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

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

By

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Contents.

I. Introduction.

The present writers have already reported two species of Ceratostomella, causing blue stains of pine wood, namely *Ceratostomella ips* Rumbold and *C. pini* Münch (Nisikado and Yamauti 1933, 1934). The third species of the blue staining fungi, *Ceratostomella piceae* Münch, is reported in this paper.

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II. Historical Review.

In Japan no valid description has been found on the blue stain of pine trees, caused by *Ceratostomella piceae* MÜNCH, although the occurrence of this sap stain of pine and spruce wood seems to have been known since long time ago. Only recently Y. TOCHINAI and M. SAKAMOTO (1934) gave a description on the blue stain of spruce wood caused by *C. piceae* in Hokkaido.

M. KASAI (1917) reported a blue stain fungus of pine and oak wood, with the name *C. pilifera* (FR.) WINTER. K. TANAKA (1926) dealt with sap stains of wood and designated the causal fungus by the above given name after KASAI.

The fungus *C. pilifera* given by them, resembles to the perithecial stage of *C. piceae*. They gave, however, no description on the conidium in Graphium-type, which is one of the most prominent characteristics of the present fungus. Therefore it seems to be duely considered that this fungus differs from *C. pilifera* given by M. KASAI, at least until the matters shall be made clear. The blue-staining fungus, reported by D. NUMATA (1931) and K. UYEDA and K. NAGAMATU (1932), seems to be *C. piceae* although it is not certain.

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

III. Symptoms.

The blue stain caused by *C. piceae* is commonly found on the cut surface of wood of pine and spruce. In a lumber yard it is found not only on pine and spruce wood, but also on many other kinds of wood. According to the writers' survey, the followings were the subject to attack by *C. piceae*: *Pinus Thunbergii* PARL., *P. densiflora* S. et Z., *P. parvifolia* S. et Z., *Chamaecyparis obtura* S. et Z., *C. pilifera* ENDL., *Picea jezoensis* CARR., *P. Glehni* MAST., *Quercus glandifolia* BLUME, *Kalopanax ricinifolia* MIQ., *Magnolia hypoleuca* S. et Z., *Prunus indica* THUNB., *Betula japonica* SIEB. and *Acer pictum* THUNB. Perhaps many more kinds of wood might be the hosts of this fungus, if surveyed more carefully.

The blue stained sap-wood of *Pinus densiflora*, "Akamatu", shows, as in Plate XXV, Fig. 1, a wedge shaped, grayish blue or dark discoloration tapering from the cortex toward the center. But the discoloration is generally much

lighter than those caused by the previously reported two Ceratostomellas, *C. pini* and *C. ips* (NISIKADO and YAMAUTI, 1933 and 1934). On the surface of the attacked woods, very prominent graphium and perithecium characteristic to *C. piceae* are produced, which may be seen by the naked eyes.

MÜNCH (1907) who described the fungus, stated that *C. piceae* was very common on the sap-wood of the spruce and the silver-fir, but did not seem to be quite sure as to what part it played in the blueing of the sap-wood. MAC CALLUM (1922) who reported this fungus in England, stated that *C. piceae* was very common there and occurred on pine wood always intermixed with other species, while on spruce it developed as pure culture. When it grew singly, it caused only faint blue and the wood remained almost unstained, even when the surface was covered with the perithecia. When it was found on blued timber, other species of fungi were invariably present.

Not only the cut wood in lumber yards, but also pine trees standing on their root are also the subject to the attack by this fungus. The disc given in Plate XXV, Fig. 1 was cut from a pine trees (*P. Thunbergii*), which was standing on its root, and still provided with quite green needles. It was only somewhat weakened as the stem was buried with sand at a sand dune in the seaside in Tottori. As shown in the Plates, the disc has wedge-shaped discolored leisions. In Saghalien it is said that many spruce trees standing on their roots are attacked by blue-staining fungi chiefly by *C. piceae*. MAC CALLUM (1922) stated that many pine trees in the forest near Edingburgh were attacked by this fungus. However, in Western Japan it is true that the fungus is observed on the lumber most commonly.

The microscopical features of the blue-staind pine wood are shown in Plate XXVI, Fig. 1—3 The hyphae of this fungus in the wood tissues are almost similar to those of *C. pini* and *C. ips*.

IV. Source of the Cultures Studied.

The culture strains of *Ceratostomella piceae* were isolated in the method given in the first report of the present contributions (NISIKADO and YAMAUTI 1933). The source of the fungus cultures studied in this investigation is given as follows:

Strain No. 727. The strain was isolated from blue-stained sap-wood of *Pinus Thunbergii* PARL., "Kuromatu", collected in Akasi Park, Pref. Hyôgo on June 12, 1932.

Strain No. 729. It was isolated from blue-stained sap-wood of *Quercus glandiflora* BLUME, collected in Kiso-Agematu, Pref. Nagano on October 12, 1932.

Strain No. 730, isolated from blue-stained wood of *Pinus densiflora* S. et Z., collected in Kiso-Agematu, Pref. Nagano on October 12, 1932.

Strain No. 731, isolated from blue-stained wood of *Pinus densiflora* S. et Z., collected in Nakatugawa, Pref. Gihu on October 12, 1932.

Strain No. 732, isolated from blue-stained wood of *Pinus parvifolia* S. et Z., collected in Nakatugawa on October 12, 1932.

Strain No. 734, isolated from blue-stained wood of *Betula japonica* SIEB., collected in Kiso-Agematu, Pref. Nagano on October 12, 1932.

Strain No. 738, isolated from blue-stained wood of *Prunus indica* THUNB., collected in Kiso-Agematu on October 12, 1932.

Strain No. 740, isolated from blue-stained wood of *Chamaecyparis pisifera* ENDL., collected in Kiso-Agematu on October 12, 1932.

Strain No. 746, isolated from blue-stained wood of *Kalopanax ricinifolium* MIQ., collected in Kiso-Agematu on October 12, 1932.

Strain No. 595. This strain was sent to the writers from Prof. J. WESTERDIJK, Holland, with the name *Ceratostomella piceae* MÜNCH.

V. Morphology of the Fungus.

1. Mycelium.

As shown in Plate XXVI, the fungus hyphae in the stained wood resemble in general to those of *Ceratostomella ips* RUMBOLD and *C. pini* MÜNCH given in the previous papers (NISIKADO and YAMAUTI 1933, 1934). They penetrate the parenchymatous cells in the medullary rays as well as the tracheids and resin ducts. They are brown or dark brown but somewhat lighter than those of the above two fungi. This fact seems to be the cause that the coloration of the wood stained by this fungus is lighter than the others. The hyphae grown on the malt-extract agar or the potato agar are of lighter color. They are 3—8 μ wide, commonly about 5 μ.

2. Conidiophores and Conidia.

According to the shape and mode of the formation, three kinds of conidiophores are found in this fungus. One of them is the conidiophore of the large Graphium-type; this is produced on the surface of the stained sap-wood. Another one is of the Cephalosporium-type, which is generally in or on the culture media, the conidia being produced on the ends of the hyphal branches in a ball. The other one is of the Cladosporium-type, and comparatively long conidia are produced verticillately and also catenulately on the ends of the hyphal branches or the conidiophores.

i) *Graphium-type:* In this type the conidia are produced on the top of a large composite conidiophore, the graphium. The conidia are produced on the surface of stained sap-wood, usually preceeding the formation of perithecia and one of the most prominent characteristics of this species. As shown in Plate XXV, Fig. 3—4, they are sometimes short and composed of only some conidiophores, but sometimes of a large bundle of more than hundreds of the conidio-

phores, the head being large and resembling the pileus of a mushroom. The head of graphium is colorless, although the base and the hyphal strands at the base are brown or dark brown.

