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

Studies on *Ceratostomella ips* Rumbold, the Cause of a Blue Stain of Pine Trees in Western Japan.

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Contents.

I. Introduction.

In January, 1931, the senior writer was asked by the Local Forestry Bureau of Osaka to study the sap stains of pine trees, which were prevalent in the western districts of Japan. Then the diseased materials of pines (*Pinus densiflora* Sieb. et Zucc. and *P. Thunbergii* Parl.) collected near Himezi, Hyôgo prefecture, were sent to the writers. The materials were infested with bark beetles, and the sap wood was blue-stained.

The sap stain of the pines not only reduced the market value of the timber by the discoloration but also caused the death of living pine trees on their roots or, at least, promoted the death considerably. Recently the disease became very prevalent in the Suma Imperial Household Forestry, the Akasi Prefectural Park and many state-forests near Himezi. By the attack of the trouble, two or three hundred years old pine trees (*Pinus densiflora* Sieb. et Zucc. and *Pinus Thunbergii* Pers.) was killed. Therefore the prevalence of the sap stain became one of the serious problems, not only on the economical points of views but also of ornamental plantation in the famous places. The writers commenced to study the trouble, so that they may find the suitable means for the control. The chief cause of death in pine trees at the above stated places, seemed to be attributed to a *Ceratostomella* fungus, associated with bark beetles. The writers' study revealed that the fungus was identical with the species *Ceratostomella ips*, newly described by C. T. Rumbold (1931) in America; also their survey showed the occurrence of many other species of *Ceratostomella* and *Graphium* in Japan, causing blue stains of sap wood in pines and other trees. But *Ceratostomella ips* Rumbold always associated with the blue stain of sap wood of the living pine trees on the roots in the above said districts. Therefore the writers' interest traced the first place to *Ceratostomella ips*.

Studies on the relation of the environmental factors and the damage of bark beetles with the occurrence of blue stains on sap wood as well as comparative studies of various forms of the blue-staining fungi are in progress in their laboratory. The present paper, however, is the first report for the series of studies on the sap stains of wood in Japan, and it deals chiefly with the blue-staining fungus, *Ceratostomella ips* Rumbold.

The writers wish to express their thanks to Messrs. K. Suzuki, K. Ônakadô and T. Matida of the Local Forestry Bureau of Osaka, for their suggestions in the investigation. The writers are greatly indebted to Professor J. Westerdijk, Baarn, Holland and Dr. C. T. Rumbold, Bureau of Plant Industry, U. S. Dept. of Agriculture, who supplied them pure cultures of the species *Ceratostomella* and related ones. Thanks are also due to Professor T. Lagerberg, Professor E. E. Hubert and Messrs. M. Kasai, H. Matsumoto, T. Kondo and T. Mine for help in the course of the investigation.

II. Historical Review.

An adequate review of Europian and American literature concerning the blue stains of sap wood caused by species of *Ceratostomella* and affiliated ones and their association with insect damages may be had by referring to the papers of MÜNCH (1907), HEDGECOCK (1906), LAGERBERG, LUNDBERG and MELIN (1927), RUMBOLD (1931), etc. Therefore only a brief review on the literature more closely connected with the writers' present investigation will be given.

In Japan, M. KASAI (1916) was the first to describe the occurence of the blue stain. His studies were made on blue-stained sap wood of oak (*Quercus glandiflora* BLUME) and pines (*Pinus* spp.) in transit. The fungus isolated by him in culture was ascribed to the species *Ceratostomella pilifera* (FR.) WINTER.

K. TANAKA (1926) mentions the general characteristics of the blue stains on the coniferous trees and the blue-staining fungus, *Ceratostomella pilifera*.

D. NUMATA (1931) reports the occurrence of a sap stain of "Ezomatu" (*Picea jezoensis* CARR.), associated with "Yatuba-kosinkui" (*Ips japonicus* NIIJIMA) in Sagalien and a blue stain of *Pinus Thunbergii* PERS. associated with "Matukawa-no-kosinkui" (*Ips proxinus* EICHH. ?) in the Akasi Park. Upon his observation of the diseases, he mentions triangular relations among the dessication, the bark beetles and the blue stains, regarding the causes of the death on living trees.

K. UYEDA and K. NAGAYAMA (1932) report the damage of "Ezomatu" (*Picea jezoensis* CARR.) caused by *Ips japonicus* NIIJIMA in Sagalien. Some useful observations were made on meteological and other environmental conditions influencing the occurrence of the trouble. They also mention the close association between the damage of the insect and the occurrence of a blue-staining fungus (*Ceratostomella* sp. ?) in sap wood.

