Contributions to the Knowledge of the Sap Stains of Wood in Japan. II.

Studies on *Ceratostomella pini* Münch, the Cause of a Blue Stain of Pine Trees.

By

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Contents.

I. Introduction.

This investigation on the sap stains of pine trees was started in co-operation with the Ôsaka Local Forestry Bureau. In the first report the results of the experiment on *Ceratostomella ips* Rumbold, the cause of a blue stain of pine trees in Western Japan, was published (Nisikado and Yamauti 1933, 1934a). This paper deals with the description of *Ceratostomella pini* Münch, the cause of another blue stain of pine wood.

As it is stated in the first report, *Ceratostomella ips* RUMBOLD attacks the pine trees standing on their root and induces them to death. *Ceratostomella pini* is also found very commonly on the wood of weakened pine trees standing on the root or of felled trees. Between the bark and trunk of the diseased pine trees very characteristic fungus layers are formed. Thus *Ceratostomella pini* is one of the most common and important blue-staining fungi. It may be safely said that most of the blue-stained pine wood, commonly found in almost all parts of Japan, is caused by this fungus.

The mycological investigations on this fungus as a ground for the establishment of preventive measures for the blueing of pine wood, are of interest from the scientific as well as the practical points of view. Therefore the writers wish to present the results, as the second contribution to the knowledge of the sap stains of wood in Japan.

The writers are obliged to Messrs K. OONAKADŌ, and T. MATIDA of the Ôsaka Local Forest Bureau, for their suggestions in the investigation. Their thanks are also due to Professor J. WESTERDIJK, Baarn, Holland, Dr. C. T. RUMBOLD, Madison, Wisconsin, U. S. A. and Professor LAGERBERG, Stockholm, Sweden.

II. Historical Review.

As the blue stain of pine wood is one of the most serious damage to the forest industry, many valuable papers have been published on this subject. However, the European and the American articles are so fully reviewed by MÜNCH (1907), HEDGCOCK (1906), LAGERBERG, LUNDBERG and MELIN (1927), and RUMBOLD (1931), that the present writers avoid the repetition here.

In Japan, the blue stain of pine wood caused by *Ceratostomella pini* MÜNCH was described only lately, although it seemed to have been known among the foresters, lumbermen and architects for a long time. M. KASAI (1917) reported a blue stain of pine and oak wood and reported *Ceratostomella pilifera*, as the cause of the blue stain, but it is different from the fungus under consideration now, especially in the perithecial shape and size. In 1933, M. KAWARA reported the occurrence of *Ceratostomella pini* as a cause of blue stains of pine wood.

The writers presented their result of comparative studies on the effects of temperature and the hydrogen-ion concentration of culture media upon the growth of *Ceratostomella ips*, *Ceratostomella pini*, and *Ceratostomella piceae*, at the general meeting of the Japanese Society of Agricultural Science held in Tokyo in April, 1934.

III. Symptoms.

The fungus under consideration affects the wood of pine trees (*Pinus densiflora* SIEB. et ZUCC. and *Pinus Thunbergii* PARL.), which are weakened by the attack

of bark-beetles or by other causes. Usually the fungus grows at first in the galleries made by the bark-beetles and then it grows into the wood and the cambium layer, namely between bark and trunk, so that the bark of infected pine trees could easily be detached from the trunks. The underside of detached bark and the surface of a trunk seem to be covered with sooty powder, which is consisted of the sclerotia and perithecia of *Ceratostomella pini*. This is a characteristic of the stain caused by this fungus, and is rarely or not observed in the cases of *Ceratostomella ips* and *Ceratostomella piceae*. Therefore the fungus is comparatively easily distinguished from the other two fungi. Not only on the surface of a trunk and on the underside of bark, but also on a cut surface of the infected wood, the sclerotia and perithecia are commonly observed.

The general appearance of the blue-stained wood caused by this fungus is almost similar to that of *Ceratostomella ips*, which was described in the first paper of this series. In the cross sections of infected wood, the blue-stained lesions are generally of wedge shape, which tapers toward the center of tree.

On the radial, longitudinal sections or splints of the blue-stained wood, many dark brown lines are observed which are very fine, and closely arranged. In the tangential, longitudinal sections or splints, many dark-colored, small dots are scattered in the discolored part. The dots are the cross ends of the discolored medullary rays.

Microscopical views of the wood strained by this fungus are given in Plate XVIII, and are almost similar to those affected by *Ceratostomella ips*.

This fungus attacks the wood of growing pine trees, when the wood drys in some degrees. The south or west side of the pine trunk, which receives the direct, strong sunshine, is more strongly attacked in general by the fungus than the north or east side. The picture, given in Plate XVII, Fig. 1, is an example of such a case. It shows the cross sections of an affected pine tree standing on the root, cut at every a meter from the base. The pine tree was growing on the southern slope in the Terayama State Forest, Iwami-mura, Ibo-gun, Hyôgo Prefecture. It had still green needles on the crown, but seemed to be slightly weakened, when it was cut down. The east side of the tree was covered with a thick bush, while the south and west sides were open. As shown in the picture (Plate XVII, Fig. 1), the west and south sides of the lowest disk (A) were completely blue-stained while the east side remained almost unchanged. Almost all the part of sap-wood in the second disk (B) was blue-stained, while in the third disk (C) only the north side remained colorless.

IV. Source of Cultures Studied.

The fungus under consideration was isolated in the manner stated in the foregoing paper of this series (NISIKADO and YAMAUTI 1933). The fungus cultures studied in the present investigation are following seven strains:

Strain No. 842. The strain was isolated from blue-stained sap-wood of "Kuromatu" (*Pinus Thunbergii* PARL.), collected at Sin-Maiko, Ibo-gun, Hyôgo Prefecture on June 7, 1933.

Strain No. 846. It was isolated from blue-stained sap-wood of "Akamatu" (*Pinus densiflora* SIEB. et ZUCC.), collected in Tatuno-mati, Ibo-gun, Hyôgo Prefecture on July 8, 1933.

Strain No. 849. It was isolated from blue-stained sap-wood of *Pinus densiflora* SIEB. et ZUCC., collected in Terayama, Iwami-mura, Ibo-gun, Hyôgo Prefecture on July 2, 1933.

Strain No. 850. It was isolated from blue-stained sap-wood of *Pinus densiflora* SIEB. et ZUCC., collected in Kyû-Siroyama, Tottori-si on June 12, 1933.

Strain No. 967. It was isolated from *Pinus densiflora* SIEB. et ZUCC., collected in Terayama, Iwami-mura, Ibo-gun, Hyôgo Prefecture on July 18, 1933.

Strain No. 707. *Ceratostomella pini* MÜNCH, the culture No. 203 of RUMBOLD. It was isolated from *Pinus ponderosa* infested with the bark-beetle, *Dendroctonus brevicomis*, collected in October 1929, near Coeur d'Alene, Idaho, U. S. A. It was sent to the writers from Dr. T. C. RUMBOLD in September 1932.

Strain No. 723. *Ceratostomella pini* MÜNCH, sent to the writers in October 1932 from Prof. J. WESTERDIJK.

V. Morphology of the Fungus.

1. Mycelium.

The fungus hyphae in the blue-stained pine wood, as shown in Plate XVIII, are generally similar to those of *Ceratostomella ips* given in the previous paper. In the cross section of wood (Plate XVIII, Fig. 1) the hyphae penetrate the cells of medullary rays. In the radial, longitudinal section, given in Plate XVIII, Fig. 2, the hyphae are observed in the parenchymatous cells of the rays. The cells, penetrated by the hyphae, are mostly brown or dark brown colored. Plate XVIII, Fig. 3 shows the longitudinal sections, where the hyphae are found in tracheids as well as in medullary rays.

The hyphae, especially matured hyphae, are brown or dark brown, although the younger ones, colorless. They vary in diameter and measured 2—6 μ and rarely 8 μ. They are septated at an interval of 35—50 μ, and constricted at the septa.

