was reproduced, and after a long time a *Valsa* form was produced which is identical with the causal fungus.

10. Drop-Cultures.

a. Germination of pycnospores.

The pycnospores were not made to germinate well in pure water. Although I sowed the spores in a drop of distilled water over and over again, I could not succeed in making them germinate well. But once in a while I noticed that a few spores put out a slender germ-tube at one end, without conspicuous swelling, but soon died for want of nutrient. I also got the same negative results with rain-water, tank-water, and various kinds of sugar in such low percentages as 1, 2, 5 or 10%. I have found the most satisfactory medium for this purpose to be a decoction made by boiling host-bark and diluted pear juice.

The time required for germination varies with the temperature. At room temperature in summer, which ranges from 20° to 28°C, the germination occurs in thirty to sixty hours. At lower temperatures the process often

requires four or five days. From these facts, we infer that infection by the pycnospores can occur only in the warm period of summer.

The process of germination begins with an enormous swelling of the spores, especially in width. The spores measuring 5.25–12.25 × 1.75–2.63 μ before germination were found, at the end of twenty-four hours in a bark decoction, to measure 6.13–14.0 × 4.72–7.0 μ; and not infrequently they reached 15.75 × 7.88 μ just before germination. In such cases, the spores took various irregular forms,— ellipsoid, ovoid, obovoid, subglobular, etc. In a few cases I noticed that some spores were divided into two cells just before germination (Pl. X, Fig. 4–5.). The spores germinate in thirty to sixty hours, throwing out one to three germ-tubes. Usually a germ tube grows out from one end, and this is followed later by a second one from the opposite end (Pl. X, Fig. 5–7.). The germinating hyphae are at first hyaline, about 3.2–4.0 μ in width and occasionally swollen in an irregular shape. The branching and septation of the germ tube or mycelium then take place; and the old hyphae often turn gradually to a yellow or light brown color after three or four days. The germinating spores and hyphae are at first granular in contents and after a while vacuolization occurs in many cases.

In the experiments described above, I took all the spores from pure cultures. Such swelling and manner of germination of the pycnospore are not infrequent in many genera belonging to the Ascomycetes. ADERHOLD (1903)[1] also described the same process in the case of *Valsa leucostoma* (PERS.) FR.; but such two-celled spores have not been observed by him. According to DE BARY (1887)[3], the cause of such enormous swelling of the spores before germination is attributed to absorption of water. On the contrary, ANDERSON and RANKIN (1914)[2] speaking about the phenomenon in the case of *Endothia parasitica* (MURR.) ANDERS., the chestnut blight fungus, write:— "The swelling of the spores is due, not merely to a mechanical imbibition of water, but also to a process of growth. Pycnospores stained just before the germ tube is started show that the increase in size is accompanied by active nuclear division, two to six nuclei then being present. The

nuclei pass out into the germ tubes almost as soon as they start. The wall, also, has increased in thickness until it almost equals the diameter of the resting spore." This is surely a very interesting observation, but I can not yet assert whether it is correct or not, at least in the case of the present fungus.

b. Germination of Ascospores.

Unlike the pycnospores, the ascospores germinate readily in pure water. After two or three days, the germinating hyphae grow weaker and weaker, and at last die in water. The time required for germination is much shorter than for the pycnospores in a nutritient solution or even in pure water. At comparatively low temperatures, ranging from 14° to 24°C, it occurs perfectly within the first twenty hours. I even observed the septation and branching of the germ tube at the end of twenty-four hours. From such facts, we can safely infer that if they are placed under more favorable circumstances, germination will take place within six or twelve hours, and that therefore infection by the ascospore is most considerable and dangerous.

Like the pycnospores, the ascospores swell before germination, but not to so great an extent as in the case of the former. The ascospores, measuring 16.0–22.0 × 3.2–4.8 μ at first, were found, just before germination in a bark decoction and pear juice, to measure 20.0–36.0 × 4.0–10.0 μ. But according to my observations, they always swell uniformly, keeping their original allantoid form. This character differs from that of *Valsa leucostoma* in which ADERHOLD (1903)[1] observed the spores to swell to ellipsoidal or globular shape. The first germ-tube usually appears at one end; the next one comes from the other end or from one side; and these are occasionally followed by another one or two, making a total of one to four germ tubes. They are most commonly thrown out from both ends, but rarely from the convex side of a spore. About 10% to 15% of the germinating ascospores are divided into two cells as in the case of the pycnospores (Pl. X, Fig. 1–2.). In 1891, such two-celled ascospores before germination were already observed

by BREFELD[3] in the case of germination of *Valsa ceratophora* TUL.

The germinating hyphae are hyaline at first, about 3.2–6.0 μ in width, and often the old parts turn light brown or light yellow. In a drop of bark decoction, it was occasionally observed that some germinating hyphae stopped their growth when they reached about 32.0–80.0 μ in length, swelling a little, and that soon the fine horn-like hyphae developed again from their swelled ends (Pl. X, Fig. 3.).

In the experiments, I obtained pure ascospores from the perithecia on the natural host by letting them eject the spores on a slide. In the process of germination of the pycno- and ascospores, the various behavior of the hyphae and changes produced in their contents are very similar. The ascospores of our present fungus do not require a period of rest, but germinate directly after maturity, if placed under favorable conditions.

c. Observations on the Hyphae in Drop Cultures.

If the surrounding conditions are favorable, the hyphae started from the germ-tube proceed with their growth in a radiating manner. But the rate of growth varies considerably with the temperature and the kinds of nutritient solutions; and in such an acid solution as fruit juice, the hyphae grow most actively. In the bark decoction, the growth is not so active, but the dense and short branching, which is said by many authors to be a young stage in the development of the pycnidium, is apt to be quickly produced. The growing hyphae are always hyaline, although their basal parts turn yellow or light brown; and the hyphae are also not of uniform diameter, but vary, according to my observation, from 2.0 to 8.0 μ.

Under the microscope, we often see the hyphae anastomosing in many ways; and in consequence, they at last make the hyphal network. After more than ten days, it is not an infrequent sight to see them entangling in a mass. Occasionally I saw a substance being secreted on the tip of the hyphal branch, which is at first hyaline and at last turns grayish yellow or dirty yellow. But the nature of this secreted substance is not yet known to me.

11. Resistant Power of the Hyphae against Solutions of Corrosive Sublimate, Copper Sulphate, and other Chemicals.

Solutions of corrosive sublimate and copper sulphate are generally used for fungicides, and occasionally soda-solutions are used for the same purpose in our country. When surgical operations of various canker-diseases are performed, they are also used to wash the exposed surface of the wound. In order to determine first their killing power against the hyphae of the present fungus, I poured each of these poisonous solutions into a test-tube, in which the hyphae had been growing up actively. After certain intervals, as indicated in the following tables, I took out the pieces of hyphae with a platinum needle, and washed them in distilled water which had been previously sterilized, and again inoculated on a new cultural medium.

Of course such experiments are not precise, because the chemicals can not sometimes touch the hyphae, owing to the air layer which is surrounding them. It was most conspicuous in the case of copper sulphate. Even if the results of my experiments were not conclusive, for the one purpose of determining their value as fungicides, we can safely summarize as follows:—

1. Corrosive sublimate has a strong sterilizing power for the hyphae of the present fungus, being effective even in such a dilute solution as 0.01 %. But as a fungicide, it is safer to use a 0.05 % or 0.1 % solution.
2. Copper sulphate and carbonate of soda did not give good results for the same purpose, probably owing to the disturbance of the air layer.
3. Caustic soda has sterilizing power for the hyphae of the present fungus, when such a concentrated solution as 10 % or 20 % is used. Even with the 5.0 % solution, we can probably obtain good results for the same purpose.