In the direct connection with the fungus under consideration only an article has been found, so far the writers are aware. C. T. RUMBOLD (1931) describes in America a new blue-staining fungus under the name *Ceratostomella ips*. She reports a close association of the blue-staining fungus with the bark beetles belonging to *Ips*.

The senior writer (NISIKADO, 1932) read a preliminary paper on this subject at the 8th general meeting of the Japanese Association for the Advancement of Science held in Nagoya in October, 1932.

III. General Features of the Blue-Stained Wood on Pines.

1. Macroscopical features.

The general features of the blue-stained wood on pines are not always the same, but vary according to the causal agencies. The writers will mention

here regarding to the blue-stained wood caused by *Ceratostomella ips* RUMBOLD, which seems to be the main cause of the death to standing pine trees in western Japan. Cross sections of the diseased wood show more or less bluish color. As shown in Plate XLVI, Fig. 1—2, the discolored lesion is generally wedge-shaped, tapering toward the center of the tree. The discolored portion spreads from the outer part or cortex toward the center of wood usually along the medullary rays, and wedge-shaped discoloration is resulted. In the advanced stages, however, the discoloration covers all over the section and the surface is uniformly blue or bluish gray. The annual rings of the autumn wood are darker colored.

In longitudinal sections of stained sap wood the general appearance differs according to the tangential and radial. In radial longitudinal sections or splints, many fine, closely arranged, dark brown lines run radially. The wood seems bluish.

In tangential, longitudinal sections or splints, bluish or brownish discoloration covers all over the surface. More close observation reveals that many small spots are closely distributed in the discolored part. The small spots are the cross cut ends of discolored medullary rays, as indicated by studies under a microscope or a magnifying lens.

Further studies show the presence of many closely arranged, small points of about 1/4 mm. in size, on the blue-stained wood. These black points are the perithecia of the causal fungus.

In the above mentioned districts, another blue stain occurs, with which the writers will give further reports in near future. Regarding this second blue stain the symptom differs somewhat from the former.

For the sakes of comparison the symptom of the second blue stain is here given briefly; viz., on radial, longitudinal sections or splints many dark colored radial lines are observed. But the dark lines are not arranged so closely as in the former, but much sparcely and are more distinct. Between these defined black lines, however, many light colored lines are observed.

Upon tangential, longitudinal splints, many distinct, more or less broad, dark lines are also observed in the longitudinal direction.

2. Microscopical features.

Small pieces of the blue-stained wood of pine, were soaked in a mixture of glycerine and alcohol overnight. Then microtome sections from paraffin imbedding as well as freehand sections were cut and studied under a microscope.

(1) TRANSVERSE SECTION.

Cross sections of blue-stained wood show dark hyphae of the fungus penetrating radially through the medullary rays, and cross sections of hyphae in resin ducts and in tracheidal cells as shown in Plate IL, Fig. 1 and Plate L, Fig. 1—2. The hyphae are dark brown and distinct; therefore the dark lines, even in

an unstained preparation they are clearly discernible, as shown in the photomicrograph in Plate IL, Fig. 1. The hyphae seem seldem to penetrate the walls of tracheidal cells transversely but longitudinally through the tracheids; therefore, the cross sections of the hyphae are observed as small black points.

In tracheidal cells the hyphae grow from a cell to its neighbouring ones through bordered pits, and very rarely through cell walls other than bordered pits. As shown in Plate IL, Fig. 1 many hyphae are seen in resin ducts. Therefore they are also a leading object of the attack of the hyphae as the parenchymatous cells of medullary rays.

(2) LONGITUDINAL SECTION.

In radial, longitudinal sections, as shown in Plate IL, Fig. 2 and Plate L, Fig. 3—4, many dark hyphae run radially through the parenchymatous cells of medullary rays; but few hyphae in the tracheidal cells, which form the upper and lower margins of the ray. The hyphae are also running lengthwise in the tracheids of the woodened tissues, as shown in Plate IL, Fig. 2 and Plate L, Fig. 4.

In the tangential longitudinal sections, as shown in Plate IL, Fig. 3, Plate L, Fig. 5 and Plate LI, Fig. 4—5, branches of the hyphae running through medullary rays extend into the tracheidal cells and grow there lengthwise.