The hyphae developed on malt-extract agar media are shown in Plate XIX, Fig. 2. The mature, old hyphae are dark brown, and the surface is covered with lighter colored, mucilaginous sheath of uneven thickness. The young hyphae are colorless or light-colored, the surface being smooth. The size of hyphae seems to vary according to the conditions under which they develop, but not to the age. The hyphae are 1 to 8 μ in diameter, and provided with septa at an interval of 20—50 μ, where they are not or slightly constricted.

2. Sclerotium.

Between the bark and trunk or on the cut surface of pine wood the sclerotium is produced as a small body very abundantly. The sclerotium is cylindrical, trapezoid or irregular polygonal, and hard and black on the outer appearance. The size varies within 140—1,000 μ, (mean of 50 measurements, 526.4 μ) in height and 100—560 μ (mean 300.8 μ) in width. (cf. Plate XVII, Fig. 2). The outer part of sclerotia consists of dark brown, comparatively small, and thick-walled pseudoparenchymatous cells, while the central part is of colorless, comparatively large and thin-walled cells. (cf. Plate XIX, Fig. 1). The cells in the outer layers are 6–20×3–8 μ and those in the inner layers are larger and attain 35×20 μ. In the pure culture, they are easily formed on such culture media like steamed pine wood and malt-extract agar.

While the sclerotium of *Ceratostomella pini* may be of use to endure unsuitable conditions, they also push up the bark from the wood and separate them. In the space between bark and wood, thus formed, the perithecia are produced among the sclerotia.

In *Ceratostomella piceae*, which will be reported in the following paper of this series, the sclerotium formation has not been found. Therefore, the perithecia of *Ceratostomella piceae* are formed only in the galleries made by the bark-beetles or on the surface of felled and cut wood. Difference of *Ceratostomella pini* and *Ceratostomella piceae* in this point seems to be the reason that *Ceratostomella pini* is very prevalent on weakened pine trees upon their root, while *Ceratostomella piceae* mostly on cut wood or lumber of pine.

3. Conidia and Conidiophores.

The conidia of this fungus are formed on the host in the space between the bark and wood, separated by the formation of sclerotia. In the older lesion, the conidium formation is not common. On the contrary, the formation of the conidia upon the culture media is very common and copious. Therefore the writers will describe the characteristics of the conidia developed in culture.

The conidiophore is at first a simple branch of hyphae and produces conidia apically, but it grows to a large bush and produces abundant conidia in a large ball as shown in Plate XX, Figs. 2–3. The young conidiophores or the apical parts of old ones are colorless, but the basal parts of large conidiophores are mostly brown colored. They are variable in size, but commonly 5 μ in length 1.5 μ in width when young. However, the larger ones, as shown in Plate XX, Figs. 2–3, attain to 30—70×20—50 μ in size, the cells of the stalks being 10—35× 3—5 μ.

The conidia are colorless, continuous, elliptical, with round ends, as shown in Plate XVII, Fig. 3 and Plate XX, Fig. 1. The contents are granular with a light-refrecting body near the both ends. The conidia are variable in size, and the results of the writers' measurements of those produced on 3% malt-extract agar and steamed block of pine at 24°C. after 2 weeks' culture are given in the following table.

Table I.

Size of Conidia of *Ceratostomella pini* Münch.

	Conidia developed on	Number measured	Range	Sextile range	Mean	Standard deviation
Length (μ)	*1) Malt-extract agar	100	2.5—6.5	3.9—5.1	4.59±0.063	0.634
	2) Steamed pine wood	100	2.5—6.5	3.7—5.2	4.42±0.067	0.668
Width (μ)	1) Malt-extract agar	100	1.8—2.8	1.9—2.3	2.09±0.019	0.193
	2) Steamed pine wood	100	1.3—2.8	1.8—2.2	2.05±0.014	0.210

Remarks: The sextile range discards the sixth of the measurements at each end of the extreme range, permitting an approximation of the frequency distribution.

* 1) means the conidia developed on malt-extract (3%) agar after 2 weeks' culture at 24°C., and 2), those developed on steamed pine block after 2 weeks' culture at 24°C.

According to the above table, the conidia developed on malt-extract agar at 24°C. measured 2.5—6.5×1.8—2.8 μ (commonly 3.9—5.1×1.9—2.3 μ; mean 4.59±0.06×2.09±0.02 μ). Those developed on the steamed pine block measured 2.5–6.5×1.3–2.8 μ (commonly 3.6–5.2×1.8–2.2 μ; mean 4.42±0.07×2.05±0.01 μ).

Inside the old large hyphae, grown in culture media, endogenous, small conidia are sometimes observed, as if the conidium formation of Endoconidiophora takes place intercalary. They are likely to be the conidia of a fungus, parasitic in the hyphae of the *Ceratostomella pini*. But it may be assumed that the endogenous conidia are produced inside an empty hyphal cell from the neighbouring, vigorous cell. The formation was observed not only in the writers' Japanese strains of *Ceratostomella pini*, but also either in the American strains or in the Dutch strains. Therefore it may be assumed to be a characteristic of *Ceratostomella pini*. The endogenous conidia are similar in shape to those of Cephalosporium type produced on the medium in balls, although they are somewhat smaller in size.

Germination of the Conidia. The conidia of the fungus germinate easily in water, and in nutrient solutions. Those germinated in 3% malt-extract agar after 24 hours' incubation at 24°C. are shown in Plate XX, Fig. 4. The conidia are elliptical and 3.7—5.2×1.8—2.3 μ when they are produced, but by the germination they swell prominently and become to globular and 6—16×3.5—6 μ in size. From one or both ends they produce one or two germ-tubes. The germ-tubes are tapered to the apex, 1.5—3 μ in width and produce secondary conidia near the apex.

4. Perithecia and Ascospores.

Perithecia of this fungus are produced numerously among the sclerotia in the space between the bark and wood of pine trees, and also on the surface. They are black, flask-shaped, and much smaller than the sclerotia, as shown in the picture in Plate XVII, Fig. 2. They are globular at base and provided with

a short beak. The base of the perithecia, developed on the host is 65—115 μ (commonly 77–97 μ; mean 88.8±0.9 μ) in height, and 55–115 μ (commonly 62–93 μ; mean 85.3±0.91 μ) in diameter. Those produced on the steamed blocks of red pine are 65–125 μ (commonly 72–110 μ; mean 97.4±1.3 μ) in height, and 55–125 μ (commonly 71—103 μ; mean 98.4±1.05 μ) in diameter. (cf. Table II.)

Table II.

Size of Perithecia and Ascospores of *Ceratostomella pini* Münch.

				Number measured	Range	Sextile range	Mean	Standard deviation
Perithecium	Base	Height (μ)	*1) Host	110	65—115	76.8—97.0	88.8±0.895	8.95
			2) Cult.	100	65—125	71.5—109.8	97.4±1.302	13.02
		Diameter (μ)	1) Host	110	55—115	61.7—92.8	85.30±0.912	9.12
			2) Cult.	100	55—125	71.0—102.6	98.40±1.050	10.50
	Beak	Length (μ)	1) Host	110	45—135	66—94	80.91±1.450	15.28
			2) Cult.	100	45—215	63—102	84.80±2.840	28.35
		Diameter: Apex	1) Host	110	3—18	3.9—10.7	7.25±0.265	2.78
			2) Cult.	100	3—18	7.5—11.8	9.45±0.225	2.25
		Diameter: Widest part	1) Host	110	13—38	14.7—22.1	18.87±0.372	3.72
			2) Cult.	100	8—38	9.7—22.5	20.95±0.581	5.81
Ascospore		Length (μ)	1) Host	150	3.8—6.3	4.3—5.3	4.74±0.031	0.39
			2) Cult.	100	3.8—6.3	4.8—5.3	4.99±0.036	0.36
		Width (μ)	1) Host	150	0.8—2.8	0.9—2.2	1.67±0.020	0.25
			2) Cult.	100	0.8—2.3	0.9—2.1	1.69±0.029	0.29

Remarks: * 1) Means the perithecium or ascospore developed on blue-stained wood of red pine (*Pinus densiflora*) in forest, and 2,) those developed on steamed block of pine wood after 3 weeks' culture at 25°C.

The perithecia developed on the host are generally smooth at the base, although those developed on the culture media are covered with brown mycelial strands at the base. The beak of perithecia developed on the culture media is generally slender and mostly not straight. The length of a beak is almost same or slightly shorter than the height of base.