The size of the graphium is variable according to the fungus strains as well as the conditions under which they are formed. The writers' measurement of the conidiophores of various strains produced on the malt-extract agar after 8 days' culture at 27°C. are given in Table I. The strains with an asterisk in Table I, have not yet produced the perithecia so far, but from the characteristics of the graphium stage they are assumed to be the same with those producing the perithecia. Therefore they have been assumed to be *C. piceae* MÜNCH.

Table I.

Size of Graphium-Conidiophore of *Ceratostomella piceae* Münch.

Result of measurement of 50 graphium, developed on malt-extract agar after 8—10 days' culture at 27°C.

Fungus strains		Length and width of graphium (μ)		Length and width of head of graphium (μ)	
Number	Host plant[1)]	Range	Mean	Range	Mean
No. 727	*Pinus Thunbergii* PARL.	220–560× 8–45	385.0×41.7	30–70×20–200	48.5×87.4
729	*Quercus glandiflora* BLUME	170–740×10–40	472.8×25.7	40–80×30–180	60.0×100.0
730*	*Pinus densiflora* S. et Z.	300–540×10–40	387.0×17.6	30–80×40–140	56.8×78.4
731*	Ditto	270–650× 8–40	419.6×15.1	20–70×30–180	45.2×85.4
732*	*Pinus parvifolia* S. et Z.	220–450×10–55	343.4×29.0	40–100×30–220	61.0×143.4
734	*Betula japonica* SIEB.	120–580× 8–40	282.6×23.3	30–100×30–180	55.6×88.0
738*	*Prunus indica* THUNB.	320–670×10–40	459.8×25.6	30–80×30–230	55.4×136.8
746	*Kalopanax ricinifolium* MIQ.	210–540×10–40	448.0×29.2	40–80×60–220	61.0×118.2
747	*Acer pictum* THUNB.	290–590×20–40	428.2×30.0	40–90×60–210	66.4×127.2

Remarks: 1) The name of the host plant, from which the strain was isolated, is here given.

According to Table I, the graphium of the strain No. 727, isolated from *Pinus Thunbergii*, "Kuromatu", measured 220—560×8—45 μ (mean 385×41.7 μ), the head being 30—70×20—200 μ (mean 48.5×87.4 μ).

The conidia developed on this type of conidiophores are colorless, elliptical, and similar to those of Cephalosporium type. They measured, as shown in Table II, 3—8×2—4 μ (mean $4.82\pm0.03\times2.50\pm0.03$ μ). (Plate XXVII, Fig. 5.)

Table II.

Size of Conidium of *Ceratostomella piceae* Münch in Three Types.

Results of measurement of 100 conidia, developed on potato-glucose agar after 10 days' culture at 25°C. The asterisk * shows, the result of 200 measurements.

Type of conidium	Fungus strains	Length of conidium (μ)		Width of conidium (μ)	
		Range	Mean	Range	Mean
Graphium-Type	No. 727	3—6	4.80±0.035	2—4	2.56±0.037
	746	3—8	4.84±0.053	2—4	2.44±0.035
	Average	3—8	4.82±0.032	2—4	2.50±0.025
Cladosporium-Type	No. 727	*4—19	*9.19±0.145	2—4	2.90±0.037
	746	*4—22	*9.06±0.136	2—4	2.90±0.032
	Average	4—22	9.13±0.099	2—4	2.90±0.024
Cephalosporium-Type	No. 727	4—12	7.19±0.084	2—4	2.80±0.027
	746	4—11	7.14±0.077	2—4	2.95±0.027
	Average	4—12	7.17±0.059	2—4	2.88±0.019

ii) *Cephalosporium-Type :* (Plate XXVII, Fig. 4.) The conidia of this type are produced on the end of young hyphae, and resemble to those of *C. ips* and *C. pini.* They are colorless, elliptical or long elliptical with round ends. They are somewhat smaller than those in Cladosporium type, although they are slightly larger than those on Graphium. The conidia produced on the potato-dextrose agar at 25°C. are 4—12×2—4 μ (mean 7.17±0.06×2.88±0.02 μ).

iii) *Cladosporium-Type :* (Plate XXVII, Fig. 1—3; Plate XXIX, Fig. 2—3.) As it will be stated later, the conidia of this type are produced on the tip of the germ-tubes developed from the conidia as well as the ascospores. The tip of germ-tubes, as a rule, swells slightly and produces two or three and sometimes many protuberances verticillately, on which the secondary conidia are produced in chains. They are colorless, spindle-shaped or elliptical, usually straight, rarely curved to one side and one or both ends are pointed. At the top of conidia, one to three or rarely four protuberances are formed, where the secondary conidia are also produced.

In the same manner the conidia are produced on the end of hyphae growing on such medium like the potato-dextrose agar. The conidia are produced in a paniculate manner. The size of the conidia in this type varies greatly. An example of the measurements of the conidia developed on the potato-dextrose agar at 25°C. is, as shown in Table II, 4—22×2—4 μ (mean 9.13±0.10×2.90±0.02 μ).

iv) *Germination of the Conidia.* The conidia germinate readily in water or in the nutrient solutions. Those germinated in 3% malt-extract solution at 24°C. after 24 hours' incubation are shown in Plate XXVII, Fig. 6. On the germination, the conidia swell prominently and become 6—15×5 μ, and spherical, elliptical or long elliptical. At the end of the germ-tubes, two to many protuberances are formed, on which the secondary conidia are produced. They are mostly of the cladosporium type.

3. Perithecia and Ascospores.

i) *Perithecia.* The perithecia are produced profusely on the cut surface of lumber or timber. As shown in Plate XXV, Fig. 2, and Plate XXVIII, Fig. 2, they are flask-shape or of Sagittaria-bulb. The base of perithecia is spherical or slightly depressed spherical, and dark brown or black. The surface is covered sometimes with mycelial strands. The perithecia produced on steamed block of pine wood, measured 105—225 μ (mean 157.1±2.34 μ) in height and 105—225 μ (mean 161.2±2.69 μ) in width, as shown in Table III.

Table III.

Size of Perithecia of *Ceratostomella piceae* Münch.

Result of measurement of 100 perithecia of strain No. 727, developed on steamed pine blocks.

		Range	Mean	Standard deviation
Base	Height (μ)	105—225	157.1±2.34	23.4
	Diameter (μ)	105—225	161.2±2.69	26.9
Beak	Length (μ)	650—1,950	1247.0±17.06	170.6
	Width (Apex) (μ)	3—18	9.6±0.047	0.471
	„ (Widest part) (μ)	5—55	26.3±0.063	0.633

The beak of the perithecium is very long, straight or slightly curved and dark brown at the base and becomes lighter color toward the apex, where it is provided with a fringe of cilia, as shown in Plate XXVIII, Fig. 2, 5, 6 and 7. The beak of perithecia developed on steamed pine block are, as shown in Table III, 650—1,950 μ (mean 124.7±17.06 μ) long, and 5—55 μ (mean 26.3±0.06 μ) wide at base and 3—18 μ (mean 9.6±0.05 μ) wide near the end. The cilia are almost colorless, 10—15 μ in a fringe, and 20—30 μ rarely 40 μ in length.

ii) *Asci and ascospores.* The perithecia push out the ascospores from the apical pore, when mature. Although the wall of ascus dissolves in water readily, the ascus of this fungus is not so hard to observe as in the case of *C. ips* or *C. pini.* Asci are usually spherical, short elliptical and contain 8 ascospores (Plate XXVIII, Fig. 3). Those developed on steamed pine wood are 4.5—10.5 μ.