As already described, the hyphae of this fungus grow at first radially through parenchymatous cells of medullary rays from the outside toward the center or to the heart wood of trees. Meanwhile branches of hyphae proceed from medullary rays into resin ducts or to tracheids, and then run through the tissues in the upper and lower directions. They grow also through bordered pits of tracheids in the tangential direction of trees. Thus the hyphae proceed from the points of the first infection to all directions. They grow, however, best to the radial direction through medullary rays, and wedge-shaped discolorations are resulted.

The hyphae of the present fungus are dark greenish brown or blackish. The infected host cells are not discolored; so the pigments of the hyphae seem to be insoluble to the solutions or water, contained in the wood cells.

The above mentioneds are the blue stain of "Akamatu" (*Pinus densiflora* SIEB. et ZUCC.) caused by the fungus under consideration. The blue stain of "Kuromatu" (*Pinus Thunbergii* PARS.) are similar to those of the former in the general features. Therefore the descriptions of the latter are omitted here.

IV. The Blue-Staining Fungus.

1. Source of cultures studied.

The cultures of the blue-staining fungus studied in the present investigation are as follows:

Strain No. 442: The strain was isolated from blue-stained sap wood of "Akamatu" (*Pinus densiflora* SIEB. et ZUCC.), sent to the senior writer on January 16, 1932, from the Himezi Local Forestry Office. The material was collected in the Terayama state-forest near Himezi; and was infested by bark beetles, which seemed to belong to *Ips*.

Strain No. 443: It is another strain of the same fungus, isolated from another material, sent by the office on the same date.

Strain No. 581: It was isolated from blue-stained sap wood of "Kuromatu" (*Pinus Thunbergii* PERS.) sent to the senior writer from Himezi Local Forestry Office on February 19, 1932.

Strain No. 683: It was isolated from blue-stained sap wood of "Akamatu" (*Pinus densiflora* SIEB. et ZUCC.), which was collected on August 18, 1932, in Nakatani-mura, Kawabe-gun, Hyôgo Prefecture, by the senior writer.

The following two strains of *Ceratostomella ips* RUMBOLD served the comparison with the writers' blue-staining fungus. These are the type cultures of RUMBOLD's species. They were sent by her to the writers with cultures of the other species.

Strain No. 705: A type culture of *Ceratostomella ips*, and the strain No. 275 of C. T. RUMBOLD. The fungus was isolated from *Pinus sylvestris* infested with bark beetles, *Ips grandicollis* and *Ips calligraphus*, collected near Chevy Chase, Maryland, in August 1930.

Strain No. 706: Another type culture of *Ceratostomella ips* of RUMBOLD, sent by her as the strain No. 255. The fungus was isolated from *Pinus echinata* infested with the bark beetle, *Ips avulsus*, collected near Asheville, North Carolina, in July 1930.

Many strains of pure cultures of *Ceratostomella* and *Graphium*, which were donated from the Centraal-Buraeu voor Schimmelcultures, Baarn, Holland, through the courtesy of Prof. JOH. WESTERDIJK, were used for comparison.

2. Isolation of fungus.

The method used by the writers for the isolation of the fungus from diseased timber was as follows: With an aid of a flamed scalpel a portion of blue-stained wood to be tested was cut obliquely or transversely, then split lengthwise. Immediately small portions of discolored wood were transferred aseptically to malt extract agar plates. Many of these gave rise to apparently the pure cultures of fungus. Upon abundant sporulation, small pieces of the fungus were used to make spore suspension from which dilution plates were poured. Transfers were made from single colonies to malt agar slants.

Sometimes portions of blue-stained timber cut as above or small pieces of the fungus were transferred aseptically to sterilized blocks of pine wood in test tubes. From the growth on sterilized blocks transfers were made to malt extract agar. Then single spore cultures were started. In many cases better results were secured by the latter method.

3. Morphology of the blue-staining fungus.

(1) Mycelium.

The mycelium of the present fungus in blue-stained wood consists of dark brown or blackish brown, 4—8 μ (average 5 μ) wide hyphae, as already described in foregoing chapter. They penetrate profusely the parenchymatous cells in medullary rays and resin ducts, and less frequently in tracheidal tissues ; also are provided with many septa at an interval of 20 to 90 μ, and somewhat constricted at the septa. (Plate IL, L and Plate LI, Fig. 4—5).