The beak of perithecia developed on the host in nature is 45—135 μ (commonly 66—94 μ; mean 80.91±1.45 μ) in length and 3—18 μ (mean 7.25±0.27 μ) in width near the apex and 13—38 μ (commonly 15—22 μ; mean 18.87±0.37 μ) wide at the thick part. The beak of those developed on the steamed block of red pine is 45—215 μ long (commonly 63—102 μ; mean 84.8±2.84 μ).

At the end of a beak, a fringe consisted of about 10 cilia is generally observed. The cilia are colorless and distinguish themselves from the colored cells of the beak. (Plate XXI, Fig. 1—2). They are 15—20 μ in length and 1.5—2.5 μ in width at the base, the ends being somewhat pointed. In the old perithecia, the cilia are generally not observed.

Ascospores. The matured perithecia push out ascospores from the end of beak. The ascospores may be retained among the fringed cilia and make a ball. Under the microscope, the perithecia may be easily broken by a slight pressure and the ascospores are pushed out from the fissure. It is difficult to observe the ascus, for its wall may dissolve in water very easily.

The ascospores are colorless, continuous, elliptical or falcate, the both ends somewhat pointed (cf. Plete XXI, Fig. 3). The contents are fine glanular and have a light-refecting body near each end. The ascospores produced on host are 3.8—6.3×0.8—2.8 μ (commonly 4.3—5.3×0.9—2.2 μ; mean 4.74×1.67 μ) and those produced on the steamed blocks of red pine, 3.8—6.3×0.8—2.3 μ (commonly 4.8—5.3×0.9—2.1 μ; mean 4.99×1.69 μ).

Germination of the ascospores. The ascospores germinate readily in water or in nutrient solutions. Those germinated in a hanging drop of 3% malt-extract solution after 24 hours' incubation at 24°C., are shown in Plate XXI, Fig. 4. Although the ascospores are falcate and 4.8—5.3×0.9—2.1 μ, when they are produced; they swell very much by the germination, and become broad fusiform of 7—10×3—4 μ. Therefore the swallen ascospores are quite different from those directly after the formation. Plate XXI, Fig. 3 and Fig. 4 show the difference of both shape of the ascospores. From one or both ends, one or rarely two colorless germ tubes are produced. They are commonly 2—3 μ and rarely 1.5—5 μ wide and septated at an interval of 15—20 μ. At the end of the germ tubes, the secondary conidia are formed.

VI. Taxonomical Consideration on the Fungus.

According to the above given morphological characteristics, the blue-staining fungus under consideration seems to be *Ceratostomella pini* MÜNCH. The most important characteristic of *Ceratostomella pini*, described by MÜNCH (1907), is a comparatively small perithecium with a short beak. LARGERBERG, LUNDBERG and MELIN (1927) also substantiate this fact.

The more prominent characteristics of the present writers' fungus and of *Ceratostomella pini*, described by previous authors, are compared as follows.

(See Table III on next page.)

The foregoing table shows that the measurements of the perithecia made by the present writers coincide with those of the previous authors. MÜNCH described the sclerotium formation between the bark and wood of infected pine trees. As to the presence of fringed cilia at the top of the perithecial beak, HEDGCOCK holds it as an important character for the classification of the species of this genus.

Table III.

Comparison in Size of Perithecium of the Present Fungus and of *Ceratostomella pini* Münch, given by Previous Authors.

Author		Height of the base (μ)	Diameter of the globe (μ)	Length of the beak (μ)
MÜNCH (1907)		70—100 (80)*	70—100 (80)	70—100 (80)
LAGERBERG etc. (1927)	Pine wood Agar		67.7—150 (80) (115)	
RUMBOLD (1931)	Agar	48—187 (97) **Commonly 82—110	44—163 (90) 76—105	19—165 (70) 53—87
	Host	56—140 (88) Commonly 75—98	51—120 (80) 69—90	19—110 (55) 41—63
KAWARA (1933)	Cult.	84—134 (111.5)	84—148 (107.2)	26—98 (49.2)
NISIKADO, etc.	Cult.	65—125 (97.4) Commonly 72—110	55—125 (98.4) 71—104	45—215 (84.8) 63—102
	Host	65—115 (88.8) Commonly 77—97	55—115 (85.3) 62—93	45—135 (80.9) 66—94

Remark: * In brachets, the mean value is given.
** This is the sextile range given by RUMBOLD.

In these characteristics, the writers' fungus resembles to *Ceratostomella pini* described by MÜNCH (1907), LARGERBERG, LUNDBERG and MELIN (1927), etc.

In regards to the size and shape of the ascospores and conidia the writers' fungus is also similar to those of *Ceratostomella pini* given by the previous writers, as shown in the following two tables.

Table IV.

Comparison in Shape and Size of Ascospores of the Present Fungus and of *Ceratostomella pini* Münch, given by Previous Authors.

Author		Shape	Length (μ)	Width (μ)
MÜNCH (1907)	Host	Gekrümmt, sichelform.	5	1.5
RUMBOLD (1931)	Cult.	Cylindrical, slightly curved and the ends round out	3—5.6 (4) 3.6—4.4	1—3.2 (1.7) 1.4—2
NISIKADO, etc.	Cult.	Falcate, carve to one side	3.8—6.3 (5) 4.8—5.3	0.8—2.3 (1.7) 0.9—2.1
„	Host	Ditto	3.8—6.3 (4.7) 4.8—5.3	0.8—2.8 (1.7) 0.9—2.2

Table V.

Comparison in Shape and Size of the Conidium of the Present Fungus and of *Ceratostomella pini* Münch, given by Previous Authors.

Author		Shape	Size (μ)
MÜNCH (1907)	Cult.	Breite, hyaline; in Büschelform gebildet	4—5×1.5
RUMBOLD (1931)	Cult.	Egg-shaped or club-shaped, usually tapering at the attached end	2.3—6×1.2—3
NISIKADO etc.	Cult.	Cylindrical, ovate, colorless. Formed in bush-form	1.5—6.5×1.8—2.8

In conclusion the writers will give here a result of the comparative study of the writers' strains of this fungus with those sent from Dr. RUMBOLD in U. S. A., in culture. The result shows that all the strains tested indicated no clear differences in the size of conidia, as shown in Table VI.

Table VI.

Comparison in Size of Conidia of the Present Writers' Strains of *Ceratostomella pini* Münch, and those Sent by Rumbold.

Culture media	Fungus stranis	Number measured	Length (μ)		Width (μ)	
			Range	Mean	Range	Mean
Potato agar	No. 707 (American)	200	3—7	4.66±0.071	2—4	2.50±0.037
	850 (Japanese)	"	3—8	4.89±0.059	2—4	2.16±0.027
	967 (Japanese)	"	3—10	4.93±0.087	2—4	2.30±0.036
Malt-extract agar	707 (American)	100	2—7	4.17±0.086	2—4	2.26±0.046
	850 (Japanese)	"	2—7	4.12±0.075	1—4	2.09±0.034
	967 (Japanese)	"	2—6	3.99±0.069	1—4	2.14±0.043

	Culture media	Fungus strains	Differences in mean (μ)	Ratio $\frac{E_{diff.}}{M_{diff.}}$
Length (μ)	Potato agar	No. 707 (Amer.) ~ No. 850 (Japan.)	0.23±0.062	3.70
		707 (Amer.) ~ 967 (Japan.)	0.27±0.075	3.62
		850 (Japan.) ~ 967 (Japan.)	0.04±0.071	0.57
	Malt-extract agar	707 (Amer.) ~ 850 (Japan.)	0.06±0.077	0.71
		707 (Amer.) ~ 967 (Japan.)	0.18±0.074	2.44
		850 (Japan.) ~ 967 (Japan.)	0.13±0.069	1.82

Table VI. (Continued.)