The results of these experiments are given in the following tables. In these tables the plus sign means the presence of the living hyphae and the minus sign means their death.

Table V. *Results of experiment with corrosive sublimate.*

Time of soakage (in minutes)	0.01 %	0.025 %	0.05 %	0.1 %
0	+	+	+	+
1	—	—	—	—
5	—	—	—	—
10	—	—	—	—
30	—	—	—	—

Table VI. *Results of experiment with copper sulphate.*

Time of soakage (in minutes)	0.5 %	1.0 %	2.0 %	3.0 %	4.0 %
0	+	+	+	+	+
1	+	+	+ —	+ —	+ —
5	+	+	+ —	+ —	+ —
10	+ —	+ —	+ —	—	—
30	+ —	+ —	+ —	—	—
60	+ —	+ —	—	—	—

Table VII. *Results of experiment with caustic soda.*

Time of soakage (in minutes)	1.0 %	2.0 %	5.0 %	10.0 %	20.0 %
0	+	+	+	+	+
1	+	+	+ —	—	—
5	+ —	+	—	—	—
10	+ —	+ —	—	—	—
30	—	—	—	—	—

Table VIII. *Results of experiment with "carbonate of soda".*

Time of soakage (in minutes)	5.0 %	10.0 %	20.0 %	30.0 %	40.0 %
0	+	+	+	+	+
1	+	+	+	+	+
5	+	+ —	+	+	+ —
10	+	+ —	+	+ —	—
30	+ —	+ —	+ —	+ —	—

12. Systematic Position and Nomenclature of the Causal Fungus.

From the morphological characters, we may easily recognize our fungus to be a species of *Valsa*. It has pycnidia of the *Cytospora* type.

The genus *Valsa* was first described by FRIES (1849) [36]; and then NITSCHKE (1867) [18], who made a new family *Valsaceae* out of it, studied the genus exhaustively in his work "Pyrenomycetes Germanici". In ENGLER and PRANTL'S "Die Natürlichen Pflanzenfamilien", LINDAU (1897) [16] divided the genus into ten subgenera. Most of these subgenera were founded by NITSCHKE and endorsed by WINTER (1887) [34] and SCHRÖTER (1908) [26]. They are distinguished from one another chiefly by the construction of the stromata. In SACCARDO'S "Sylloge Fungorum", however, only two subgenera, *Euvalsa* and *Leucostoma*, are treated as belonging to this genus and all other subgenera were raised to independent genera. On the ground of the morphological characters of the stromata, I have come to the conclusion that the present fungus is a species of *Euvalsa*. NITSCHKE divided this subgenus further into two groups, *Monostichae* and *Circinatae*, and our fungus belongs to the latter, which corresponds to *Macrosporae* in SACCARDO'S system.

Up to the present time, more than fifteen *Valsa* and still more *Cytospora* were found both saprophytically and parasitically on the twigs and branches of the various species of *Prunus* in the world; but most of them differ in their systematic position and also in other essential characteristics from our fungus. With some of them, owing to the brevity of their descriptions, I could scarcely make any comparison with our causal fungus.

Valsa leucostoma (PERS.) FR. which causes the disease of many drupaceous trees, known as "die back", throughout Europe, Australia and America, not only has the "conceptaculum" between the stroma and the host tissue, but differs also from our present fungus in many other essential points, as shown in the following table:—

Table IX.

	Valsa leucostoma	The Causal Fungus
Number of perithecia in a stroma	3—10 (to 20 after some authors)	Commonly 15—40
Asci	48—56×6—8 μ (35—45×7—8 μ after STEVENS, 40—62×5—8 μ after ADERHOLD.)	60—96×8.8—16 μ
Ascospores	10—18×2.5—3.5 μ (9.3—14×2.3—2.6 μ (mostly 10—12×2.5 μ) after ADERHOLD, 9—12×2—2.5 μ after STEVENS.)	10—28×3.2—7.2 μ (Commonly 18—22×4.0—4.8 μ)
Pycnospores	4—5×1 μ (3.5—9.3×0.87—2.3 μ (Commonly 4.5—6×1—1.5 μ) after ADERHOLD, 5.5—7.5×1.0—1.5 μ after MCALPINE.)	3.5—15.75×1.0—2.63 μ (Commonly 7.0—8.75×1.4—1.75 μ)

Valsa Prunastri (PERS.) FR., reported as the cause of a serious disease of apple, plum and other drupaceous trees in England, belongs to the subgenus *Eutypella*, which was raised by SACCARDO to an independent genus, and is too distinct from our fungus for the purpose of comparison.

Valsa ambiens (PERS.) FR., which is described as widely distributed on various kinds of trees and which belongs systematically to the same subgenus, seems to be nearly allied to our causal fungus. But one can still find various differences, as seen in the following table, especially in the measurement of the pycnospores and the structure of the stromata.

Table X.

	Valsa ambiens	The Causal Fungus
Size of stromata	1.5—3.0 mm. in diameter at base.	3—5 mm. in breadth, 2—4 mm. in height, and 1—1.5 mm. in depth.
Number of perithecia in a stroma	4—20	Commonly 15—40
Construction of stromata	Lower half of the stroma consists mostly of the host-tissue.	The greater part of the stroma consists of the mycelial tissue, and the host-cells are scattered in it.
Asci	oblong or clavate, 40—88×8—16 μ, 4—8 spored.	Cylindrical or rarely clavate, 60—96×8.8—16 μ, always 8 spored.
Ascospores	(16—24×3—6 μ in 8 spored asci, 24—36×5—8 μ in 4 spored asci after WINTER and SCHROETER.) (16—18×3—4 μ in 8 spored asci, 20—26×5—6 μ in 4 spored asci after SACCARDO.)	10—28×3.2—7.2 μ (Commonly 18—22×4.0—4.8μ)
Pycnospores	5—7 × 1 μ	3.5—15.75×1.0—2.63 μ (Most commonly 7.0—8.75×1.4—1.75 μ

Further we were fully convinced that these two fungi are quite different, when we examined and compared our fungus with the European specimen of *Valsa ambiens* preserved in the Herbarium of our University.

As we have not been able to find any species of *Valsa* or *Cytospora* which agrees exactly with the present fungus, either in mycological or phytopathological literature, we consider the fungus in question to be quite new to science. The species may be characterized as follows:—

Valsa japonica MIYABE et HEMMI sp. n.

Stromata scattered, produced at first under periderm, then erumpent, forming lenticel-like openings, which are slightly elongated horizontally, conical or wart-like with round or elliptical bases, gray or brownish-gray with many black ostioles of perithecia on the surface, gray or yellowish gray, subcoriaceous in the inside, consisting for the most part of the mycelial tissue, with a few host cells scattered deeply in it, about 3–5 mm. in average breadth, 2–4 mm. in average height and 1–1.5 mm. in depth; perithecia immersed, subglobose with long neck, compactly arranged in concentric circles, 15–40 in a stroma, 350–580 μ in diameter; asci cylindrical or rarely clavate, subsessile or short stalked, 60–96 × 8.8–16.0 μ, hyaline, eight-spored, evanescent in old perithecia; spores mostly biseriate, sometimes irregularly arranged, allantoid with rounded ends, almost hyaline, slightly curved, 10.0–28.0 × 3.2–7.2 μ (commonly 18.0–22.0 × 4.0–4.8 μ).