The mycelium developed in culture varies according to the media on which it develops. The hyphae grown on or near the surface of malt extract agar (composed of 3% malt extract, and 2% agar) are 2—3 μ and sometimes 5—6 μ in width. When old they are thick, dark brown or olivaceous black and discernible to naked eyes. The medium in which these hyphae grow, seems jet black. The hyphae submerged in medium are of a light color or sometimes entirely colorless, especially so the hyphae growing deep in the medium. Their presence in the middle of agar is perceived by the development of the dark colored perithecia, but on such a less suitable medium, like "Miduame" agar (composed of 3% "Miduame", a kind of rice jelly, and 2% agar) the color tone of the hyphae is generally light. These hyphae are slender and measure about 2 μ. The hyphal cells are not long, produce budding-like branches, which are roundish, spherical or pear-form. (Plate LII).

When the fungus is inoculated on boiled blocks of pine wood, the hyphae slightly color or sometimes remain entirely colorless. In these cultures, the growth of the hyphae is perceived sometimes only through the formation of dark colored perithecium. The mycelial growth on boiled blocks of oak wood is much better, the colonies being black or jet black. The hyphae developed on this medium are comparatively thick and show a somewhat budding-like form. Young hyphae measure 3—5 μ in thickness. When old, however, they are thick-walled, dark brown and attain 10 to 15 μ in width.

(2) Conidial stage.

i. Conidia and Conidiophores.

When a bit of hyphae of the present fungus is transferred on a medium such as malt extract agar and kept at from 24 to 27°C for 2 or 3 days, copious conidia formation may be observed. The conidia develop at first submerged near the surface, but then they develop also on the surface of medium or on the wall of the culture tubes.

The conidia are borne on erect or slanting hyphae which branch from the mycelium growing in or over the medium. They are produced terminally and detached from the tips, but groups together in an agglutinated mass about that parts of the conidiophores, in a form which resembles *Cephalosporium* (Plate LIV, Fig. 1—3). The conidiophores grow much longer, branch, and their base gradually turn brown, while the slender tips, with conidia, remain hyaline. (Plate

LIV, Fig. 4—6 and Plate LV, Fig. 1—3). Under suitable conditions the conidial mass on a conidiophore becomes larger and produces a large ball, as shown in Plate LIII, Fig. 1. The conidia are, however, never produced on a *Graphium*-like conidiophore, as in the conidial stages of many species of *Ceratostomella*. When young the conidiophore is a simple branch of hyphae, 2—3 μ wide, 20—60 μ long and one or two septated. An old complicated conidiophore attains sometimes 90—100 μ in length, and its thickest cells 5—8 μ in width. (Plate LIV, Fig. 5—6).

The conidia vary in shape from obovate to elliptical or cylindrical with rounded ends. They are hyaline, but often become vacuolate or guttulate when old. (Plate LIV, Fig. 1—3 and Plate LV, Fig. 1 and 4).

Table I.

Variations and Constants for Width of Conidia of *Ceratostomella ips* Rumbold, Developed on Various Culture Media.

Conidia Developed on	Width of Conidia (μ)						Total
	1.5	2	2.5	3	3.5	4	
(1) Pine Wood	9	61	26	4	—	—	100
(2) "Ame" Agar	17	50	61	54	18	—	200
(3) 〃	3	40	63	68	25	1	200
(4) Malt Agar	—	36	86	160	16	2	300
Sum	29	187	236	286	59	3	800

Conidia Developed on	Mean (μ)	Standard Deviation (μ)	Variation Coefficient
(1) Pine Wood	2.13±0.033	0.33±0.011	15.72±0.77
(2) "Ame" Agar	2.46±0.036	0.51±0.017	20.69±0.73
(3) 〃	2.65±0.031	0.44±0.015	16.70±0.58
(4) Malt Agar	2.77±0.023	0.40±0.011	14.36±1.27
Sum	2.58±0.015	0.43±0.007	16.67±0.29

Remarks: 1) Small conidia developed on a sterilized block of pine wood after about 3 weeks' culture at 27°C.
2) Small conidia developed on 3% "Midu-Ame" (rice jelly) agar after 4 weeks' culture at 27°C.
3) Large conidia developed on the above medium.
4) Conidia developed on 3% malt extract agar after 10 days' culture at 27°C.