	Culture media	Fungus strains	Differences in mean (μ)	Ratio $\frac{E_{diff.}}{M_{diff.}}$
Width (μ)	Potato agar	No. 707 (Amer.) ~ No. 850 (Japan.)	0.35±0.031	11.1
		707 (Amer.) ~ 967 (Japan.)	0.20±0.035	5.73
		850 (Japan.) ~ 967 (Japan.)	0.15±0.031	4.79
	Malt-extract agar	707 (Amer.) ~ 850 (Japan.)	0.17±0.038	4.43
		707 (Amer.) ~ 967 (Japan.)	0.12±0.042	2.84
		850 (Japan.) ~ 967 (Japan.)	0.05±0.037	1.37

According to the results of the foregoing comparisons, the fungus under consideration may be safely identified as *Ceratostomella pini* MÜNCH.

Host plants and the distribution : The present fungus has been found on *Pinus* spp. and *Pecea* spp. in Europe and in America. In Japan the fungus has been observed on *Pinus densiflora* SIEB. et ZUCC. and *Pinus Thunbergii* PARL. so far. The localities and dates of the specimens, examined by the present writers in this investigation, are given here. The figure in the brackets shows the strain number of pure culture isolated from the specimen.

On *Pinus densiflora* SIEB. et ZUCC. "Akamatu".

Terayama, Mitu-mura, Ibo-gun, Hyôgo Pref., 7, VI, 1933 (strain No. 841); *Do.*, 18, VII, 1933 (No. 967); Sin-maiko, Ibo-gun, *Do.*, 18, VII, 1933; Tatuno, *Do.*, 8, VI, 1933 (No. 846); Syosya-zan, Sosa-mura, Sikama-gun, Hyôgo Pref., 19, VII, 1933 (No. 847); Kyû-siroyama, Tottori-si, 12, VI, 1933 (No. 850) and Iwakuni, Kuka-gun, Yamaguti Pref., 15, I, 1934 (No. 980).

On *Pinus Thunbergii* PARL. "Kuromatu".

Akasi Park, Hyôgo Pref., 20, V, 1932; Sin-maiko, Ibo-gun, *Do.*, 7, VI, 1933 (strain No. 842) and Hamanomiya, Onoe-mura, Kako-gun, *Do.*, 8, VI, 1933 (No. 848).

VII. Physiology of the Fungus.

Among the physiological characteristics, the influence of hydrogen-ion concentration and temperature upon the growth of the present fungus will be treated in the separate papers, in comparison with those of *Ceratostomella ips* and *Ceratostomella piceae*. Therefore the writers will report here the following points :

1. Characteristics of the Fungus on Culture Media.

Ceratostomella pini MÜNCH grows well on all the culture media more commonly used and the cultural experiments were carried out with them. The culture media used were prepared in the manner given in the first report of this series contributions (NISIKADO and YAMAUTI 1933). The summarized characteristics of *Ceratostomella pini* on culture media are shown in Table VII and VIII. Table VII

gives the results after 7 days' culture at 25°C., and Table VIII, those after 3 weeks' culture. The radial growth of colonies is expressed by the mean value of their diameter, but sometimes by the number of plus signs.

Tabla VII.

Summarized Characteristics of *Ceratostomella pini* Münch on Culture Media. I.

Results after one week's culture at 25°C.

Culture media	Fungus strains	Radial growth of colonies	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of	
					Color name	Degree	Conidium	Perithecium
Malt-extract agar	No. 842	70 mm.	+	T	Avellaneous	+	+	+
	846	62	+	T	"	+	++	−
	849	74	+	T	"	+	+	−
	850	73	+	T	Wood brown	+	+	−
	967	74	+	T	"	+	+	−
	707	62	−	T	Colorless		++	−
	723	45	−	T	"		++	−
Rice-straw decoction agar	No. 842	82	++	Ct	Dark olive	++	++	+++
	846	86	++	Ct	Buffy brown	+	+++	+++
	849	86	+	Ct	"	++	+++	++
	850	83	++	Ct	Dark olive	+	+++	+
	967	91	++	C	Clove brown	++	+++	+++
	707	75	−	Ct	Colorless		+++	−
	723	76	−	T	Deep olive	+	+	−
Bouillon agar	No. 842	63	+	C	Colorless		+	−
	846	60	−	C	"		+	−
	849	60	−	C	"		++	−
	850	70	−	C	"		+	−
	967	55	−	C	"		+	−
	707	35	−	T	"		++	−
	723	50	−	T	"		+	−
Onion-soja agar	No. 842	97	++	C	Dark olive	++	++	++
	846	87	−	C	Buffy brown	±	++	−
	849	94	++	C	Olive brown	++	++	+++
	850	98	−	C	Clove brown	++	++	−
	967	98	+	C	"	+++	++	+
	707	65	−	C	Colorless		+	−
	723	70	−	C	"		++	−

Table VII. (Continued.)

Culture media	Fungus strains	Radial growth of colonies	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of	
					Color name	Degree	Conidium	Perithecium
		mm.						
Dried apricot agar	No. 842	68	+	T	Buffy brown	+	+	−
	846	85	+	T	Dark olive	±	++	+
	849	80	+	T	Buffy brown	+	++	−
	850	94	+	T	„	+	+	−
	967	86	+	T	Dark olive	++	−	−
	707	76	−	T	Natal brown	+	++	−
	723	70	−	T	Colorless		++	−
Potato agar	No. 842	99	+	Ct	Wood brown	±	+++	−
	846	80	++	Ct	Dark olive	±	+++	++
	849	73	+	Ct	„	+	+++	+
	850	76	+	Ct	„	+	+++	+
	967	88	+	Ct	„	+	++	+
	707	82	−	Ct	Deep olive	+	++	−
	723	90	−	Ct	„	+	++	−
Steamed block of pine wood	No. 842	+++	−	Tt			+	+
	846	+++	−	Tt			+	+
	849	+++	−	Tt			+	+
	850	+++	−	Tt			+	+
	967	+++	−	Tt			+	+
	707		+	Tt			++	+
	723		−	Tt			+	−
Steamed block of oak wood	No. 842	+++	−	Tt			++	++
	846	+++	−	T			+++	++
	849	+++	−	Tt			++	++
	850	+++	+	Tt			++	+
	967	+++	−	Tt			++	++
HOPKIN's agar	No. 842	39	−	Tt	Colorless		+	−
	846	60	−	Tt	„		+	−
	849	54	−	Tt	„		+	−
	850	60	−	Tt	„		+	−
	967	55	−	Tt	„		+	−

Table VII. (Continued.)

Culture media	Fungus strains	Radial growth of colonies	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of	
					Color name	Degree	Conidium	Perithecium
		mm.						
Currie's agar	No. 842	24	−	Tt	Colorless		+	−
	846	47	−	Tt	"		+	−
	849	25	−	Tt	"		+	−
	850	40	−	Tt	"		+	−
	967	36	−	Tt	"		+	−
Steamed potato cylinder	No. 842	++	−	C	Clove brown	++	++	−
	846	++	−	C	"	++	++	−
	849	++	+	C	Buffy brown	++	++	−
	850	++	+	C	Clove brown	++	++	−
	967	++	−	C	"	++	+	−
Steamed rind of water-melon	No. 842	++	++	Ct	Clove brown	++	+++	+++
	846	++	++	Ct	"	++	+++	++
	849	++	++	Ct	"	++	++	+++
	850	++	++	Ct	"	++	++	++
	967	++	+	Ct	"	++	++	+++

Remarks: In the columns of the formation of aerial mycelium, conidium and perithecium the plus sign means the formation, the more the plus signs the better formation and minus sign, no formation.

* In this column, T means that the colonies thin and C, compact.

** The color names are given after RIDGEWAY's Color Standard. The number of plus signs in the color degree shows the breadth of the colored parts of the colonies.

Table VIII.

Summarized Characteristics of *Ceratostomella pini* Münch on Culture Media. II.

Results after 3 weeks' culture at 25°C.

Culture media	Fungus strains	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of	
				Color name	Degree	Conidium	Perithecium
Malt-extract agar	No. 842	+	T	Clove brown	++	+++	++
	846	+	T	Natal brown	++	+++	++
	849	+	T	Clove brown	+++	+++	++
	850	+	T	"	+++	++++	+++
	967	+	T	"	+++	+++	+++
	707	−	T	Deep olive	++	++	−
	723	+	T	Buffy brown	++	++	−

Table VIII. (Continued.)