Pycnidia: stromata scattered, produced at first under periderm as subspherical or ellipsoidal cushions, then erumpent, turning to conical or wart-like shape, average about 2.2 mm. in breadth, 1.5 mm. in height and 1.0 mm. in depth; internally divided into cavities with a single common exit; pycnospores hyaline, cylindrical, rounded at both ends, generally curved but occasionally straight, 3.50–15.75 × 1.00–2.63 μ (most commonly 7.00–8.75 × 1.4–1.75 μ), oozing out in reddish curls when moist; basidia hyaline, simple or branched, variable in length but measuring 14–28 × 1.75–2.1 μ.

Hab. On the bark of *Prunus yedoensis* MATSUM.

Prov. Ishikari; Sapporo and its vicinity (I & II.* from 1913 to 1915. K. MIYABE, S. ITO, T. HEMMI), Asahikawa (I. Sept. 26, 1914. T. HEMMI).

Prov. Oshima; Hakodate (II. Feb. 14, 1915. S. NISHIDA).

On the bark of *Prunus sachalinensis* KOIDZ.

Prov. Ishikari; Sapporo and its vicinity (I & II. Apr.—Nov. 1914. T. HEMMI).

On the twigs of *Prunus Koidzumii* MAKINO.

Prov. Ishikari; Sapporo (I. May 18, 1915. T. HEMMI).

On the bark of *Prunus serrulata* LINDLEY.

Prov. Ishikari; Sapporo (I. August 15, 1914. T. HEMMI).

On the bark of *Prunus serrulata* LINDL. var. *nobilis* (KOIDZ.) f. *Yokihi*.

Prov. Ishikari; Sapporo (II. May, 1915. I. NAMIKAWA).

On the twigs of *Prunus kurilensis* MIYABE.

Prov. Ishikari; Sapporo (I & II. May 12, 1915. S. ITO).

On the bark of *Prunus Mume* S. et Z.

Prov. Ishikari; Sapporo (I & II. 1914–1915. K. MIYABE, S. ITO, T. HEMMI).

On the bark of *Prunus Persica* S. et Z.

Prov. Ishikari; Sapporo (I & II. Feb. 8, 1915; I & II. Apr. 8, 1914. T. HEMMI).

13. Inoculation Experiments.

These experiments were undertaken over and over again in order to determine the parasitic habit of this fungus. Although I had failed in many cases with these inoculations, I succeeded at last under a special condition to produce the disease artificially. The most important reason for such failures seems to be the powerful resisting power of the seedlings, which were used in these experiments for convenience' sake.

* I = Pycnidial stage. II = Ascosporous stage.

Experiments I.

It is rather difficult in making a series of inoculations in the field to provide conditions which correspond to those of natural infection, and also by such experiments to draw the conclusion that the disease is caused by the fungus used.

From April to June, 1914, I inoculated the fungus on *Prunus yedoensis*, using various methods in the field. But all these inoculations resulted in failure with one exception, that is, when I inoculated with the germinating ascospores in the apple juice into a wound and covered it up with moist cotton and paraffine paper for a few days. After two months I found the characteristic appearance of the disease around the inoculated point. The failures of other inoculations were due chiefly to the want of moisture or to the washing away of the spores by a heavy rain.

Experiments II.

In this case, the seedling trees of *Prunus yedoensis*, a little more than two years old, grown in pots, were used. In July, 1914, the inoculations were made partly with pycnospores from artificial cultures, and partly with ascospores from a natural host. Ordinarily, a small slit in the bark was made with a sharp sterilized scalpel, and the spores were introduced with a sterilized needle. The wound was covered with moist absorbent cotton, and then all was bound up with paraffine paper. The seedlings, inoculated by the same method, were partly placed in a moist condition under a bell-glass for a few days. As checks, uninjured sound bark and lenticels were also inoculated. Although in all these experiments the moisture was supplied in sufficient quantity, in no case was the infection accomplished.

Experiments III.

Again in September (28–30), 1914, the seedlings of *Prunus yedoensis*, a little more than two years old, grown in pots, were used for inoculation. In these experiments, for the place of inoculation, a small portion of the bark

was burned in addition to the same methods used in Experiment II. The experiment was based upon the idea that the fungus may first require the dead cells for the penetration of its germ-tubes. Upon such a hypothesis, infection experiments were performed by ADERHOLD [1] with great success in the case of *Valsa leucostoma*. To keep it moist, I placed the pot under a bell-glass for one week. The results are given in the following table.

Table XI.

Inoculated bark	Inoculum	Results
Smooth bark	Pycnospores from culture	–
Lenticel	Pycnospores and mycelium from culture	–
Cut and burnt bark	Pycnospores from culture	+
Cut and burnt bark	Mycelium from culture	+
New cut wound	Diseased bark	–
New cut wound	Ascospores from natural host	–
New cut wound	Pycnospores from culture	–
New cut wound	Mycelium from culture	–
New cut wound	Pycnospores and mycelium from culture	–

The seedling which had been inoculated with the mycelium to its burnt portions suddenly wilted in about two weeks after inoculation, and the seedling which was inoculated with the pycnospores to a similar wound also suddenly wilted in about three weeks after inoculation. But even on the same seedling, the twigs above a burnt wound which was treated in the same way, with the exception that no spores were introduced into the wound, did not show any change for a long time. In the former case, the cork layer of the infected portion was raised in numerous little blisters. Sections through such a dead portion showed the tissues of the bark as well as the water ducts of the woody portion to be traversed by the mycelium. Under each blister was a compact mass of the mycelium, which is no doubt a young stage in the development of the stroma. But in no blisters were pycnidia developed, whose non-formation is chiefly due to the resistibility of the young host.

Experiments IV.

As a result of the above experiments, it was necessary to try inoculations on a large branch. On January 19th, 1915, a large branch of *Prunus yedoensis*, about four centimeters in diameter, was cut and put in water in the laboratory; and the water was renewed every day in order to prevent the multiplication of micro-organisms in it. On about the 20th of February the branch was in full blossom. On the day when the branch was cut, it was inoculated with the fungus in various ways, and on the 13th of February the results were as follows:—

Table XII.

Inoculated portion		Inoculum	Results
(1)	New cut wound with scalpel	Ascospores from natural host	Bark slightly sunken and gum flowed out
	,,	Check	No change
(2)	New cut wound with saw	Pycnospores and mycelium from culture	No change
	,,	Check	No change
(3)	Burnt wound	Ascospores from natural host	Bark sunken, gum flowed out and stromata developed
	,,	Pycnospores and mycelium from culture	Bark sunken, gum flowed out and stromata developed
	,,	Check	No change

In these experiments, the wounds were kept moist by covering them with moist cotton, and then binding them up with paraffin paper for a week.

In the first case, the portion around the inoculated point stopped its growth, forming an elliptical sunken spot; and the gum was exuded from the inoculated wound. But in such a sunken area, the progress of the disease soon ceased, and no stroma of the fungus developed in it. But on the contrary, the sunken area of the third case gradually increased its extent and the stromata of the fungus were developed sparingly upon it. When the branch was in full-bloom, thirty-five days after the inoculations, it began to wilt, showing a diseased appearance. In April, numerous small pustules of the fungus stromata appeared, scattered throughout the surface of such a dead

branch, showing the symptom of a girdled branch. Similar experiments were also undertaken with smaller branches, and the same results were obtained.