Ascospores, as shown in Plate XXVIII, Fig. 4, are colorless, reniform or long elliptical, straight or slightly curved to one side, one or both ends being pointed. They are 2.8—4.8×0.8—2.3 μ (mean 3.7—1.4 μ) as shown in Table IV.

Table IV.

Size of Asci and Ascospores of *Ceratostomella piceae* Münch.

Result of measurement of asci of strain No. 727, developed on steamed pine blocks.

		Number measured	Range	Mean	Standard deviation
Ascis	Length (μ)	50	4.5—10.5	6.98±0.159	1.122
	Width (μ)	50	4.5—10.5	6.26±0.132	0.934
Ascospore	Length (μ)	100	2.8—4.8	3.7±0.041	0.414
	Width (μ)	100	0.8—2.3	1.41±0.026	0.259

iii) *The germination of the ascospores.* The ascospores germinate readily in water or in the nutrient solutions. Those germinated in 3% malt-extract solution at 24°C. after 24 hours' incubation are shown in Plate XXIX, Fig. 1—2. On the germination they swell prominently, especially in width and become globular, measuring 5—8×4—5 μ. They produce one or two germ-tubes from one or both ends. The germ-tubes are comparatively thick and attaining above 6 μ. At the end of germ-tubes they produce conidia in Cladosporium-type and sometimes in Cephalosporium-type.

VI. Taxonomical Consideration on the Fungus.

From the above given morphological description, it is clear that the present fungus belongs to the genus Ceratostomella. According to the shape and size of the perithecia, the fungus must be a member of the so-called Pilifera group named by MÜNCH (1907). As it produces the conidia on large conidiophore in Graphium-type and in Cephalosporium-type as well as Cladosporium-types, it seems to be *C. piceae* MÜNCH.

C. piceae, isolated by MÜNCH (1907) from the species known as *C. pilifera* (FR.) WINTER, has a perithecium resembling to those of *C. cana*, *C. coerulea* and *Endoconidiophora coerulescens*. The characteristics of the group "Pilifera" described by MÜNCH are as follows:

Perithecien kohlschwarz, kugelig, an der Ansatzfläche etwas abgeplattet, zuweilen schwach behaart, 160—240 μ im Durchmesser (selten 150—260 μ), mit einem 0.8—1.2 mm langen, 20—30 μ dicken, schwarzen, unregelmässig etwas gebogenen Schnabel, an dessen Ende ein Kranz farbloser, 20—50 μ langer

Wimpern die in einem Schleimtropfen suspendierten Sporen trägt. Diese sind farblos, zylindrisch, beiderseits abgerundet, schwach gekrümmt, 3.5—4.5 μ lang, 1.5—2 μ dick. Asei 5—6 μ im Durchmesser. (Vergl. die ähnliche Figur 20.)

LAGERBERG, LUNDBERG and MELIN (1927), state that *C. piceae* differs from *C. coerulea* in shape. Those of the former species is spherical and not flattened in the ventral part. The cilia of the fringe are much shorter in the former. The above given characteristics of *C. piceae* noted by MÜNCH and LAGERBERG etc. coincide with those of the present fungus.

The important characteristics of the writers' fungus and *C. piceae* given by previous authors are compared as follows:

Table V.

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

			MÜNCH (1907)	LAGERBERG (1927)	NISIKADO etc.
Perithecium	Base	Diameter (μ)	160—240 (150–260)	192—224	105—205 (161)
		Height (μ)		1,060—1,970 (1,500)	105—225 (157)
	Beak	Length (μ)	800—1,200	32 14—16	650–1,950 (1,247)
		Diameter (μ)	20—30	10.7—21.4	5—55 3—18
	Cilia	Length (μ)	20—50	3.2 2.4	20—30 (40)
		Diameter (μ)			
Ascus	Size (μ)		5—6		5—10×5—9
Ascospore	Size (μ)		3.5—4.5×1.2—2	2.3—4.6×1.6	3.0—4.5×1—2
Conidium	Graphium (μ)		3.5—4×1.7	3.2–4.8×1.6–1.9	3—8×2—4 (4.8×2.5)
	Cladosporium (μ)				4—22×2—4 (9.2—2.9)
	Cephalosporium (μ)		15×4	8—12.8×3.2—4	4—11×2—4 (7.2—2.9)
Conidiophore	(Graphium)		1 mm. long	2 mm. long	140–740×8–55 μ

Remarks: In brackets, mean values are given.

According to the above statement, the fungus under consideration may be safely identified as *Ceratostomella piceae* MÜNCH.

Host-plants and distribution: The present fungus seems to attack the wood of many kinds of trees in Japan. The host-plants, collected by the writers are given here. The figures in the brackets show the strain number of pure culture isolated from the specimen. Almost all the specimens were collected by the senior writer unless otherwise noted.

On *Pinus densiflora* S. et Z. "Akamatu".

Nakatugawa-mati, Ena-gun, Pref. Gihu, 12, X, 1932 (strain No. 731); Sinmaiko, Mitu-mura, Ibo-gun, Pref. Hyôgo, 7, VI, 1933 (No. 845); Akasaki-mati,

Tôhaku-gun, Pref. Tottori, 15, VI, 1933 (No. 852) and Syôsya-zan, Sosa-mura, Sikama-gun, Pref. Hyôgo, 19, VII, 1933 (No. 968).

On *Pinus parvifolia* S. et Z. "Hime-ko-matu".

Agematu, Nisitikuma-gun, Pref. Nagano, 12, X, 1932 (strain No. 732).

On *Pinus Thunbergii* PARL. "Kuromatu".

Kyû-syôzan, Tottori-si, 12, VI, 1933 (strain No. 851); Akasi Park, Pref. Hyôgo, 12, VI, 1932, by Y. TAKAHASI (Nos. 727, 728); Sin-maiko, Mitu-mura, Ibo-gun, Pref. Hyôgo, 7, VI, 1933 (No. 843); Higasi-yama, Kôbe-si, 21, VII, 1933 (No. 972); Tetukkai-zan, Suma, Kôbe-si, 21, VII, 1933.

On *Chamaecyparis obtusa* S. et Z. "Hinoki".

Syôsya-zan, Sôsa-mura, Sikama-gun, Pref. Hyôgo, 19, VII, 1933 (strain No. 969).

On *Chamaecyparis pisifera* ENDL. "Sawara".

Agematu, Nisitikuma-gun, Pref. Nagano, 12, X, 1932 (strain Nos. 740, 742).

On *Picea Glehni* MAST. "Aka-ezomatu".

Sapporo, Hokkaidô, VIII, 1931, by S. KAMEI.

On *Picea jezoensis* CARR. "Ezomatu".

Sapporo, Hokkaidô, VIII, 1931, by S. KAMEI.