Culture media	Fungus strains	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of	
				Color name	Degree	Conidium	Perithecium
Rice straw decoction agar	No. 842	++	Ct	Clove brown	+++	+++	+++
	846	++	Ct	„	++	+++	+++
	849	+	Ct	„	++	+++	++
	850	+	Ct	„	+++	+++	+++
	967	++	Ct	„	+++	+++	++
	707	−	Ct	„	++	+++	−
	723	+	T	Buffy brown	+++	+++	−
Bouillon agar	No. 842	−	C	Colerless		+++	−
	846	−	C	„		++	−
	849	−	C	„		+++	−
	850	−	C	„		+++	−
	967	−	C	„		+++	−
	707	−	Ct	„		+++	−
	723	−	Ct	„		++	−
Onion-soja agar	No. 842	+	Ct	Natal brown	++	++	+++
	846	−	C	„	+	++	+
	849	+	C	„	++	+++	+++
	850	+	C	„	++	+++	+
	967	+	C	„	++	++	++
	707	−	C	Colorless		++	−
	723	+	C	„		+++	−
Dried apricot agar	No. 742	+	T	Clove brown	++	+++	+++
	846	+	T	„	+++	+++	+++
	849	−	T	„	++	++	++
	850	+	T	„	+++	+++	+++
	967	+	T	„	+++	+++	++
	707	+	T	„	+++	+++	+
	723	+	T	Buffy brown	+++	+++	−

Table VIII. (Continued.)

Culture media	Fungus strains	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of	
				Color name	Degree	Conidium	Perithecium
Potato agar	No. 842	−	Ct	Blackish violet gray	++	++++	++
	846	−	Ct	Army brown	+	++++	++
	849	−	C	Blackish violet gray	++	++++	++
	850	+	Ct	"	++	++++	+
	967	+	C	"	++	++++	++
	707	−	C	Clove brown	++	+++	−
	723	+	Ct	Deep olive buff	++	++	−
Steamed block of pine wood	No. 842	−	Tt	Clove brown	+	+++	+++
	846	−	Tt	"	+	+++	+
	849	−	Tt	"	+	++	+++
	850	−	Tt	"	+	++	+++
	967	−	Tt	"	+	++	+++
	707	+	Tt	"	+	++	+++
	723	−	Tt	"	+	++	−
Steamed block of oak wood	No. 842	−	T	Bone brown	++	+++	+++
	846	−	T	"	++	++	+++
	849	+	Tt	"	++	++	+++
	850	+	T	"	++	++	+++
	967	+	T	"	++	++	+++
Hopkin's agar	No. 842	−	Tt	Colorless		+	−
	846	−	Tt	"		+	−
	849	−	Tt	"		+	−
	850	−	Tt	"		+	−
	967	−	Tt	"		+	−
Currie's agar	No. 842	−	Tt	Colorless		+	−
	846	−	Tt	"		+	−
	849	−	Tt	"		+	−
	850	−	Tt	"		+	−
	967	−	Tt	"		+	−
Steamed potato cylinder	No. 842	+	C	Black	++	+++	+
	846	−	C	"	++	++++	+
	849	−	C	"	+++	+++	−
	850	−	C	"	+++	++++	−
	967	−	C	"	+++	++++	+

Table VIII. (Continued.)

Culture media	Fungus strains	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of	
				Color name	Degree	Conidium	Perithecium
Steamed rind of water-melon	No. 842	+	Ct	Black	|||	|||	|||
	846	+	Ct	„	|||	||||	|||
	849	+	Ct	„	|||	||||	||
	850	+	T	„	|||	|||	||||
	967	+	T	„	|||	||	|||

Remarks: In the columns of the formation of aerial mycelium, coindium and perithecium, the plus sign means the formation, the more the plus signs the better formation and miuns sign, no formation.

* In this column, T means that the colonies are thin and C, compact.

** The color names are given after RIDGWAY'S Color Standard. The number of plus signs in the color degree shows the breadth of the colored parts of the colonies.

2. Effect of Free Oxygen on the Growth of the Fungus.

Effect of free oxygen on the mycelial growth and on the conidium germination was tested with the pyrogallic acid method after BUCHNER. As stated in the first report of this series, one gram of pyrogallol and 10 cc. of 10% water solution of caustic potash were added to a glass tube of 100 cc. capacity. Then a culture tube inoculated with the fungus was immediately introduced into it, and the tube was stoppered and sealed. The tubes thus prepared were kept at 24°C., and the result after 3 and 7 days culture was examined. The result, given in Table IX, shows that the fungus does not grow at all without free oxygen.

Table IX.

Effect of Free Oxygen on the Mycelial Growth of *Ceratostomella pini* Münch.

Temperature tested: 24°C.

Fungus strains	After 3 days		After 7 days	
	Without free oxygen	Control	Without free oxygen	Control
No. 850	No growth	Good growth, colonies 20.5 mm. in diameter	No growth	Colonies cover all over the surface of the medium.
No. 967	No growth	Good growth, colonies 30.5 mm. in diameter	No growth	Ditto
No. 707	No growth	Good growth, colonies 18.9 mm. in diameter	No growth	Ditto

In regards to the effect of free oxygen to the conidium germination an experiment, similar to that of the first report, was carried out. As shown in Table X, the conidium and also ascospore show no germination under anaerobic condition.

Table X.

Effect of Free Oxygen on Germination of Conidia and Ascospores of *Ceratostomella pini* Münch.

Results after 3 days' culture at 24°C.

	Fungus strains	Without free oxygen	Control
Conidium	No. 850	No germination	Good germination. Secondary conidia were produced
	No. 967	No germination	Ditto
	No. 707	No germination	Ditto
Ascospore	No. 850	No germination	About an half number of the ascospores germinated
	No. 967	No germination	Ditto
	No. 707	No germination	Ditto

VIII. Sterilization Experiments of the Fungus.

1. Thermal Death Point of the Conidium.

In this experiment the thermal death points of the conidia of *Ceratostomella pini* were determined in the following manner. The concentrate conidium suspension, prepared from the conidia developed on culture media, was added to 5 cc. of sterilized distilled water in test tubes of 1.5 cm. in diameter. The test tubes were inserted into hot water, in a water thermostat. After a duration of 5, 10, 15 and 20 minutes, respectively, 2 platinum loopfuls, 2 mm. in diameter, were transfered from these tubes to 2% malt-extract solution. They were then kept in an incubator, set at 27°C. for 7 days. According to the results which are given in Table XI, the conidia of this fungus lose the vitality by a treatment at 54°C. for 5 minutes or at 52°C., for more than 15 minutes.

(See Table XI on next page.)

2. Experiments to Kill the Conidium by Disinfectants.

In the experiment to kill the conidia by disinfectants the following method was used: Wheat seeds in Petri dishes were sterilized in a hot-air sterilizer at 150—160°C. for 30 minutes, to which conidium suspension of the fungus was added. The Petri dish was shaken for a few minutes, to attach the conidia to the surface of the wheat seeds.

Table XI.

Thermal Death Point of Conidia of *Ceratostomella pini* Münch.

Fungus strain	Period of immersion	Control	Temperature of water (C.) tested					
			44°	46°	48°	50°	52°	54°
No. 850	5 Minutes	+	+	+	+	+	+	−
	10 „	+	+	+	+	+	−	−
	15 „	+	+	+	+	+	−	−
	20 „	+	+	+	+	+	−	−
No. 967	5 Minutes	+	+	+	+	+	+	−
	10 „	+	+	+	+	+	+	−
	15 „	+	+	+	+	+	−	−
	20 „	+	+	+	+	+	−	−

Remarks: In this table the plus sign means that the conidia were not killed by the treatment and minus sign, killed.

Solutions of disinfectants to be tested were diluted in various strength with sterilized distilled water aseptically, to which the sterilized wheat seeds, covered with the conidium to be tested, were added. They were kept at 27°C. in an incubator. At various intervals, the wheat seeds were transferred from the solutions, into 3% malt-extract solution. Then the formation of the fungus colonies in the test tubes were examined after 3 or 7 days' incubation at 27°C. The results are given in Table XII.

Table XII.