Besides these experiments, I also inoculated the same fungus on the branch of *Prunus Cerasus*, using the same method, but the results were negative.

Experiments V.

In this case, I used two comparatively aged potted trees of *Prunus Mume* which were at that time in full-bloom in a green house, and on each tree small burnt areas and slit wounds by a scalpel were made just before inoculation. In the injured portions of the one I inoculated the mycelium from a culture which was isolated from *P. Mume*, on February 12, 1915. On another one I inoculated the mycelium from a culture which was isolated from *Prunus yedoensis* on the same day. To keep it moist, I placed the pot under a bell-glass for one week, and then it was placed for a long time in a warm laboratory.

On the first tree, the bark of an inoculated branch changed its color around the burnt portion, then gum flowed out, and at last many small pustules of the fungus stromata appeared rather scatteringly, while the checks indicated no change. The discolored portion of the bark gradually extended its area, which was sunken slightly, but up to the end of June the branch was still in foliage, except a small twig which was branched near the infected portion. From July to September, the upper portion of the branch from the infected point died gradually, and the fungus stromata also produced scatteringly all over its surface. On the other branch which was inoculated in the slit wounds, the results were negative.

On the second tree, the bark in the vicinity of both burned and injured portions on which inoculations had been made, first changed color, then gum flowed out, and at last the upper two twigs died, when there appeared also small pustules of the fungus stromata. The wilted flowers remained clinging

on the dead twig for a long time (Pl. VIII, Fig. 3.). But the lower branches were still in foliage up to the end of September, although the diseased area had slowly been extending.

Experiments VI.

From January to April, 1915, I several times tried various kinds of inoculation on the three-year seedlings of *P. yedoensis* and also the comparatively old potted trees of a kind of *P. serrulata*. And the conclusion was reached that this fungus most easily infects the hosts at the old wounds which have a layer of dead cells on the exposed surface. In these experiments the pots were placed under bell-glass in order to keep them moist for a week. The details of these experiments are shown in the following table:—

Table XIII.

Inoculated portion	Time of inoculation	Inoculum	Results
1. Sound buds of seedling	Jan. 26	Pycnospores from culture	— but infected buds wilted
2. New slit wounds on seedling	Jan. 26	Ascospores from natural host	—
3. Older wounds on seedling	March 13	Mycelium from culture	+
4. New slit wounds on aged potted tree	Febr. 12	Mycelium from culture	—
5. Burnt portion on aged potted tree	Febr. 12	Pycnospores from culture	—
6. Burnt portion on aged potted tree	April 16	Pycnospores from culture	+

In the first case, it seemed to me that the germ-tubes of the inoculated spores had the power to kill the buds. But on the sound bark, the infection never took place. In the third case, four slit wounds were given with a sterilized scalpel on the bark of the three-year-old seedlings, about two months before inoculation. On March 13th, 1915, mycelium with a little piece of the cultural medium was inoculated in the upper three wounds, and the lowest wound was treated as a check. About the 9th of April, the upper-most shoot wilted, and at the end of the same month the second shoot wilted, developing at the same time many small pustules of the young stage of the

fungus stromata on the bark. Its appearance on the 1st of May is shown in the photograph of Pl. VIII, Fig. 2. In the sixth case, the upper twigs wilted about the middle of May, and the pustules of the stromata began to be produced at the end of the same month.

Experiments VII.

In April, 1915, several inoculation experiments were made with mycelium from cultures, isolated from various host plants, on the burned bark of the seedlings of *P. yedoensis*. The results of these experiments are as follows:—

Table XIV.

Host of inoculated fungus	Time of inoculation	Results
Prunus Persica S. et Z.	April 9	On May 10 the uppermost shoot wilted and on about June 4 the stromata developed.
Prunus sachalinensis Koidz.	April 9	On May 3 the uppermost shoot wilted and at the end of May stromata developed.
Prunus Mume S. et Z.	April 30	Negative
Prunus Cerasus L.	April 16	Negative
Prunus yedoensis Matsum.	April 9	On May 1 the uppermost two shoots wilted and at the end of May stromata developed.

14. Entrances and Promoting Agencies of the Disease.

Judging from the results of the inoculation experiments, we may safely infer that *Valsa japonica* is the direct cause of this canker-disease, and that the fungus is a wound parasite requiring for its infection various kinds of wounds. When the spores germinate in a wound, the mycelium derived from the germ tubes thrives on the injured and dead cells until it has produced a mass of mycelium. Then, gradually accumulating strength as it increases, the mycelium *en masse* pushes out into the living tissue of the bark. Single hyphae do not seem to possess the power of penetrating into the living cells, but the invasion is accomplished by the force of mass action. Starting from

an infected point, the hyphae grow and spread in all directions, completely destroying parenchyma, collenchyma and cambium cells as they go. These facts are not open to question, judging from the results of the inoculation and anatomical studies. I shall now give a brief explanation, based upon my own observations, of the mode of the entrance of the hyphae, and its promoting agencies.

a. Crotch of branches.

The crotches of branches have many chances to get cracked by wind, snow and various other causes. There are commonly many rumples which are composed of dead tissues, especially in the case of old branches. Such places are also under such a condition as to remain moist for a comparatively long time. It is therefore probable that such crotches serve as places of entrance of the parasite. Indeed, I have observed many cases in which the disease spreads into both branches, starting from a crotch, as shown in the photograph in Pl. VII, Fig. 1.

In the case of *Valsa Mali*, it was also suggested by Prof. K. MIYABE and other observers that the crotch infection of the fungus takes place in the apple tree.

b. Dead twigs.

I do not hesitate to suggest a dead twig as a channel of the entrance of the disease, since I often find it at the center of cankers, especially of young cankers, as shown in the photograph in Pl. VII, Fig. 2 and Fig. 6.

Although the cause of the death of the twigs was not investigated by me, winter injury may in most cases be the cause of its death in a cold, snowy country such as Hokkaidō. Indeed in spring, one can find numerous dead twigs on the tree, especially on *Prunus Mume*. But unskilful pruning and injuries caused by men, and by birds and other animals may occasionally be promoting agencies of such death.

c. Buds.

As already shown by the inoculation experiment, the germ-tubes and hyphae of this causal fungus have the power to kill the buds of the host plant, although I failed to secure infection of the bark of the twig.

In the case of *Valsa leucostoma* PERS., the causal fungus of the "die-back" of the peach tree, ROLFS (1907)[23] also suggested it to be a channel of entrance of the disease.

d. Insects.

Insects are found in great numbers both in and on the bark of the host plants in the vicinity of Sapporo, and it is reasonable to believe that they may crawl over sticky spore horns and carry the spores away to deposit them on wounds, and thus start new cankers. Indirectly, insects may be connected with the spread of the disease by making wounds in the bark, where the spores may gain entrance after having been carried by some other agents. I am now of the opinion that this is the way in which insects are most closely related to the dissemination of the disease. In the fall of 1914, I found many larvae of *Sesia hector* BUTL., commonly known by the name of "*Kosukashiba*" in Japan, feeding on the diseased portions in almost all infected parts of the smooth bark on trunks. According to Profs. MATSUMURA and NIIJIMA, *Sesia hector* BUTL. is the most common noxious insect, whose larvae inhabit the bark and wood of the trees, which belong to the genus *Prunus*, in Japan. It is also written that this insect is found mostly on the trunks of aged trees and causes the gummosis of the host plant. The fact that the present disease is very common on the trunks of old trees shows beyond all doubt that infection on such a smooth bark originated for the most part in the exit holes of this insect.