As shown in Table I, the width of the conidia developed in various culture conditions shows no large variation, ranges 1.5—4 μ and is 2.58±0.015 μ in mean. But the conidia developed on malt extract agar are somewhat thicker than those

developed on the host and the former measures $2.77\pm0.023\,\mu$ and the latter $2.13\pm0.033\,\mu$ in width, respectively. Regarding the length there exist two kinds of conidia: viz., the larger and the smaller ones. The larger conidia measure $7—18\,\mu$ (mean $11.42\pm0.097\,\mu$) and the smaller ones $3—9\,\mu$ (mean $4.82\pm0.045\,\mu$) as shown in Table II. The former type of conidia develops chiefly upon the surface of medium, while the latter is formed not only superficially but also submerged in medium. The both types of conidia can be readily mixed together. An example of the writers' measurement was $3—17\,\mu$ (mean $8.15\pm0.116\,\mu$) long for mixed type of the conidia, as shown in Table II.

Table II.

Variations and Constants for Length of Conidia of *Ceratostomella ips* Rumbold, Developed on Various Culture Media.

Conidia Developed on	Length of Conidia (μ)																Total
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
(1) Pine Block	1	43	**53**	3	—	—	—	—	—	—	—	—	—	—	—	—	100
(2) "Ame" Agar	11	55	**74**	48	9	2	1	—	—	—	—	—	—	—	—	—	200
Sum Sm. = (1)+(2)	12	98	**127**	51	9	2	1	—	—	—	—	—	—	—	—	—	300
(3) "Ame" Agar	—	—	—	—	4	10	17	45	**48**	30	18	16	6	2	2	2	200
(4) Malt Ext. Agar	—	—	—	—	2	10	26	27	**45**	36	27	15	9	1	1	1	200
Sum La. = (3)+(4)	—	—	—	—	6	20	43	72	**93**	66	45	31	15	3	3	3	400
(5) Malt Ext. Agar	1	**39**	34	26	24	26	34	**36**	25	21	15	9	5	3	2	—	300
(6) 〃	2	24	37	**52**	44	33	23	**27**	27	15	8	3	2	3	—	—	300
Sum Mix. = (5)+(6)	3	63	71	**78**	68	59	57	**63**	52	36	23	12	7	6	2	—	600

Conidia Developed on	Mean (μ)	Standard Deviation (μ)	Variation Coefficient
(1) Pine Block	4.58 ± 0.047	0.47 ± 0.022	10.35 ± 0.49
(2) "Ame" Agar	4.94 ± 0.067	0.95 ± 0.032	19.17 ± 0.67
Sum Sm. = (1)+(2)	4.82 ± 0.045	0.78 ± 0.022	16.18 ± 0.46
(3) "Ame" Agar	11.47 ± 0.137	1.94 ± 0.066	16.94 ± 0.59
(4) Malt Ext. Agar	11.36 ± 0.137	1.94 ± 0.066	17.08 ± 0.58
Sum La. = (3)+(4)	11.42 ± 0.097	1.94 ± 0.047	16.99 ± 0.42
(5) Malt Ext. Agar	8.44 ± 0.170	2.95 ± 0.081	34.95 ± 1.10
(6) 〃	7.86 ± 0.158	2.73 ± 0.075	34.72 ± 1.07
Sum Mix. = (5)+(6)	8.15 ± 0.116	2.84 ± 0.055	34.85 ± 0.76

Remarks: 1) Small conidia developed on a sterilized block of pine wood after about 3 weeks' culture at 27°C.

2) Small conidia developed on 3% "Midu-Ame" (rice jelly) agar after 4 weeks' culture at 27°C.

3) Large conidia developed on the above medium.

4—6) Conidia developed on 3% malt extract agar after 10 days' culture at 27°C. The three measurements were made independently from different cultures

ii. Germination of Conidia.

The conidia of the fungus under consideration germinate readily at a moderate temperature in tap water or in nutrient solutions. The conidium germination in 2% malt extract solution after 24 hours' incubation at 24°C. is shown in Plate LIII, Fig. 2. Before the germination conidia swell remarkably and attain 4—6 μ in width. Increase in the conidial length is not so remarkable. The germ tubes are hyaline, 1.5—4 μ wide and septated at an interval of 10—40 μ. Secondary conidia are readily formed not only on the tips of elongated germ tubes but also directly on conidia. Sometimes they are produced in chains or in a budding form. The secondary conidia vary in size, but usually are 3—6 μ long and 1.5—3 μ wide.

(3) PERITHECIAL STAGE.

i. Perithecium.