Germicidal Efficiency of Disinfectants against Conidia of *Ceratostomella pini* Münch.

Temperature of the solutions tested: 27°C.
Fungus strain studied: No. 967.

Corrosive sublimate, $HgCl_2$.

Period of immersion	Dilution						Control
	1 : 1,000	1 : 2,000	1 : 4,000	1 : 6,000	1 : 8,000	1 : 10,000	
1 hour	−	−	±	±	+	+	+
3 hours	−	−	−	±	±	±	+
6 „	−	−	−	−	±	±	+
24 „	−	−	−	−	−	−	+

(Continued to the next page.)

Uspulun

Period of immersion	Dilution					Control
	1:100	1:200	1:400	1:800	1:1,600	
1 hour	−	−	+	+	+	+
3 hours	−	−	+	+	+	+
6 „	−	−	+	+	+	+
24 „	−	−	−	+	+	+

Copper sulphate, $CuSO_4$ $5H_2O$

Period of immersion	Dilution					Control
	1:25	1:50	1:100	1:200	1:400	
1 hour	−	+	+	+	+	+
3 hours	−	−	+	+	+	+
6 „	−	−	−	+	+	+
24 „	−	−	−	+	+	+

Formalin

Period of immersion	Dilution					Control
	1:50	1:100	1:200	1:400	1:800	
1 hour	−	−	−	+	+	+
3 hours	−	−	−	−	−	+
6 „	−	−	−	−	−	+
24 „	−	−	−	−	−	+

Remarks: In this table, the puls sign shows that the conidia were not killed by the treatment and minus sign, killed.

According to the results given in Table XII, the solution of corrosive sublimate is very strong in the germicidal efficiency against the conidia of the fungus. One hour's treatment in 1:6,000 solution of corrosive sublimate at 27°C. killed almost all the conidia. Three hours immersion in 1:10,000 solution of the same showed the similar efficiency. With formalin, a treatment in 1:200 solution for 1 hour, or 1:800 solution for 3 hours was effective enough to kill the conidia. Copper sulphate solutions were comparatively weak in the germicidal efficiency and a treatment of 3 hours in 1:50 solution or 6 hours in 1:100 solution was necessary to kill the conidia.

3. Experiments to Check the Fungus Growth by Disinfectants.

Further the writers examined the checking efficacy of disinfectants against the fungus, only to check the growth. The disinfectants were diluted with sterilized 1% malt-extract solution aseptically in test tubes. The tubes were inoculated with a bit of culture of *Ceratostomella pini* and kept at 27°C. for 7 days. According to the result, which is given in Table XIII, the fungus grows no more in 1 : 50,000 to 1 : 100,000 solution of corrosive sublimate or 1 : 10,000 solution of copper sulphate, iron sulphate, ' germisan ' and ' uspulun ' respectively.

Table XIII.

Checking Efficiency of Solutions of Disinfectants against Mycelial Growth of *Ceratostomella pini* Münch.

Nutrient solution used for dilution: 1% malt extract solution.
Temperature of the solutions tested: 27°C.
Fungus strains studied: No. 850, No. 967.

Fungus strains	Disinfectants	Dilution								Control
		1:1,000	1:5,000	1:10,000	1:50,000	1:100,000	1:500,000	1:1,000,000	1:5,000,000	
No. 850	Copper sulphate	−	−	−	+	+	+	+	+	+
	Iron sulphate	−	−	−	+	+	+	+	+	+
	Corrosive sublimate	−	−	−	−	−	+	+	+	+
	Germisan	−	−	−	+	+	+	+	+	+
	Uspulun	−	−	−	+	+	+	+	+	+
No. 967	Copper sulphate	−	−	−	+	+	+	+	+	+
	Corrosive sublimate	−	−	−	−	+	+	+	+	+
	Uspulun	−	−	−	−	+	+	+	+	+

Remarks: In this table, the plus sign shows that the fungus colonies appeared in the solutions tested and minus sign, no growth.

IX. Summary.

1) The present paper is the second report on the sap stains of wood in Japan, and deals with the blue-staining fungus of pine wood, *Ceratostomella pini* MÜNCH.

2) In Japan, the fungus is generally found on " Akamatu " (*Pinus densiflora* SIEB. et ZUCC.) and " Kuromatu " (*Pinus Thunbergii* PARL). It attacks not only

the felled trees, but also those standing on their roots and weakened by the attack of bark-beetles or by other causes.

3) As in the case of *Ceratostomella ips*, already stated in the first report, the hyphae of present fungus penetrates through the parenchymatous cells of medullary rays from the cortex toward the center, while they grow through the resin ducts and the tracheids longitudinally and through bordered pits in the tangential direction.

4) The fungus develops small, short-beaked perithecia, among their comparatively large, irregular-shaped sclerotia. Conidia are produced apically in a ball of Cephalosporium-type, but not so large a ball as in the case of *Ceratostomella ips* RUMBOLD. The conidiophores, in Graphium-type as seen in *Ceratostomella piceae*, have not yet been observed.

5) The ascospores are falcate and the conidia, long elliptical. On the germination, however, they swell prominently and become short elliptical or sometimes globular.

6) The fungus grows very well on culture media, and the growth rate at a moderate temperature is much larger than that of *Ceratostomella ips* RUMBOLD and *Ceratostomella piceae* MÜNCH.

7) The fungus conidium loses the vitality by a treatment in water at 52°C. for 15 minutes or at 54°C., for 5 minutes, and also by one hour immersion in 1 : 6,000 solution of corrosive sublimate or in 1 : 200 solution or formalin.

8) The fungus growth in 1% malt-extract solution was checked by addition of corrosive sublimate in the strength of 1 : 100,000 or of copper sulphate, iron sulphate and 'uspulun' in 1 : 10,000, respectively.

9) Without free oxygen neither the conidium germination nor the mycelial growth takes place.

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Explanation of the Plates.

Plate XVII.

Fig. 1. Transverse sections of "Akamatu" (*Pinus densiflora* SIEB. et ZUCC.), attacked by *Ceratostomella pini* MÜNCH. The pine tree was felled in the Terayama State Forest in Iwami-mura, Ibo-gun, Hyôgo Prefecture on July 18, 1933. The disc A was cut at the base of the tree near the earth; the disc B, at a distance of a meter above the disc A; C a meter higher than B; and the other discs, D, E and F were cut in the same order.

Fig. 2. Sclerotia and perithecia of *Ceratostomella pini* MÜNCH, developed inside the bark of an diseased pine tree (*Pinus densiflora*). The flask-shaped small bodies are the perithecia and the larger ones, sclerotia. (×100)

Fig. 3. Conidia of *Ceratostomella pini* MÜNCH, develeped on 3% malt-extract agar. (×2,000)

Plate XVIII.

Sections of blue-stained sap-wood of pine tree (*Pinus densiflora*), attacked by *Ceratostomella pini* MÜNCH. Showing dark hyphae penetrating the cells in medullary rays and tracheids. The figures were drawn from water mounted preparations with an aid of camera lucida under Zeiss K. 10× and Apochromat 40×; and were reduced to one half of original size. (×250)

Fig. 1. Transverse section of blue-stained sap-wood of pine. Showing dark hyphae in the cells of medullary rays.

Fig. 2. Radial, longitudinal section of blue-stained sap-wood of pine.

Fig. 3. Tangential, longitudinal section of blue-stained sap-wood of pine.

Plate XIX.

Fig. 1. Section of a sclerotium of *Ceratostomella pini* MÜNCH, produced inside the bark of a pine tree. Showing the pseudoparenchymatous layers near the surface. (×500)

Fig. 2. Hyphae of *Ceratostomella pini* MÜNCH, developed in malt-extract agar at 27°C. Showing the variousness in diameter of hyphae and the uneven surface of hyphal sheath. (×500)

Fig. 3. Conidia of *Ceratostomella pini* MÜNCH (Cephalosporium type), developed on 3% malt-extract agar. (×1,500)

Fig. 4. Conidia sown in malt-extract solution for the germination experiment. They swell and begun to germinate, some ones producing secondary conidia. (×1,500)

Plate XX.