On *Acer pictum* THUNB. "Itaya-kaede".

Agematu, Nisitikuma-gun, Pref. Nagano, 12, V, 1932.

On *Betula japonica* SIEB. "Sirakaba".

Agematu, Nisitikuma-gun, Pref. Nagano, 12, X, 1932.

On *Kalopanax ricinifolium* MIQ. "Hari-giri".

Agematu, Nisitikuma-gun, Pref. Nagano, 12, X, 1932.

On *Magnolia hypoleuca* S. et Z. "Hoho-no-ki".

Agematu, Nisitikuma-gun, Pref. Nagano, 12, X, 1932.

On *Prunus indica* THUNB. "Mizu-zakura".

Agematu, Nisitikuma-gun, Pref. Nagano, 12, X, 1932.

On *Quercus glandifolia* BLUME. "Ko-nara".

Agematu, Nisitikuma-gun, Pref. Nagano, 12, X, 1932.

VII. Physiology of the Fungus.

The relations between the mycelial growth of *C. piceae* MÜNCH and the culture temperature as well as the hydrogen-ion concentration of culture media will be stated in separate papers in comparison with those of *C. pini* and *C. ips*. Therefore only the following two items are here dealt with.

1. Characteristics of the Fungus on Culture Media.

According to the method described in the first paper of this series, the present fungus was grown on various culture media. The growth at 25°C. after one and three weeks incubation, respectively, is shown in the following two tables:

Table VI.

Summarized Characteristics of *Ceratostomella piceae* 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 conidium	
					Color name	Degree	Graphium	Cephalosporium
		mm.						
Malt-extract agar (3%)	No. 727	35	−	T	Colorless	−	++	+
	729	38	+	T	Ecru-drab	++	++	+
	734	35	+	T	Colorless	−	++	+
	740	35	+	T	„	−	++	+
	746	42	+	T	Drab	++	+++	+
	595	18	+	T	Dark olive	++	−	++
Rice-straw decoction agar	No. 727	33	++	C	Clove brown	++	+++	+
	729	32	+++	Ct	Pale drab-gray	+++	+	++
	734	32	+++	Ct	„	+++	+++	+
	740	32	++	Ct	Drab-gray	++	++++	+
	746	30	++++	Ct	Hair brawn	+	++++	+
	595	14	++	C	Deep grayish olive	++	−	+++
Dried apricot agar	No. 727	35	+++	Ct	Vinaceous buff	+++	+++	+
	729	36	+++	T	Ecru-drab	++	++	+
	734	36	++	T	Vinaceous buff	++	+++	+
	740	38	+	T	Light drab	+	+++	+
	746	35	++	T	Natal brown	++	++	+
	595	17	++	C	Deep grayish olive	++	−	++++
Onion-soja agar	No. 727	34	+	C	Colorless	−	−	++++
	729	35	+	C	„	−	−	++++
	734	35	+	C	„	−	−	++++
	740	34	+	C	„	−	+	++++
	746	32	++	C	„	−	−	++++
	595	13	+++	C	Deep grayish olive	+++	−	+++

Table VI. (Continued.)

Culture media	Fungus strains	Radial growth of colonies	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of conidium	
					Color name	Degree	Graphium	Cephalosporium
Potato-glucose agar	No. 727	42 mm.	###	Ct	Smoke gray	###	####	++
	729	39	####	Ct	"	++	++	++
	734	42	####	Ct	"	###	####	+
	740	42	###	Ct	Deep grayish olive	++	###	+
	746	33	####	Ct	"	++	++	+
	595	20	++	C	"	###	—	####
Bouillon agar	No. 727	24	###	Ct	Colorless	—	—	###
	729	24	+	T	"	—	—	###
	734	26	++	T	"	—	—	###
	740	27	+	Ct	"	—	+	###
	746	25	++	Ct	"	—	—	###
	595	13	+	C	Buffy brown	+	—	###
Hopkin's agar	No. 727	+	—	Tt	Colorless	—	—	—
	729	+	—	Tt	"	—	—	—
	734	+	—	Tt	"	—	—	—
	740	+	—	Tt	"	—	—	—
	746	+	—	Tt	"	—	—	—
Currie's agar	No. 727	+	—	Tt	Colorless	—	—	—
	729	+	—	Tt	"	—	—	—
	734	+	—	Tt	"	—	—	—
	740	+	—	Tt	"	—	—	—
	746	+	—	Tt	"	—	—	—
Steamed potato cylinder	No. 727	###	++	C	Colorless	—	+	####
	729	###	++	C	"	—	###	###
	734	++	+	C	"	—	+	+
	740	###	++	C	Smoke gray	++	###	+
	746	###	++	C	"	++	+	+
Steamed rind of water-melon	No. 727	####	+	T	Fuscous	+	####	###
	729	####	+	T	Colorless	—	####	++
	734	####	+	Tt	"	—	####	+
	740	####	+	T	"	—	#####	++
	746	###	++	C	Smoke gray	++	+	+

Table VI. (Continued.)

Culture media	Fungus strains	Radial growth of colonies	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of conidium	
					Color name	Degree	Graphium	Cephalosporium
Steamed block of pine	No. 727	###	—	Tt	Colorless	—	##	+
	729	##	—	Tt	„	—	##	+
	734	##	—	Tt	„	—	##	+
	740	##	—	Tt	„	—	##	+
	746	##	—	Tt	„	—	##	+
Steamed block of oak	No. 727	###	—	Tt	Colorless	—	##	+
	729	###	—	Tt	„	—	##	+
	734	##	—	Tt	„	—	###	+
	740	##	—	Tt	„	—	##	+
	746	##	—	Tt	„	—	##	+

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 the formation and minus 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.

Table VII.

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

Results after three weeks' culture at 25°C.

Culture media	Fungus strains	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of		
				Color name	Degree	Graphium conidium	Cephalosporium conidium	Perithecium
Malt-extract agar (3%)	No. 727	—	T	Buffy brown	+	###	+	—
	729	+	T	Clove brown	##	##	+	+
	734	+	T	„	##	##	+	##
	740	—	T	Olive brown	+	###	+	—
	746	+	T	Clove olive	##	##	+	+
	595	+	C	Dark olive	##	—	##	—

Table VII. (Continued.)

Culture media	Fungus strains	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of		
				Color name	Degree	Graphium conidium	Cephalosporium conidium	Perithecium
Rice-straw decoction agar	No. 727	+	T	Clove brown	+	###	+	−
	729	###	Ct	Olive brown	+	###	##	−
	734	##	T	„	##	###	+	−
	740	+	T	„	+	###	+	−
	746	###	T	Grayish olive	###	###	+	−
	595	##	C	Deep olive	###	−	###	−
Dried apricot decoction agar	No. 727	+	T	Olive brown	+	###	+	##
	729	##	Ct	„	##	###	+	+
	734	+	T	Dark olive	##	###	+	##
	740	+	T	„	##	###	+	+
	746	##	T	Clove brown	##	###	+	##
	595	###	C	Dark olive	###	−	###	−
Onion-soja agar	No. 727	##	Ct	Dark olive	###	####	####	−
	729	##	Ct	Buffy brown	##	###	###	−
	734	+	Ct	„	##	###	###	−
	740	##	Ct	Dark grayish olive	##	###	###	−
	746	###	Ct	Dark olive	+	###	###	−
	595	###	C	„	###	−	###	−
Potato-dextrose agar	No. 727	##	Ct	Deep grayish olive	##	###	##	−
	729	###	Ct	„	###	##	##	+
	734	##	Ct	„	###	###	+	+
	740	+	Ct	„	##	###	+	−
	746	###	Ct	Dark olive	##	##	+	+
	595	###	C	„	###	−	###	−
Bouillon agar	No. 727	##	Ct	Colorless	−	−	###	−
	729	+	Ct	„	−	−	###	−
	734	+	Ct	„	−	−	###	−
	740	+	Ct	„	−	+	###	−
	746	+	Ct	„	−	−	###	−
	595	−	C	Deep olive	+	−	##	−

Table VII. (Continued.)