There are, besides, two or three kinds of bark borers in the vicinity of Sapporo, found on the living and dead trees of the hosts.

e. Pruning.

It is a very rare practice in Japan to prune *Prunus yedoensis* and other Flowering Cherry trees, while it is quite common to prune *Prunus Mume* and *Prunus Persica*. The cut surface of the branches is most likely to become a place of entrance for the disease. An instance of such infection is shown in the photograph in Pl. VII, Fig. 3.

f. Rain and Wind.

As the pycnospores appear to be sticky, there is no evidence that they are transmitted by wind except where they are washed down into the dust and blown about with it. But rain dissolves the mucilaginous matrix of the spore horns, and the pycnospores may be splashed to other trees that are in close proximity to diseased ones; particularly they are washed down from twig infections to the lower parts of the tree where they lodge in wounds and produce cankers. But the ascospores can be caught up by the wind and carried for considerable distances and may well be responsible for a large part of the infection, for they are forcibly ejected into the air. Also the ascospores, which sometimes ooze out instead of being forcibly ejected, may be carried down in the same manner as the pycnospores.

15. Relation between the Present Disease and Gummosis.

Gummosis is a disease common to a number of trees, and is specially prevalent in the genus *Prunus*. The name gummosis is applied to a condition in which an exudation of the gummy substance takes place through the bark. The gummosis of *Prunus* has been the subject of study by many investigators, but it was most thoroughly studied by BUTLER (1911)[4]. Dr. S. KUSANO (1911)[15] also studied the gummosis at the basal portion of the witches' broom in *Prunus serrulata*.

It is generally said that such plants are susceptible to gummosis whenever conditions are favourable for the active growth of the cambium. It is also said that the disease may be produced either autogenously or by external agents.

Although the external agents causing gummosis must be many, it has been proved by various authors that this phenomenon is produced by a variety of parasitic fungi. In the case of our disease, it is always accompanied by the gum flows as already stated; and the fact that in the inoculation experiments the gum flowed out most vigorously only from the injured bark in which the infection occurred, shows the gum flow of our disease to be surely produced, or at least its quantity to be increased, by the fungus attack.

The cross-sections of the diseased branch always show many gum pockets, arranged in one or several rows, in the spring wood of the annual rings which were formed after the fungus attack, especially of the callus wood. Such gum pockets are always produced in the embryonic woody tissue developed specially from the cambium layer. As has already been shown by BUTLER (1911)[1] and other authors, the cambium lays down centripetally cells rich in granular protoplasm, and the tissue thus formed constitutes the embryonic woody tissue. But such embryonic woody tissue is later buried deeply in wood, in consequence of the formation of normal woody tissue. By absorption of water, the membranes of the embryonic wood cells increase in bulk and turn into a semi-fluid gummy substance. The gum thus formed is accumulated in small cavities or pockets. The gummosis now spreads more and more deeply into the circumambient tissues. The cells bordering the pocket are sloughed off from the subjacent cells, which become convex on their free ends and finally loosened and freed by a process exactly similar to that which brought about the first formation of the gum cavity and which may continue until all the tissue capable of gummy degeneration has been destroyed. In young gum pockets, cells will sometimes be observed floating in the gum. But these cells immediately vanish upon the addition of water. In general, the medullary ray cells seem to remain longest unchanged into the gum. The above observations agree entirely with those of BUTLER.[4] I have occasionally observed that the cambium itself is destroyed and changed into the gum, and that the gum pockets are thereby produced also in the inner bark.

The existence of the gum in the normal tissue, especially in vessels, of

the diseased branch requires special attention, as it has an important bearing upon the disease. In general, the gum appears in the wood vessels of the diseased branch in extraordinary amounts, as shown in Pl. X, Fig. 11–12. The gum also gradually replaces the starch and other contents in the medullary ray cells, wood parenchyma and other tissues; and the wood vessels are slowly plugged up by the deposits of the gum. In consequence, the water supply is cut off from the distal portion of the tree, causing the death of a large branch or the whole tree when it is affected by this disease. The fact that a branch of the tree, having a small canker of this disease, wilts in a comparatively early stage, may also be caused by the gum-formation.

16. Anatomical and Histological Studies of the Diseased Branch.

The observations and investigations recorded below were undertaken with a view to determine the nature of the changes produced in the bark and sap-wood of *Prunus yedoensis*, when it is invaded by this fungus. For this purpose a comparative study of normal and diseased tissues was made. Special attention was also paid to determine the distribution of the mycelium in the host tissues. In the microscopical studies free hand sections of fresh material were used when possible; but sections requiring more uniformity were made by means of a Thoma-Jung sliding microtome, the material having been previously imbedded in celloidin. In making studies of the fungus hyphae in the host tissues, the use of the stain known as "PIANEZE III b" [32] has been found more or less satisfactory in differentiating the fungus from the plant substratum.

Starting from a point of infection, the hyphae grow out, destroying parenchyma, collenchyma and at last cambium cells as they go. The diseased branch ceases, therefore, its growth on the attacked side, and the callus formation soon begins to appear from the edge of the canker. The width of the annual ring becomes thereby irregular, and in consequencc, the cross sections show various shapes.

In 1914, KEEFER[13] reported his microchemical and histological studies of the effect of the fungus (*Endothia parasitica*) on the bark and the wood of the chestnut canker. I tried the same studies on our cherry canker, but the results were not so conspicuous as in the case of the chestnut tree, chiefly owing to the disturbance caused by the destruction of the cell-walls by the fungus and the formation of the gum. But I got, on the whole, the following results:—

The primary cortex. The cork cells are practically unaffected by the fungus, with the exception of the rupture which is apparently produced by mechanical pressure from below. No hyphae were seen penetrating the cell walls or passing between them. Perhaps the only change to be observed was with respect to the arrangement of the tissue. At points where the stromata are forming beneath the periderm, there is a bulging of the layer, followed by a complete rupture during the maturation of the perithecia.

The sclerenchyma, which have highly lignified walls enclosing a very narrow empty cell lumen, are also not affected by the fungus,—the structure, size, arrangement, and chemical nature being unchanged.

The collenchyma and the thin walled parenchyma composed of cells having more or less pure cellulose membrane, are destroyed by the fungus, and the mycelium is penetrating and ramifying throughout those tissues. As KEEFER has shown, I have also found that the cellulose cell walls become partially lignified. The degree of lignification, however, is very poor in my case. Such lignified cells are only sparingly found by a microchemical test. As to the change in the cell contents, I could not get clear results, but it is apparent, according to the test, that the tannin is increased more or less in those cells.

The bast zone. This is chiefly composed of sclerenchyma in the form of bast fibers and sieve tubes, as well as of phloem parenchyma and medullary rays. The sieve tubes and the parenchyma, which have more or less pure cellulose cell-walls, are easily destroyed by the fungus. Those cells are so strongly dissolved that their original shapes are lost, and the mycelium is

found penetrating and ramifying among those tissues. The lignification of the cell-walls occurs also more or less, and is rather rare as in the case of the primary cortex.