Fig. 1. Conidium of *Ceratostomella pini* MÜNCH, formed on malt-extract agar. (×1,500)

Fig. 2—3. Conidiophores of *Ceratostomella pini* MÜNCH. Showing much branched conidiophores. (×1,500)

Fig. 4. Germination of conidia of *Ceratostomella pini* MÜNCH, in 3% malt-extract agar after 24 hours' incubation at 25°C. (×1,500)

Plate XXI.

Fig. 1. Perithecia of *Ceratostomella pini* MÜNCH, developed on a steamed pine block. Showing mature (in the center) and premature ones (at the sides). (×400)

Fig. 2. Apex of perithecial beaks of *Ceratostomella pini* MÜNCH. Showing fringed cilia around the apex. (×1,000)

Fig. 3. Ascospores of *Ceratostomella pini* MÜNCH, developed on a steamed pine block. (×1,500)

Fig. 4. Germination of ascospores of *Ceratostomella pini* MÜNCH, in 3% malt-extract agar after 24 hours' incubation at 24°C. (×1,500)

PLATE XVII.

Fig. 1.

Fig. 2.

Fig. 3.

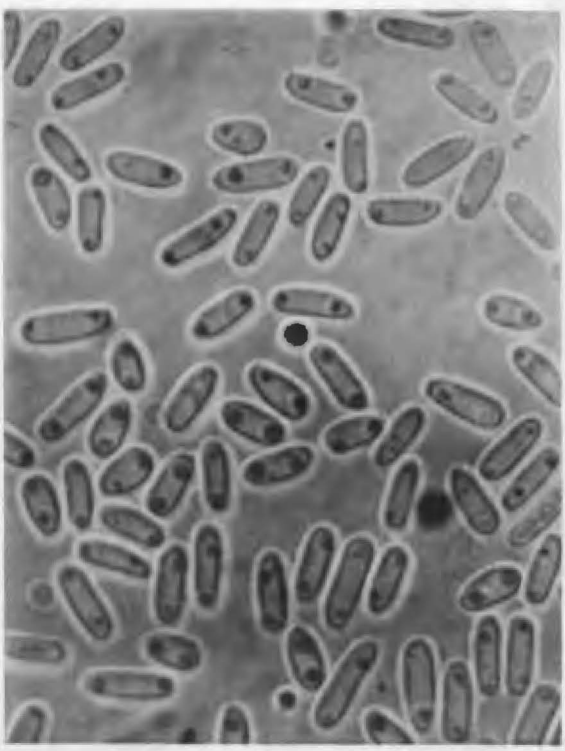


PLATE XVIII.

Fig. 1.

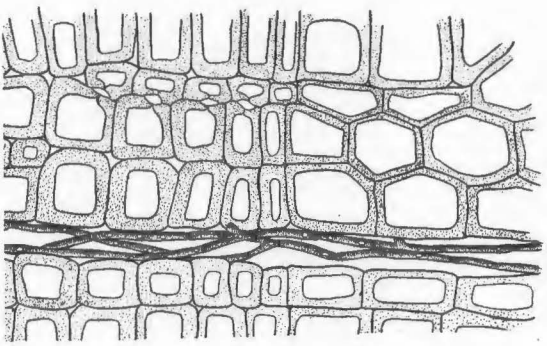


Fig. 2.

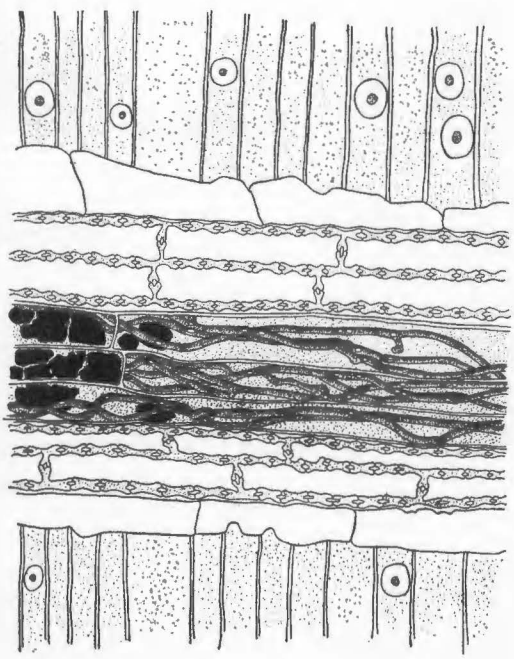


Fig. 3.

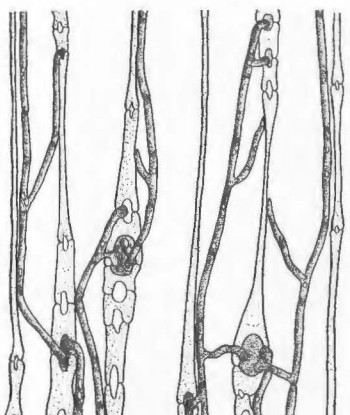
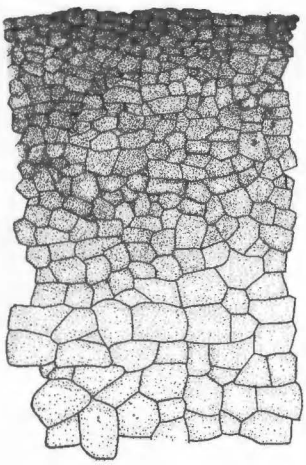


PLATE XIX.

Fig. 1.



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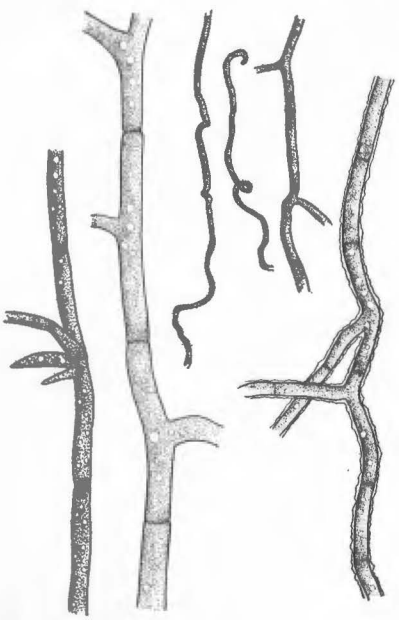

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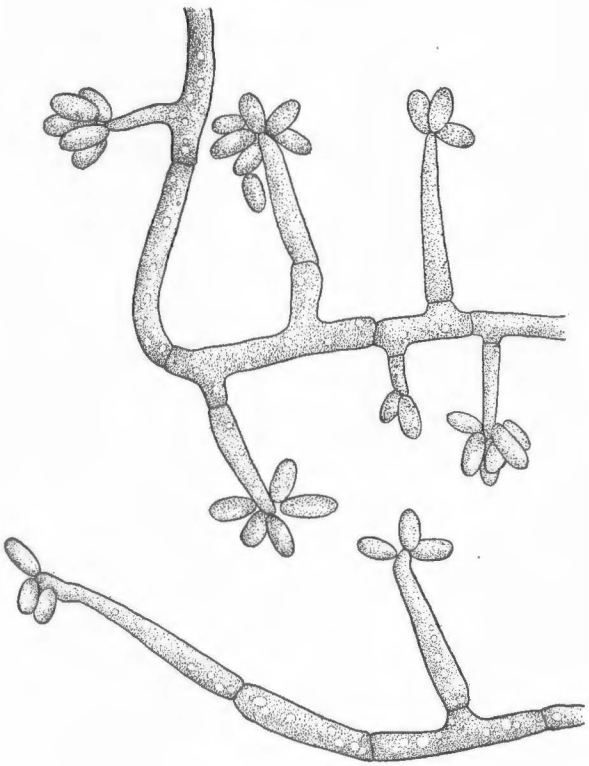


Fig. 4.

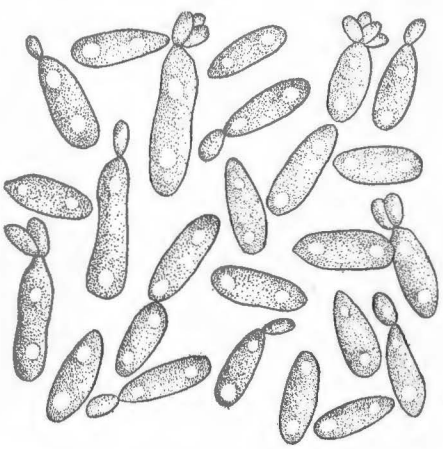


PLATE XX.