Culture media	Fungus strains	Formation of aerial mycelium	Characteristics of colonies*	Color of colonies**		Formation of		
				Color name	Degree	Graphium conidium	Cephalosporium conidium	Perithecium
Hopkin's agar	No. 727	−	Tt	Colorless	−	−	+	−
	729	−	Tt	"	−	−	+	−
	734	−	Tt	"	−	−	+	−
	740	−	Tt	"	−	−	+	−
	746	−	Tt	"	−	−	+	−
Currie's agar	No. 727	−	Tt	Colorless	−	−	+	−
	729	−	Tt	"	−	−	+	−
	734	−	Tt	"	−	−	+	−
	740	−	Tt	"	−	−	+	−
	746	−	Tt	"	−	−	+	−
Steamed potato cylinder	No. 727	−	C	Olive brown	+	+	+	−
	729	+	C	"	+	++	+	−
	734	+	C	"	+	+++	++	−
	740	−	Ct	Dark grayish olive	+++	++++	+	−
	746	+	Ct	Clove brown	+	++	++	−
Steamed rind of water-melon	No. 727	+	T	Dark olive	+	++++	++	−
	729	−	T	Colorless	−	++++	++	−
	734	+	T	"	−	+++	+++	−
	740	+	T	Dark olive	++	++++	+++	+
	746	−	T	Colorless	−	++++	++	−
Steamed block of pine	No. 727	−	Tt	Colorless	−	+++	+	−
	729	−	Tt	"	−	+++	+	++
	734	−	Tt	"	−	+++	+	++
	740	−	Tt	"	−	+++	+	+
	746	−	Tt	"	−	+++	+	++
Steamed block of oak	No. 727	+	Tt	Buffy brown	+	+++	+	+
	729	+	Tt	Clove brown	++	++	+	++
	734	+	Tt	"	++	+++	+	+++
	740	+	T	"	++	++++	+	++
	746	+	T	"	++	++++	+	++

Remarks: The same is this table with Table VI.

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

Effect of free oxygen on the growth of the fungus was tested by the method given in the foregoing report of this series. The test-tube culture of malt-extract agar inoculated with this fungus was inserted into a large tube, in which the free oxygen was absorbed by alkaline solution of pyrogallol. Thus prepared test-tubes were kept at 24°C. for three and seven days, respectively. The result, which is given in Table VIII, shows that the fungus can not grow at all without free oxygen.

Table VIII.

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

Temperature tested: 24°C.

Fungus strains	After 3 days		After 7 days	
	Without free oxygen	Control	Without free oxygen	Control
No. 727	No growth	Good growth, colonies 16 mm. in diameter	No growth	Colonies 34 mm. in diameter
746	No growth	Good growth, colonies 17 mm. in diameter	No growth	Colonies 35 mm. in diameter

In regard to the conidium germination, the effect of free oxygen was tested. It does not take place under anaerobic condition.

VIII. Disinfection Experiments of the Fungus.

1. Thermal Death Points of the Conidia.

The fungus conidia, developed on the culture, were collected and suspended in water. The conidium suspension was immersed in water at various temperature, after the method shown in the previous paper. According to the result, which is given in Table IX, the conidia lose the vitality with 10 minutes' immersion in water at 52°C. or 15 minutes at 50°C.

(See Table IX on next page.)

2. Experiments to Kill the Conidia by Disinfectants.

In this experiment, one or two drops of the concentrated conidium suspension were added to the solutions of corrosive sublimate, copper sulphate, uspulun, formalin in various strength. After one, three, six and 24 hours' immersion, two loopfuls of the solutions were transferred to 3% malt-extract agar. After one week incubation, the formation of the fungus colonies was examined. The results are given in Table X.

Table IX.

Thermal Death Points of Conidia of *Ceratostomella piceae* Münch.

Fungus strains	Period of immersion	Control	Temperature of water (C.)					
			44°	46°	48°	50°	52°	54°
No. 727	5 minutes	+	+	+	+	+	−	−
	10 „	+	+	+	+	±	−	−
	15 „	+	+	+	+	−	−	−
	20 „	+	+	+	+	−	−	−
No. 746	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.

Table X.

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

Temperature of the solutions tested: 27°C.

Corrosive sublimate, $HgCl_2$.

Fungus strains	Period of immersion	Dilution						Control
		1 : 1,000	1 : 2,000	1 : 4,000	1 : 6,000	1 : 8,000	1 : 10,000	
No. 727	1 hour	−	−	±	+	+	+	+
	3 hours	−	−	−	+	+	+	+
	6 „	−	−	−	−	−	+	+
	24 „	−	−	−	−	−	−	+
No. 746	1 hour	−	−	±	+	+	+	+
	3 hours	−	−	−	+	+	+	+
	6 „	−	−	−	−	±	+	+
	24 „	−	−	−	−	−	−	+

(Continued to the next page.)

Cupper sulphate, $CuSO_4 5HO$.

Fungus strains	Period of immersion	Dilution					Control
		1 : 25	1 : 50	1 : 100	1 : 200	1 : 400	
No. 727	1 hour	−	+	+	+	+	+
	3 hours	−	−	+	+	+	+
	6 „	−	−	+	+	+	+
	24 „	−	−	−	+	+	+
No. 746	1 hour	−	+	+	+	+	+
	3 hours	−	−	+	+	+	+
	6 „	−	−	±	+	+	+
	24 „	−	−	−	+	+	+

Uspulun.

Fungus strains	Period of immersion	Dilution					Control
		1 : 100	1 : 200	1 : 400	1 : 800	1 : 1,600	
No. 727	1 hour	−	−	+	+	+	+
	3 hours	−	−	−	+	+	+
	6 „	−	−	−	−	+	+
	24 „	−	−	−	−	+	+
No. 746	1 hour	−	−	±	+	+	+
	3 hours	−	−	±	+	+	+
	6 „	−	−	−	+	+	+
	24 „	−	−	−	−	+	+

Formalin.

Fungus strains	Period of immersion	Dilution					Control
		1 : 100	1 : 200	1 : 400	1 : 800	1 : 1,600	
No. 727	1 hour	−	−	+	+	+	+
	3 hours	−	−	+	+	+	+
	6 „	−	−	±	+	+	+
	24 „	−	−	−	−	−	+
No. 746	1 hour	−	−	+	+	+	+
	3 hours	−	−	+	+	+	+
	6 „	−	−	−	+	+	+
	24 „	−	−	−	−	−	+

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

3. Experiments to Check the Fungus Growth by Disinfectants.

Checking efficacy of some disinfectants against the fungus growth was also examined in the present studies. The disinfectants were diluted with sterilized malt-extract solution aseptically in test-tubes. After inoculating the fungus in these solutions and keeping for 7 days at 27°C., the fungus growth was examined. According to the result, given in Table XI, the fungus growth was not observed in 1 : 10,000 solution of corrosive sublimate or 1 : 5,000 solution of copper sulphate.