In nature perithecia of this fungus develop chiefly near the surface of infected pine wood, then they become usually erumpent. On splints or shaved planes of wood, they are discernible, when closely inspected under a magnifying lens. Sometimes they are produced also deep in the wood. The perithecia are of flask-shape and black in color. (Plate XLVII, Fig. 2). The neck or beak of a perithecium varies in length, but not so long as those produced in culture. The length of beak is usually about twice the height of the basal part of a perithecium. At the end of a beak no fringe-like appendages are observed, although they are one of the leading characteristics of the genus. As shown in Table IV, the beaks of the perithecia developed on the host in nature are 80—400 μ (mean 215.0±7.60 μ) in length. The width ranges 30—50 μ, but varies according to the part of the beaks and it measures 34.70±0.56 μ at the end and 40.0±0.25 μ at the widest part. (Table V).

Table III.

Variations and Constants for Size of the Base of Perithecia of *Ceratostomella ips* Rumbold, Developed on the Host and in Culture.

	Perithecia Developed on	Size of Perithecia (μ)													Total
		120	140	160	180	200	220	240	260	280	300	320	340	360	
Height	(1) Host	5	7	**32**	26	14	14	2	—	—	—	—	—	—	100
	(2) "Ame" Agar	—	—	5	11	13	**28**	18	15	4	5	1	—	—	100
	(3) Pine Block	2	38	**72**	43	42	3	—	—	—					200
Diameter	(1) Host	5	14	21	**29**	14	10	7	—	—					100
	(2) "Ame" Agar	—	—	2	7	15	17	**27**	18	3	6	3	2	—	100
	(3) Pine Block	1	29	**68**	43	45	12	2	—	—	—				200

	Perithecia Developed on	Range (μ)	Mean (μ)	Standard Deviation (μ)	Variation Coefficient
Height	(1) Host	120—240	177.8±2.69	26.91±1.20	15.00±0.69
	(2) "Ame" Agar	160—310	227.5±3.48	34.82±1.56	15.31±0.67
	(3) Pine Block	120—220	169.4±1.38	19.21±0.65	11.34±0.39
Diameter	(1) Host	110—250	179.6±3.10	30.96±1.38	17.00±0.78
	(2) "Ame" Agar	160—340	238.1±3.85	38.49±1.72	16.17±0.74
	(3) Pine Block	130—240	174.6±1.67	23.59±0.79	13.51±0.46

Remarks: 1) Perithecia developed on the host wood, *Pinus densiflora* SIEB. et ZUCC., sent from the Himezi Local Forestry Office.
2) Perithecia developed on 3% "Midu-Ame" (rice jelly) agar after 4 weeks' culture at 20—25°C.
3) Perithecia developed on a sterilized block of pine wood after 3 weeks' culture at 27°C.

Table IV.

Variations and Constants for Length of the Beak of Perithecia of *Ceratostomella ips* Rumbold, Developed on the Host and in Culture.

Perithecia Developed on	Length of Beaks of Perithecia (μ)															Total
	100	200	300	400	500	600	700	800	900	1000	1100	1200	1300	1400	1500	
(1) Host	15	48	28	9	—	—	—	—	—	—	—	—	—	—	—	100
(2) "Ame" Agar	—	—	5	35	40	18	2	—	—	—	—	—	—	—	—	100
(3) Pine Block	—	—	—	—	—	5	22	39	61	57	48	34	16	15	3	300

Perithecia Developed on	Range (μ)	Mean (μ)	Standard Deviation (μ)	Variation Coefficient
(1) Host	80—400	215.0±7.60	75.99±3.63	35.34±1.76
(2) "Ame" Agar	270—630	456.1±6.63	66.29±3.16	15.15±0.69
(3) Pine Block	560—1540	955.8±2.26	39.12±1.08	4.10±0.11

Remarks: 1) Perithecia developed on the host wood, *Pinus densiflora* SIEB. et ZUCC., sent from the Himezi Local Forestry Office.
2) Perithecia developed on 3% "Midu-Ame" (rice jelly) agar after 4 weeks' culture at 20—25°C.
3) Perithecia developed on a sterilized block of pine wood after 3 weeks' culture at 27°C.

Table V.

Variations and Constants for Width of the Beak of Perithecia of *Ceratostomella ips* Rumbold, Developed on the Host and in Culture.