No change of importance were noticed in the sclerenchyma. A partial process of lignification takes place also in the medullary ray cells when they are approached by the mycelium. In the case of the chestnut canker, KEEFER[13] reported that the cells of the medullary rays are not individually affected or broken, but there is an increase in the number of medullary ray cells, due probably to a stimulating action by the fungus. In our case also, the medullary rays seem to remain for a long time without being injured by the fungus, but at last they also are destroyed. I did not, however, find an increase in the number of the medullary ray cells, as KEEFER [13] observed.

The cambium. The cambium is easily killed by the fungus attack, and a partial lignification takes place in the wall of the cambium upon the invasion of the fungus. No hyphae were seen penetrating the cell-walls or going between them as reported by KEEFER [13].

The woody portion. As already stated, in the woody portion, the embryonic tissue and the gum pockets are formed as the result of the disease. Although the mycelium is seen even in the deeper portion, the walls of the other woody elements apparently show no change. According to my studies, the mycelium of this fungus is commonly seen in the wood vessels, medullary ray cells, and occasionally in the wood parenchyma, but it is not so numerous as to plug up those cells (Pl. X, Fig. 10–12.). An experiment was made to determine to what depth the fungus really does penetrate into the wood. New diseased logs were selected and cut into moderate sizes. The exposed surface was passed through the flame of an alcohol lamp to guard against contamination. They were at once placed in moist sterilized Petri-dishes. After one or two days, the white mycelium grew out even from a deep portion of the wood, and occasionally from the central portion of the branch of about five centimeters in diameter. The mycelium gradually turned to the proper yellowish-brown color, and in the course of a few weeks even stromata

were produced on the cut surface. The presence of the mycelium in the deep wood is also easily proved under a microscope in the stained sections.

17. Prevention and Cure.

(a) Cut off all dead or diseased branches, and burn them up as soon as possible. We can not cure them by a surgical operation as in the case of (b), for the mycelium of the causal fungus is living deeply in the woody portion. If the cut surfaces are large, paint them at once.

(b) If the diseased portion is on the large limb or the trunk, we can cure them only in the case of the young stage of the canker. Carve off the affected bark and even the woody tissue of the diseased portion with a sharp knife or with other cutting instruments as thoroughly as possible. Wash the cut surface at once with a powerful fungicide such as 0.05 or 0.1 % corrosive sublimate, and, after a few days, paint the surface with a thick paint. In this operation, the cut bark and woody tissue must be burnt up at once, without leaving them in the garden or the orchard.

(c) Take all possible care to prevent external injuries to the healthy trees.

(d) Take care to prevent injuries to the bark by insects, such as *Sesia hector*.

(e) Cut off all dead twigs and other dead portions, and keep the trees clean.

(f) To prevent the infection of the disease, spray the trees with the Bordeaux Mixture or some other fungicide before the leaves or the flowers come out in the spring. But in the case of ornamental trees, a dirty fungicide such as the Bordeaux Mixture should be avoided.

18. General Summary.

1. The present canker disease was first noticed in Sapporo in 1913 and its distribution is at present limited to Hokkaidō.
2. The canker disease is not only common on *Prunus yedoensis*, *P. sachalinensis* and *P. Mume*, but it is also found on a number of other species of *Prunus*. The identity of the fungi which were found parasitic on *P. yedoensis*, *P. sachalinensis*, *P. Mume* and *P. Persica* was proved by cultural and inoculation experiments.
3. There are two different types of symptoms in the diseased branch. The one is the cankered type and the other is the girdled one, which never forms a canker.
4. The causal fungus belongs to the subgenus *Euvalsa* and is new to science. It is described under the name of *Valsa japonica* MIYABE et HEMMI.
5. In the saprophytic condition, *Valsa japonica* seems to be almost omnivorous. On a comparatively high acid medium such as fruit-juice, the fruiting pustules are not generally produced, in spite of the vigorous growth of its mycelium. The fruiting pustules are, however, most readily produced on the host-bark-decoction-agar, oat-juice-agar, and also on the sterilized twigs.
6. The mycelium of the present fungus on artificial cultures turns yellow, greenish-yellow or light brown color with age.
7. The fungus produces a blackening of the media and grows more luxuriantly on cultures containing a low percentage of tannic acid than without it. But the high percentages of tannic acid inhibit its growth entirely. The fruiting of the fungus is also stimulated by low percentages of tannin.
8. Corrosive sublimate has the strongest sterilizing power for the hyphae of the fungus, and a 0.05% or 0.1% solution is the most effective. But copper sulphate and carbonate of soda are not effective for the same purpose.

9. Judging from the results of the inoculation experiments, we can infer that *Valsa japonica* is the direct cause of this disease. But the fungus requires a wound to secure infection most easily, and for that purpose, the wound must not be new. It requires a layer of dead cells on the exposed surface, on which a mass of mycelium is first formed, and by accumulated strength it penetrates the living tissues below.
10. This disease is always accompanied with gummosis. As to the process of the gum formation, I entirely agree with BUTLER and other investigators.
11. The causal fungus grows in the bark, destroying the cellulose cell-walls of collenchyma, parenchyma, sieve tubes and at last the medullary ray cells. But it produces no change on the lignified membrane of sclerenchyma. In the woody portion, the hyphae penetrate into the wood vessels, medullary ray-cells and wood parenchyma.

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20. Explanation of Plates.

The microscopical drawings were done with the aid of a camera lucida.

Plate VII.

Fig. 1. A young stage of the canker at the crotch of *Prunus yedoensis.*

Fig. 2. Cankered branch of *Prunus yedoensis* caused by *Valsa japonica.*

Fig. 3. Diseased branch of *Prunus Mume* caused by *Valsa japonica.*

Fig. 4. Cankered branch of *Prunus yedoensis* caused by *Valsa japonica.*

Fig. 5. Diseased branch of *Prunus yedoensis* caused by *Valsa japonica.*

Fig. 6. Diseased branch of *Prunus Mume* caused by *Valsa japonica.*

Plate VIII.

Fig. 1. Diseased branch of *Prunus sachalinensis* caused by *Valsa japonica.*

Fig. 2. Result of inoculation experiment on the seedling of *Prunus yedoensis* with the causal fungus isolated from the same host. (Inoculated in the old slit wounds.)

P .....Inoculated point.

C.....Check wound.

Fig. 3. Result of inoculation experiment on *Prunus Mume* with the causal fungus isolated from *Prunus yedoensis.*

P .....Inoculated point.

C.....Check wound.

Plate IX.

Fig. 1. Section of matured ectostroma and pycnidium. *(Prunus yedoensis).* Zeiss A × 2.

Fig. 2. Pycnospores. *(Prunus yedoensis).* Zeiss F × 4.

Fig. 3. Section of stroma showing perithecia and a pycnidium. *(Prunus Mume).* Leitz 1 × 3.

Fig. 4. Ascospores and germinating ascospores in the cavity of perithecia. *(Prunus Mume).* Zeiss F × 2.

Fig. 5. Section of matured entostroma and perithecia. *(Prunus yedoensis).* Leitz 1 × 3.

Fig. 6. Ascospores. *(Prunus yedoensis).* Zeiss F × 2.

Fig. 7. Asci and ascospores. *(Prunus yedoensis).* Zeiss DD × 4.