Table XI.

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

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. 727	Cupper sulphate	−	−	+	+	+	+	+	+	+
	Corrosive sublimate	−	−	−	+	+	+	+	+	+
	Uspulun	−	−	−	+	+	+	+	+	+

Remarks: 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 third report on the sap stains of wood in Japan, and deals with the blue-staining fungus of pine wood, *Ceratostomella piceae* MÜNCH.

2) In Japan the fungus is found on wood not only of *Pinus densiflora* S. et Z. (=Akamatu, Japanese name), *Pinus parvifolia* S. et Z. (=Hime-ko-matu), *Pinus Thunbergii* PARL. (=Kuromatu), *Picea Glehni* MAST. (=Aka-ezomatu) and *Picea jezoensis* CARR. (=Ezomatu) but also of *Chamaecyparis obtusa* S. et Z. (=Hinoki), *Chamaecyparis pisifera* ENDL. (=Sawara), *Acer pictum* THUNB. (=Itaya-kaede), *Betula japonica* SIEB. (=Sirakaba), *Kalopanax ricinifolium* MIQ. (=Hari-giri), *Prunus indica* THUNB. (=Mizu-zakura) and *Quercus glandifolia* BLUME (=Ko-nara).

3) The hyphae of this fungus penetrate, like those of *Ceratostomella ips* and *C. pini*, through the parenchymatous cells of medullary rays from the cortex toward the center, while they grow through the resin ducts and tracheids longitudinally and through bordered pits in the tangential direction.

4) This fungus produces comparatively large, long-beaked perithecia, and for this reason it is different from *C. pini*. The ascospores are reniform and not truncately cylindrical, in this regard the fungus distinguishes itself from *C. ips*.

5) This fungus produces the conidia in the following three types: (1) Graphium, (2) Cladosporium and (3) Cephalosporium. The conidia in the first type are produced on large composite conidiophores, the synnemata, or the graphium. Those of the second type are comparatively large, long elliptical, and formed on the end of young hyphae verticillately. Further the conidia are also formed in a ball as in the genus Cephalosporium. These conidia are mostly found in the medium and resembling to those of Graphium type in shape.

6) The ascospores and the conidia swell prominently, on the germination.

7) The fungus grows well on the culture media, and the growth rate at a moderate temperature is much smaller than that of *C. pini* and *C. ips*. Without free oxygen neither the conidium germination nor the mycelial growth takes place.

8) The conidia as well as the ascospores are killed by a treatment in water at 52°C. for 10 minutes or 50°C. for 15 minutes, and also by one hour's immersion in 1 : 4,000 solution of corrosive sublimate or 1 : 200 solution of formalin and uspulun. The fungus can no longer grow in malt-extract agar, containing corrosive sublimate or uspulun in the strength of 1 : 10,000 or copper sulphate in 1 : 5,000.

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Explanation of the Plates.

Plate XXV.

Fig. 1. A transverse section of "Kuromatu" (*Pinus Thunbergii* PARL.), attacked by *Ceratostomella piceae* MÜNCH. The pine tree was growing in a sand dune in the Banzan State Forest in Pref. Tottori, and cut on June 12, 1933. Showing the blue stain of the sap-wood.

Fig. 2. Two perithecia of *Ceratostomella piceae*, produced on pine wood, collected at Akasaki, Pref. Tottori, on June 15, 1933. (×75)

Fig. 3. Two graphia of *C. piceae*, developed on pine wood (*Pinus densiflora*), collected at Nakatugawa-mati, Pref. Gihu. (×150)

Fig. 4. Heads of the same ones. Showing the large bundles of numerous conidiophores. (×350)

Plate XXVI.

Sections of blue-stained sap-wood of pine tree (*Pinus densiflora*), attacked by *Ceratostomella piceae* MÜNCH. Showing the 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 the original size. (×250)

Fig. 1. Transverse section of blue-stained sap-wood of pine. Showing the 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 XXVII.

Conidia and conidiophores of *Ceratostomella piceae* MÜNCH, developed on potato-dextrose agar, and the conidium germination. The figures were drawn from water-mounted preparations with an aid of camera lucida under Zeiss K 10× and Apochromat 90×, and were reduced to 0.68 times of the original size. (×1,500)

Fig. 1-2. Conidia and conidiophores of *C. piceae*, in Cladosporium-type. The conidia are produced verticillately and catenulately on the conidiophores.

Fig. 3. Conidia in Cladosporium-type.

Fig. 4. Conidia and conidiophores in Cephalosporium-type. The conidia are produced in balls on tips of the hyphal branches.

Fig. 5. Conidia produced on a graphium, developed on potato-dextrose agar.

Fig. 6. Germination of conidia, in 3% malt-extract solution after 24 hours' incubation at 24°C.

Plate XXVIII.

Perithecia, asci, ascospores and conidia of *Ceratostomella piceae* MÜNCH, drawn from water-mounted preparations with an aid of camera lucida.

Fig. 1. Conidia in Cladosporium-type, developed on potato-dextrose agar. (×1,500)

Fig. 2. Two perithecia. (×150)

Fig. 3. Asci. (×1,500)

Fig. 4. Ascospores. (×1,500)

Fig. 5-7. Pieces of the perithecial beaks. (×500)

Plate XXIX.

Germination of the ascospores of *Ceratostomella piceae* MÜNCH. The figures were drawn from water-mounted preparations with an aid of camera lucida under Zeiss K 10× or K 20× and Apochromat 40×.

Fig. 1. Germinations of ascospores in 3% malt-extract solution after 14 hours' incubation at 27°C. (×1,500)

Fig. 2. Germinations of ascospores in 3% malt-extract solution after 20 hours' incubation at 27°C. (×700)

Fig. 3. Secondary conidia produced on the germ-tubes from ascospores, kept in 3% malt-extract solution at 27°C., and their germination. (×1,500)

PLATE XXV.

Fig. 1.

Fig. 2.

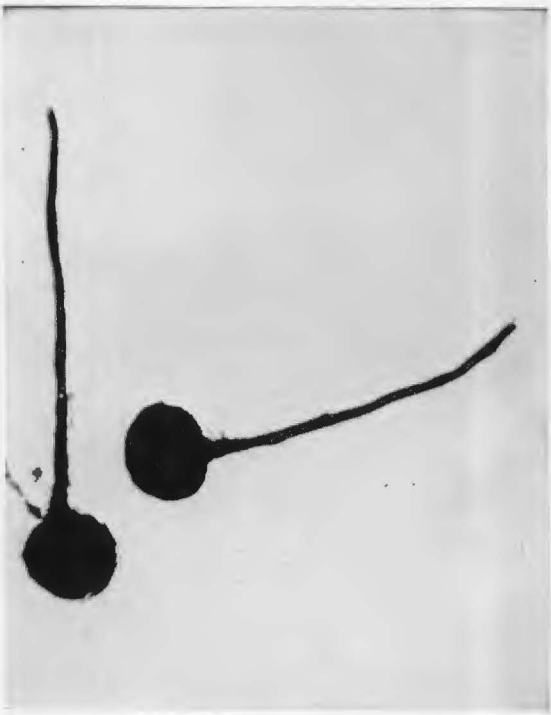


Fig. 3.