Perithecia Developed on	Parts Measured	With of Perithecial Beak (μ)								Total
		20	30	40	50	60	70	80	90	
(1) Host	End	—	56	41	3	—	—	—	—	100
〃	Widest Part	—	3	94	3	—	—	—	—	100
(2) "Ame" Agar	End	—	—	97	3	—	—	—	—	100
〃	Widest Part	—	—	—	14	55	26	4	1	100
(3) Pine Block	End	3	178	19	—	—	—	—	—	200

Perithecia Developed on	Parts of the Beaks measured	Mean (μ)	Standard Deviation (μ)	Variation Coefficient
(1) Host	End	34.7±0.556	5.56±0.25	16.02±0.73
〃	Widest Part	40.0±0.245	2.45±0.11	6.12±0.28
(2) "Ame" Agar	End	40.3±0.170	1.70±0.08	4.23±0.19
〃	Widest Part	65.4±0.119	1.19±0.05	1.86±0.081
(3) Pine Block	End	30.8±0.228	3.22±0.11	10.15±0.35

Remarks: 1) Perithecia developed on the host wood, *Pinus densiflora* SIEB. et ZUCC., sent from the Himezi Local Forestry Office.
2) Perithecia developed on 3% "Midu-Ame" (rice jelly) agar after 4 weeks' culture at 20—25°C.
3) Perithecia developed on a sterilized block of pine wood after 3 weeks' culture at 27°C.

The perithecium proper or the base of a perithecium is spherical, and measures 120—240 μ (mean 177.8±2.69 μ) in height and 110—250 μ (mean 179.6± 3.10 μ) in diameter (Table III). The base of a perithecium is sparcely covered with flexible, dark colored hyphae, although not provided with bristle-like appendages (Plate XLVI, Fig. 3).

The Perithecium developed in culture somewhat differs in shape from those developed on the host in nature. On boiled blocks of pine wood in test tubes, the perithecia are produced pretty well. The shape is regular and nearly constant (Plate XLVI, Fig. 4 and Plate XLVII, Fig. 1). Therefore the writers will describe the shape of those produced on this medium in some length. The first indication of the perithecial formation on the mycelium is the formation of a dark colored knotted mass. In the center of the mass the young perithecium develops as a glanular black body, without a beak. From the upper side of the young perithecium, after it has grown to about its normal size, a long tube or

beak is projected. As shown in Plate XLVI, Fig. 4, and Plate XLVII, Fig. 1, the basal part of a perithecium is complete spherical, covered with mycelial strands, but not provided with bristles as in many other species of the genus. They measure 120—220 μ (mean 169.40±1.38 μ) in height and 130—240 μ (mean 174.6±1.67 μ) in diameter, as shown in Table III.

The beak is slender and black in color at the base, but lighter toward end, which is nearly colorless. The end is not provided with fringe-like appendage. The beak measures 560—1540 μ (mean 955.8±2.26 μ) in length (Table IV). The width of the beak varies with the part of the beak, widest near the base. The width at the end is 20—40 μ (mean 30.8±0.23 μ) (Table V). The beaks consists of long parallel cells. The cells near the end of a perithecial beak developed on a sterilized block of pine wood measure 14—25 μ (mean 19.97±0.21 μ) in length and 1.5—3.5 μ (mean 2.33±0.04 μ) in width. (Table VI).

Table VI.

Variations and Constants for Length and Width of the End Cells of Perithecial Beaks of *Ceratostomella ips* Rumbold, Developed on a Sterilized Block of Pine Wood at 27°C.

Length (μ)	14	15	16	17	18	19	20	21	22	23	24	25	Total
Frequency	1	1	2	7	15	15	18	10	14	8	1	2	100

Width (μ)	1.5	2	2.5	3	3.5	Total
Frequency	1	43	46	9	1	100

	Mean	Standard Deviation	Variation Coefficient
Length (μ)	19.97±0.210	2.10±0.10	10.53±0.50
Width (μ)	2.33±0.035	0.35±0.02	14.92±0.71

ii. Asci and Ascospores.

When ascospores are ejected from a perithecium they are combined with slime sheath in a long string, the ascus-wall being dissolved. The string is not broken for a long time in such a medium like acetic acid solution. Sometimes, thin-walled, faint asci, as shown in Plate LVI, Fig. 1—3, are founded. They may be long elliptical, ovoid or irregular in shape, although it is impossible to describe a certain form as a representative of asci. They seem to contain 8 ascospores, but it is not quite certain, as the ascus-wall dissolves readily, when a ascus is ejected out of a perithecium. The ascospores are covered with slime and combined

together. If a group of them is dried up, so it becomes a hard ball and pretty difficult to dissolve them into water. Therefore, it is somewhat difficult to isolate single spores from spore-groups, in which the ascospores have been ejected beforehand and then dried up into a ball.