Fig. 8. Section of stroma in young stage. *(Prunus yedoensis).* Leitz 1 × 3.

Fig. 9. Conidiophores and pycnospores. *(Prunus yedoensis).* Zeiss F × 4.

Plate X.

Fig. 1–3. Germinating ascospores. *(Prunus yedoensis).* Zeiss DD × 4.

Fig. 4–7. Stages in the germination of pycnospores. *(Prunus yedoensis).* Zeiss DD × 5.

Fig. 8. Hyphal branches in drop culture. Zeiss DD × 4.

Fig. 9. Longitudinal section of the neck of a perithecia. *(Prunus yedoensis).* Zeiss A × 4.

Fig. 10. Cross sections of the wood vessels showing the hyphae in their cavities. *(Prunus yedoensis).* Zeiss DD × 4.

Fig. 11. Longitudinal section of a wood vessel showing the hyphae and gummy substance in its cavity. *(Prunus yedoensis).* *g.* Gummy substance. Zeiss DD × 4.

Fig. 12. Showing the hyphae in the medullary ray cells and gummy substance in the wood vessel. *(Prunus yedoensis).* *g.* Gummy substance. Zeiss DD × 4.

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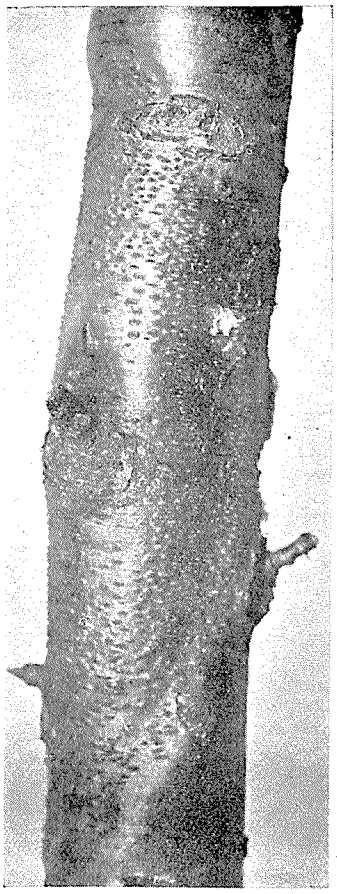

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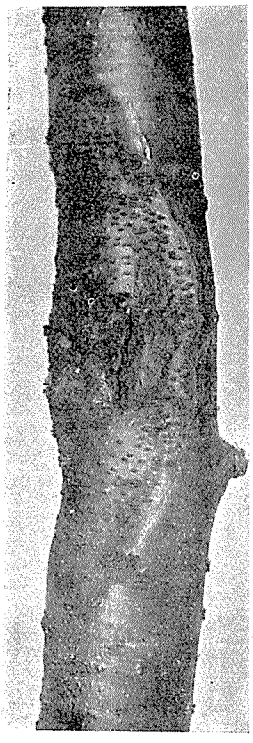

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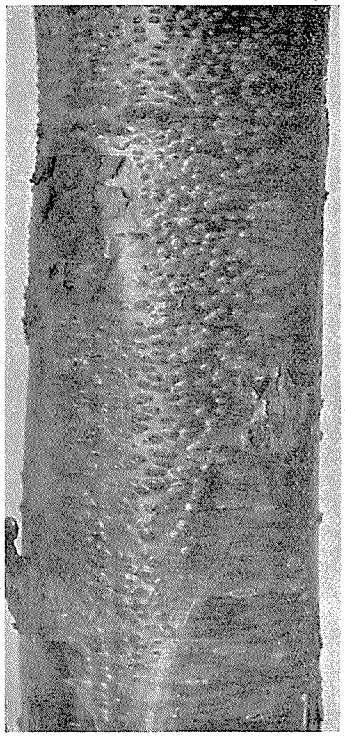

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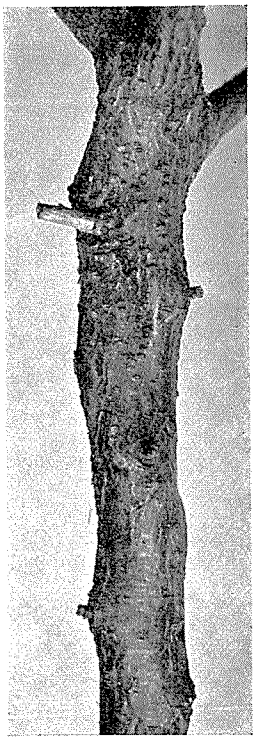

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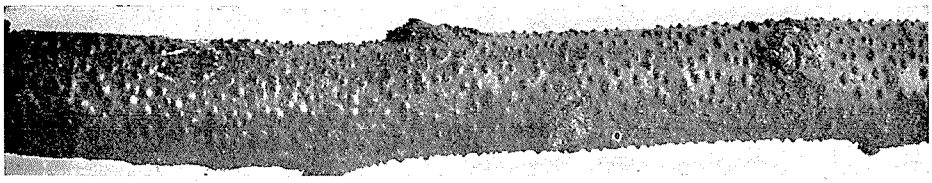

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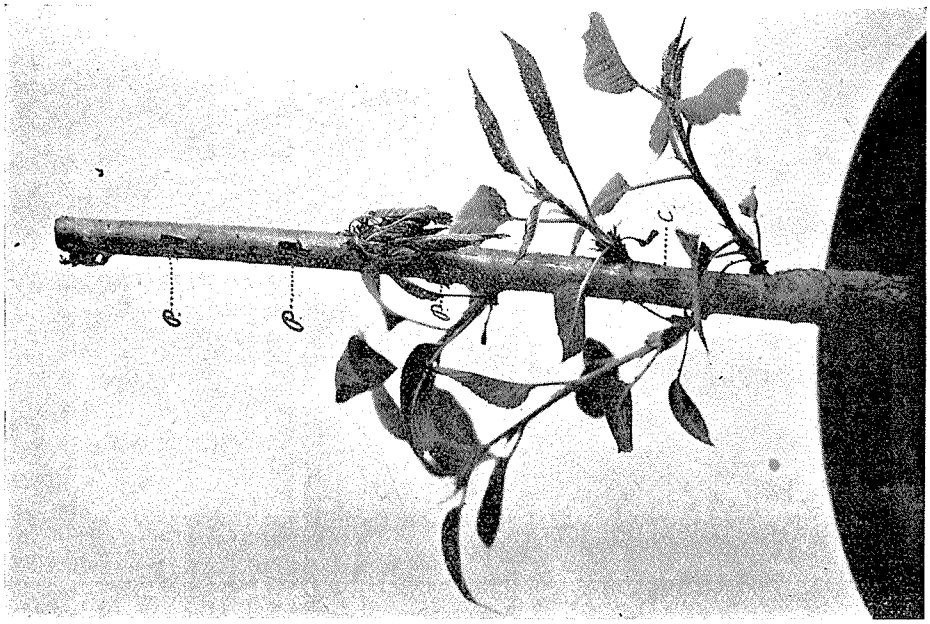