Fig. 4.

PLATE XXVI.

Fig. 1.

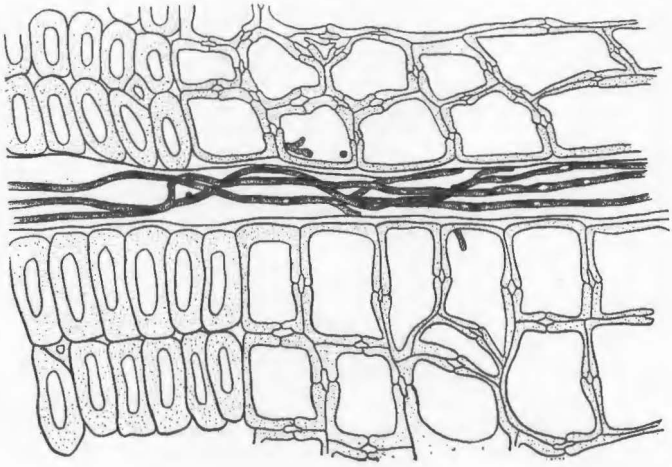


Fig. 2.

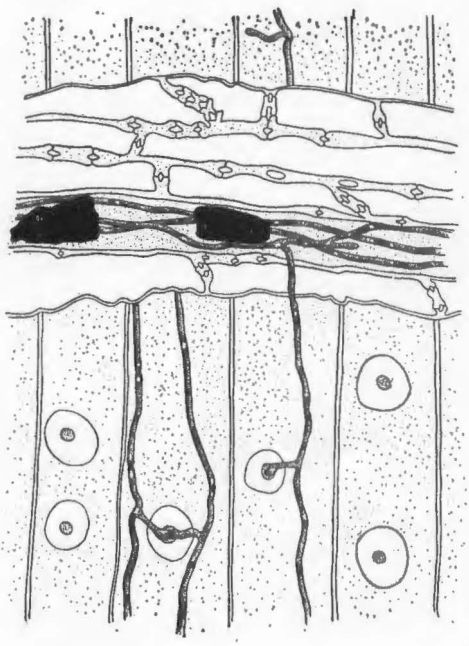


Fig. 3.

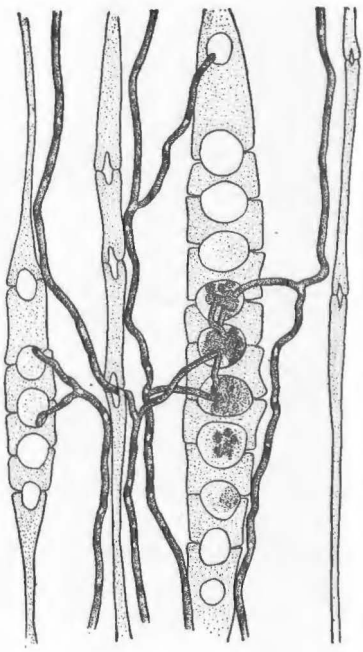


PLATE XXVII.

Fig. 1.

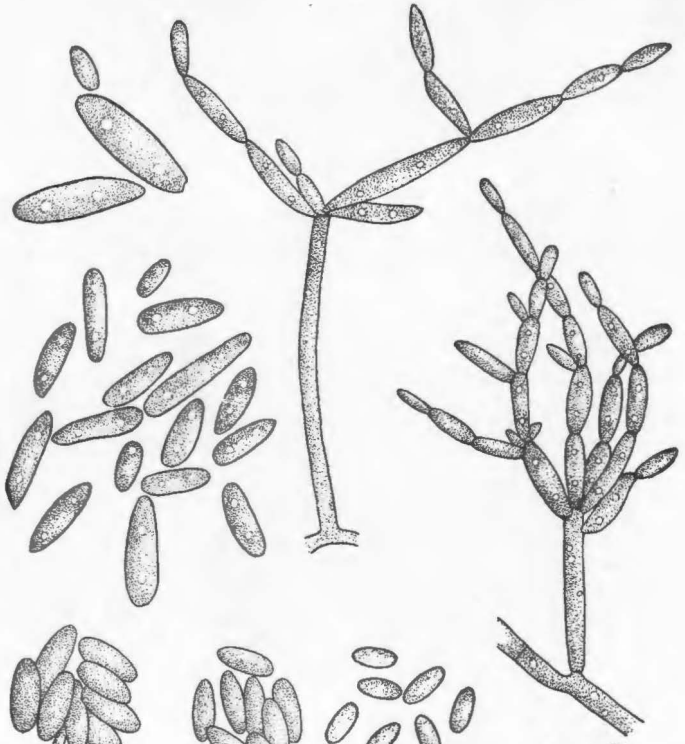


Fig. 3.

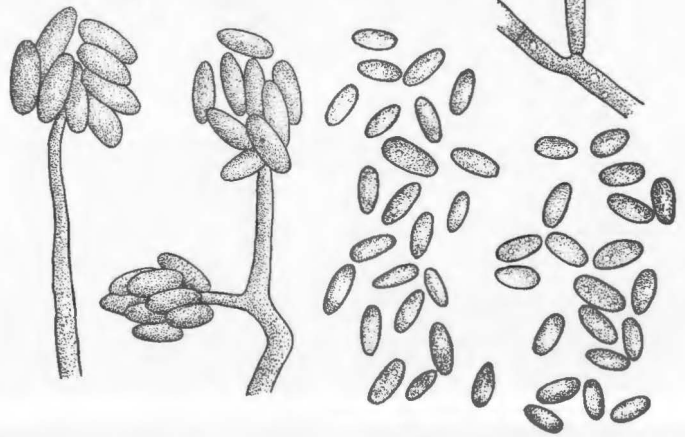


Fig. 4.

Fig. 5.

Fig. 6.

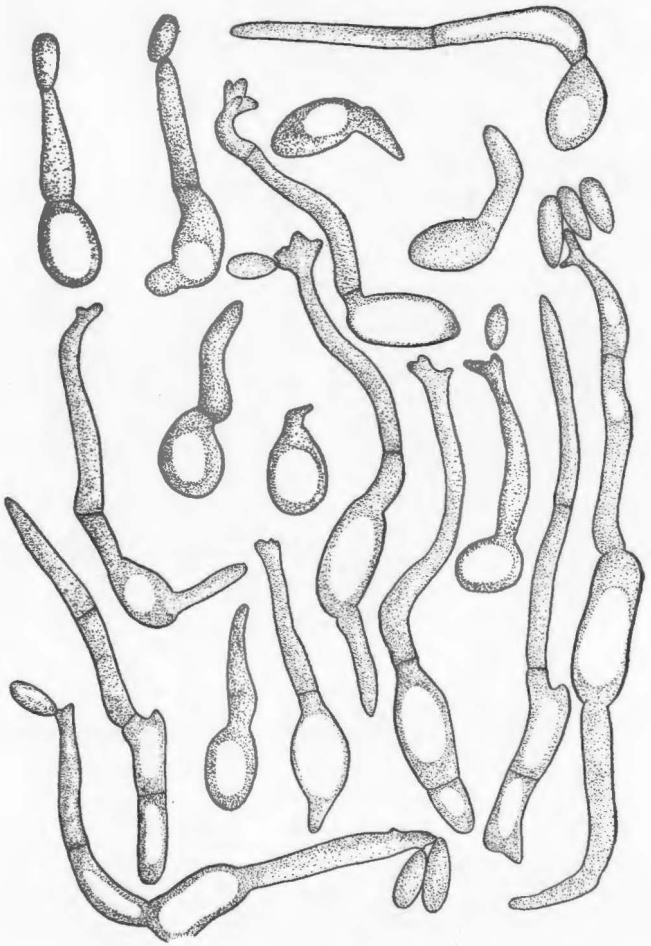


Fig. 2.