Fig. 1.

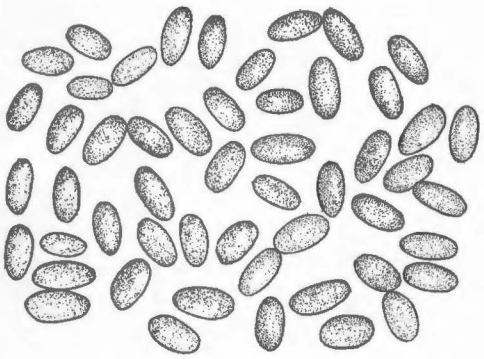


Fig. 3.

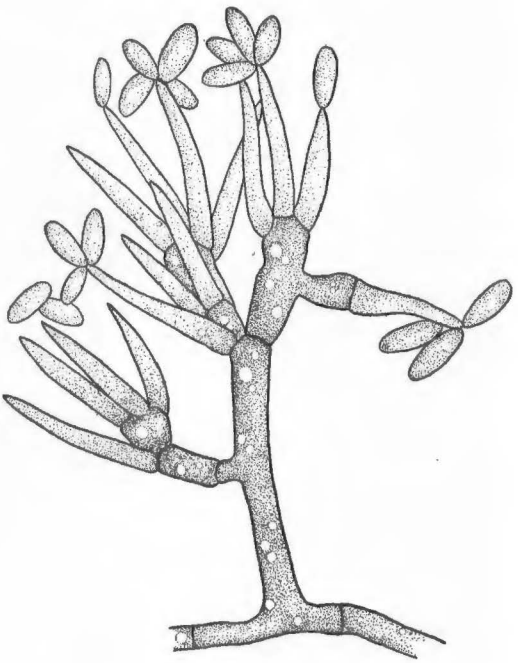


Fig. 2.

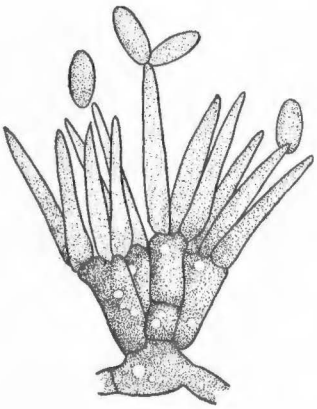


Fig. 4.

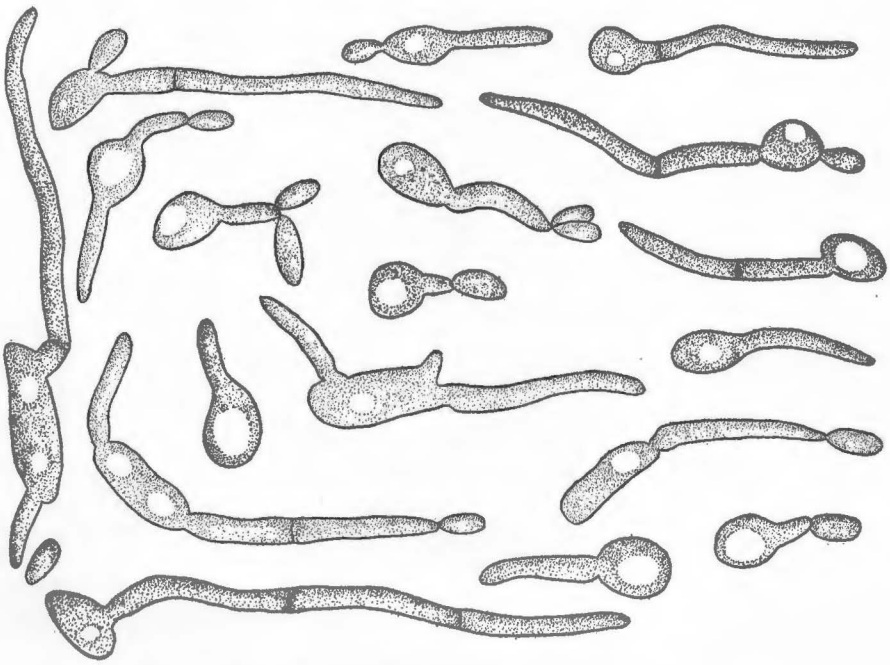


PLATE XXI.

Fig. 1.

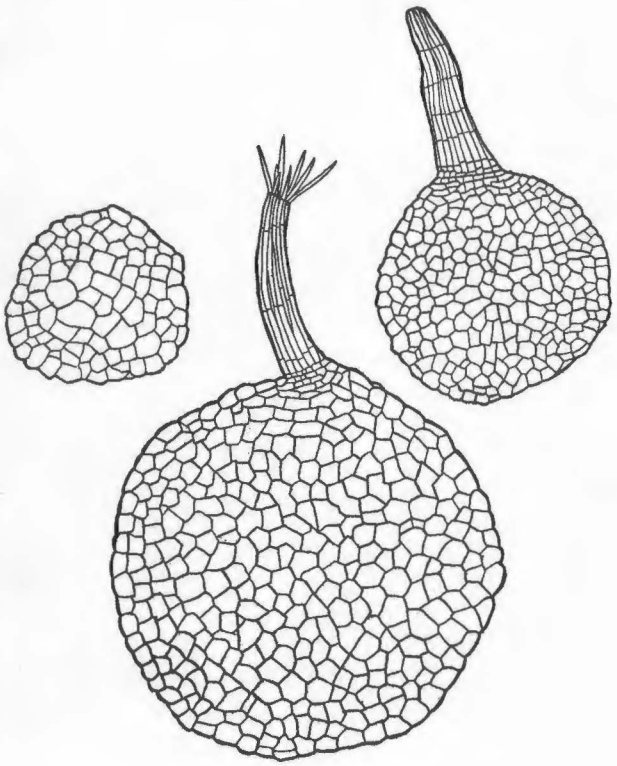
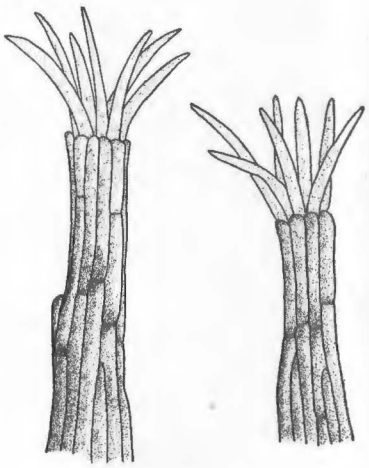


Fig. 2.



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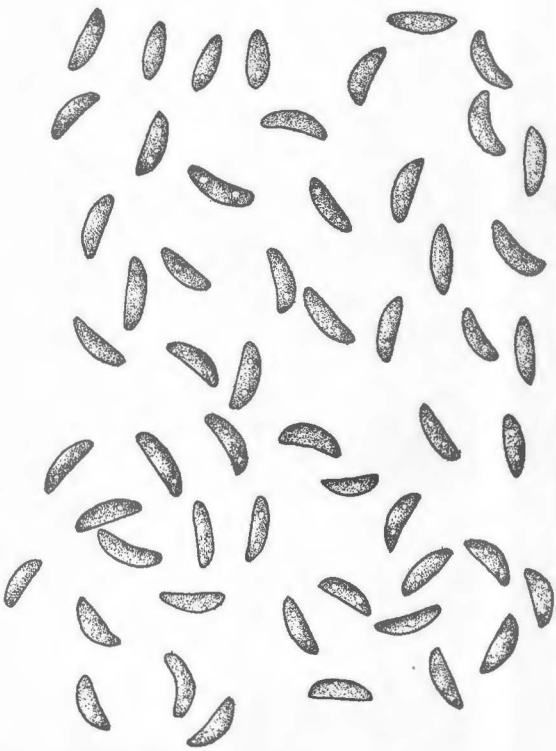

Fig. 4.