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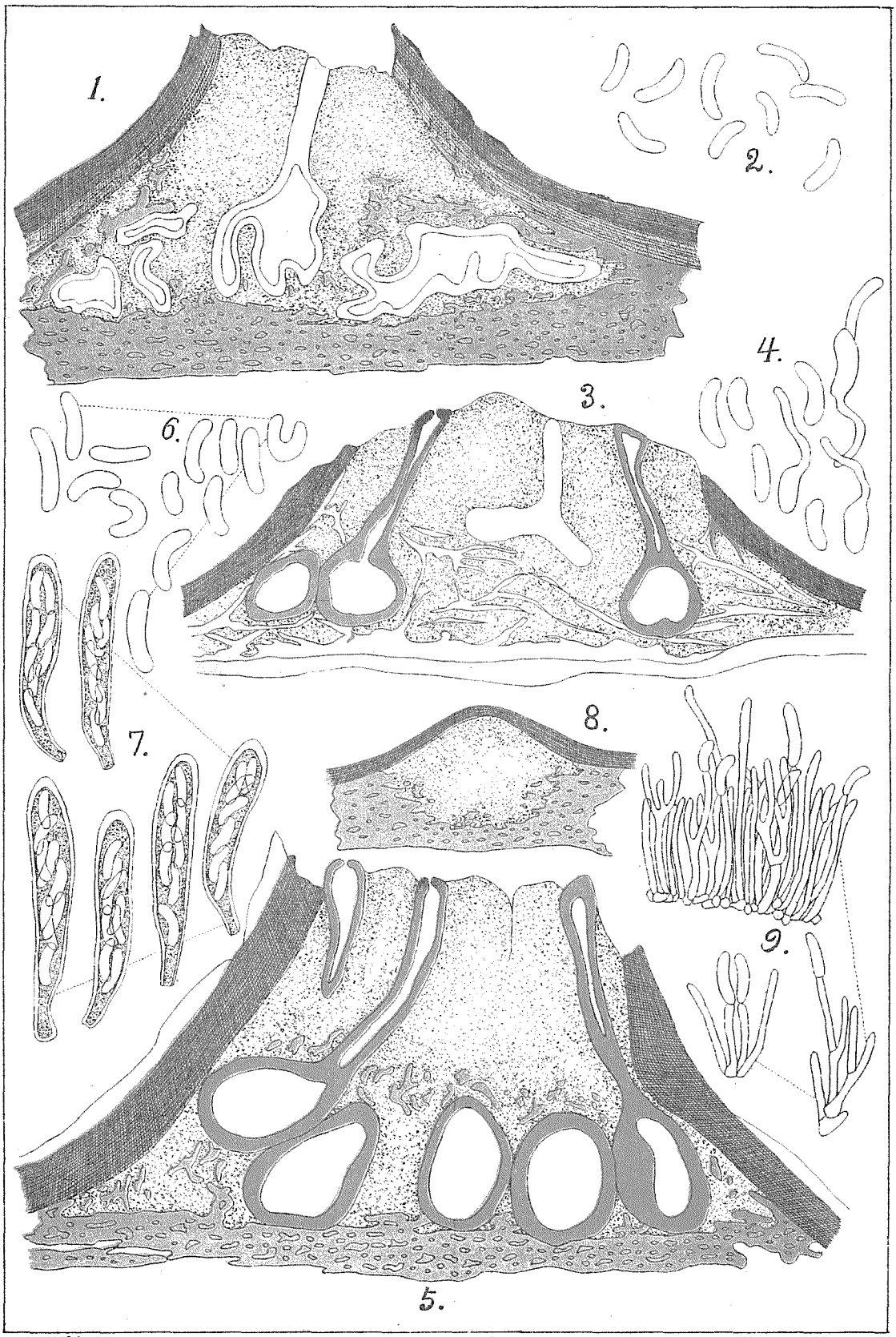

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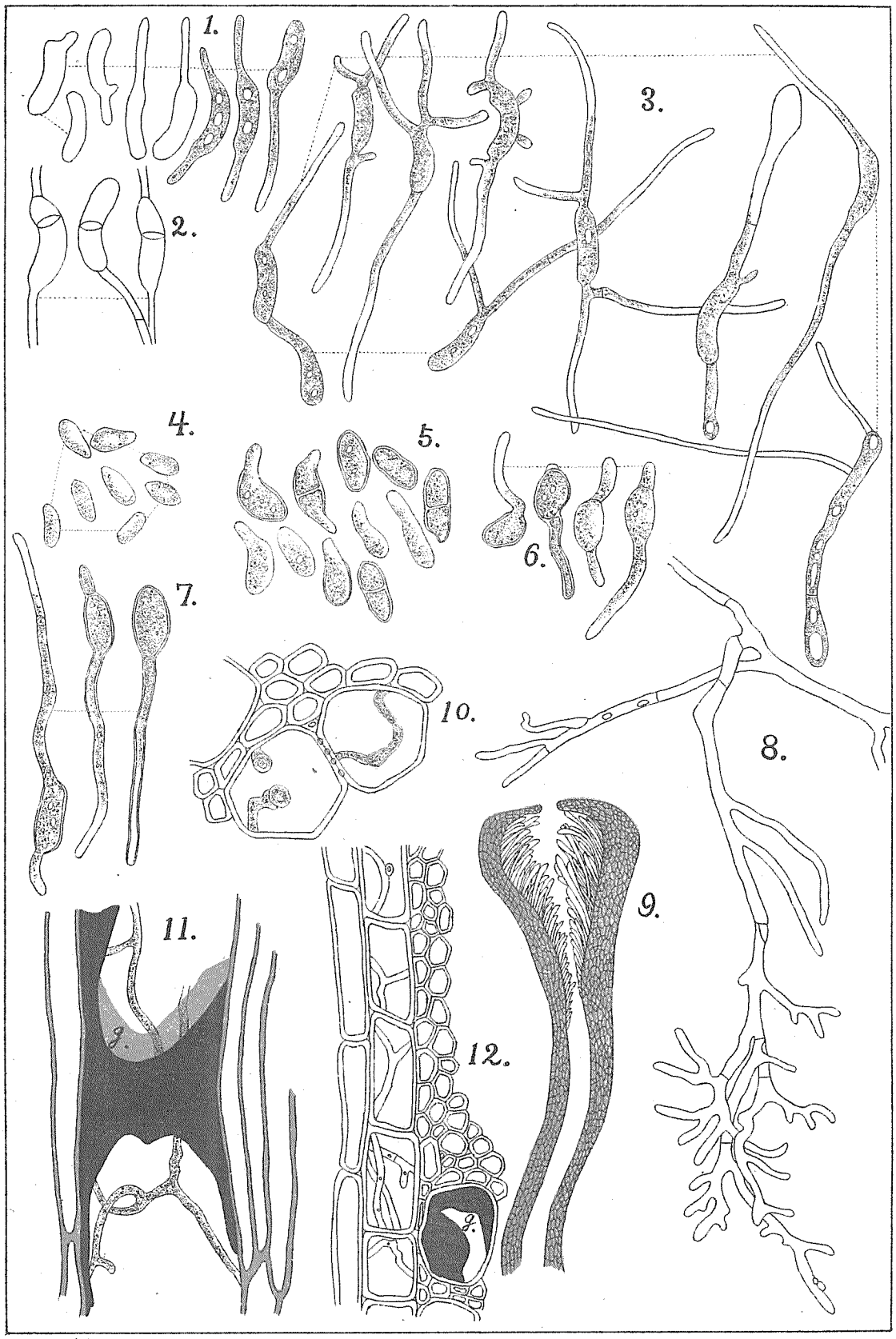

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