PLATE XXVIII.

Fig. 1.

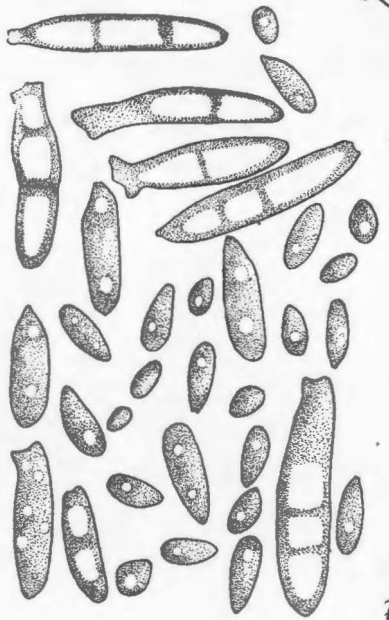
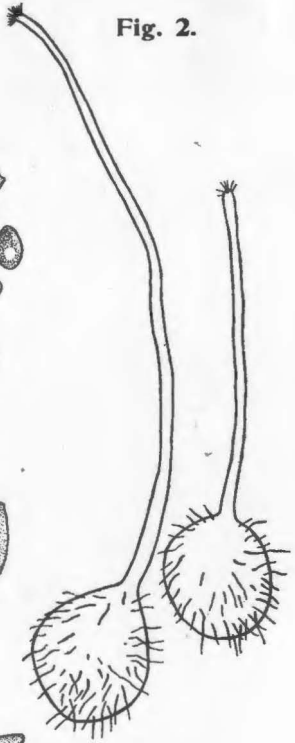


Fig. 2.



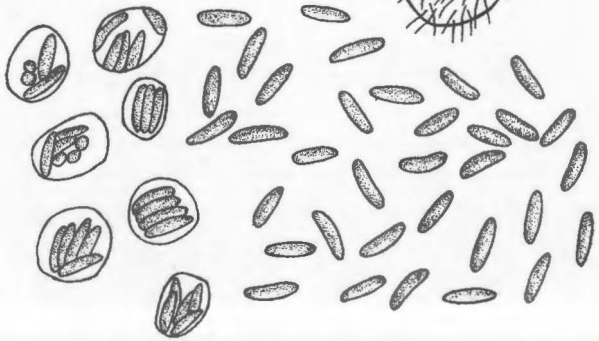


Fig. 3.

Fig. 4.

Fig. 5.

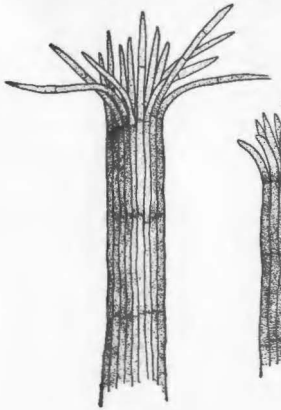


Fig. 6.

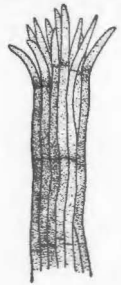


Fig. 7.

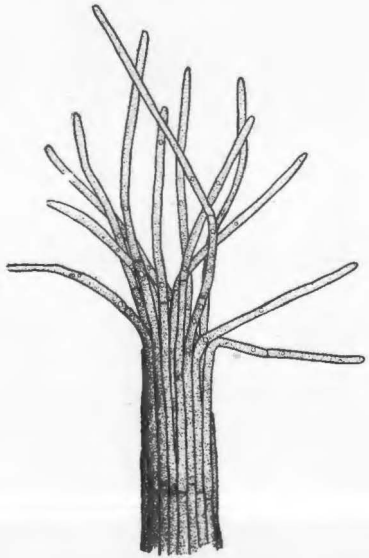


PLATE XXIX.

Fig. 1.

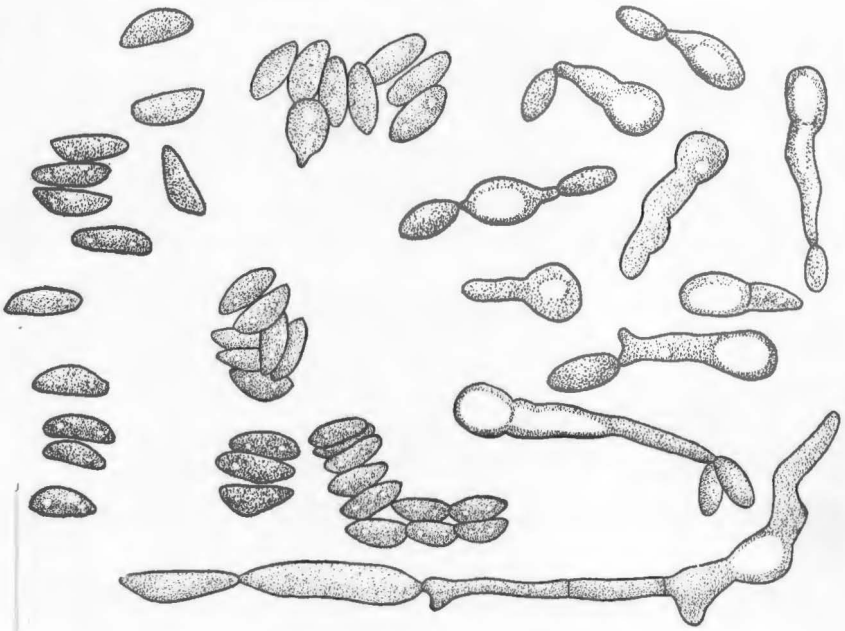


Fig. 2.

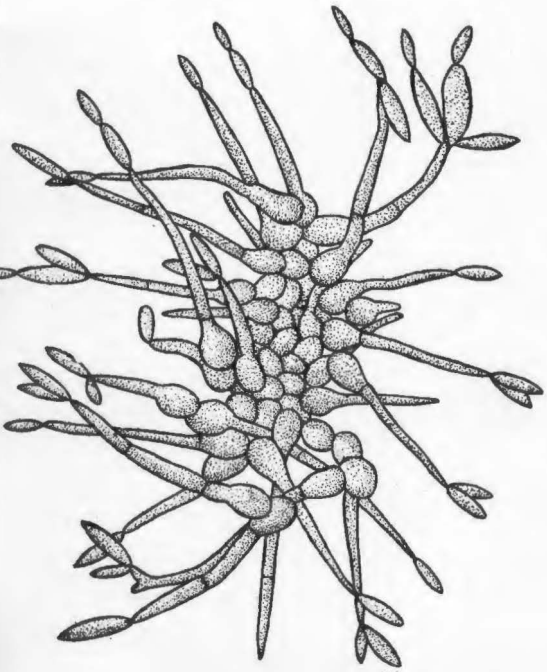


Fig. 3.