As to the arrangement of the asci and ascospores in a perithecium, microtome sections were studied. Perithecia developed on a sterilized block of pine wood were fixed in CARNOY'S mixture, and then imbedded in paraffin and cut. The sections were stained with methylen blue and eosin after the RIDGEWAY'S method. In the preparations thus prepared, the wall of a perithecium was bluish black. In the cavity, the mature ascospores closed together without any order. The shape of asci was impossible to observe. (Plate XLVII, Fig. 3).

Table VII.

Variations and Constants for Length and Width of Ascospores of *Ceratostomella ips* Rumbold, Developed on the Host and in Culture.

	Ascospores Developed on	Size of Ascospores (μ)										Total
		1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	
Length	(1) "Ame" Agar	—	—	—	19	43	53	**47**	38	—	—	200
	(2) Pine Block				—	1	22	19	**45**	11	2	100
	Sum	—	—	—	19	44	75	66	**83**	11	2	300
Width	(1) "Ame" Agar	14	71	**79**	36	—	—	—	—	—	—	200
	(2) Pine Block	—	41	**44**	15	—	—	—	—	—	—	100
	Sum	14	112	**123**	51	—	—	—	—	—	—	300

	Ascospores Developed on	Mean	Standard Deviation	Variation Coefficient
Length (μ)	(1) "Ame" Agar	3.93±0.053	0.53±0.018	13.54±0.46
	(2) Pine Block	4.78±0.054	0.54±0.025	10.24±0.49
	Sum	4.21±0.031	0.53±0.014	12.59±0.35
Width (μ)	(1) "Ame" Agar	2.36±0.041	0.41±0.014	17.38±0.60
	(2) Pine Block	2.37±0.035	0.35±0.017	13.35±0.69
	Sum	2.36±0.023	0.39±0.011	16.53±0.47

Remarks: 1) Ascospores developed on 3% "Midu-Ame" (rice jelly) agar after 4 weeks' culture at 20—25°C.

2) Ascospores developed on a sterilized block of pine wood after 3 weeks' culture at 27°C.

The ascospores are colorless, cylindrical with truncated ends; therefore they seem, under a microscope, to be rectangular, as shown in Plate III, Fig. 4 and Plate LVI. In the photomicrograph in Plate III, Fig. 4, glanular contents of the ascospores are perceptible. Sach rectangular ascospores are very rare within the species of this genus, so far the writers are aware.

The size of ascospores developed in culture ranges 3—6 μ in length and 1.5—3 μ in width, but slightly varies according to the medium. The mean length of ascospores developed on sterilized pine blocks is $4.78\pm0.05\,\mu$ and on rice jelly agar $3.93\pm0.05\,\mu$; the mean width of the former being $2.37\pm0.04\,\mu$ while the latter $2.36\pm0.03\,\mu$ respectively. (Table VII).

iii. Germination of ascospores.

The ascospores of the present fungus germinate readily in tap water or in nutrient solutions at a moderate temperature. The ascospore germination in 2% malt extract solution after 30 hours' incubation at 24°C. are shown in Plate LVII, Fig. 1. Before the germination ascospores swell more or less, and the ends become round and plump. The germ tubes are hyaline, 1.5—4 μ wide, septated at an interval of 10 to 40 μ and similar to those form conidia. Secondary conidia are also readily formed on the tips of elongated germ tubes. The growth of germ tubes and the conidium formation at their tips after 2 days' incubation in the same solution are shown in Plate LVII, Fig. 2. In this figure the conidium formation in *Cephalosporium*-like clusters is very distinct.

4. Taxonomical consideration on the blue-staining fungus.

According to the above given characteristics, the complete stage of the fungus under consideration seems without doubts to belong to the genus *Ceratostomella*. As regards the blue stains on sap wood, many species of the genus *Ceratostomella* and the closely related genera have already been described.

In America Hedgcock (1906) described 8 species of the genus *Ceratostomella*, relating to the blue stains of wood. The key to these 8 species of *Ceratostomella* given by him in the 17th annual report of Missouri Botanical Garden, p. 112, is as follows:

1. Conidia borne in short, branching moniliform chains on upright hyphae.
   * Beaked ostiolum more than twice the height of the perithecium.
     † Fringe to ostiolum terminal.
       § Terminal filamants short and often thickend.
         Perithecium smooth or sparcely hirsute. *C. pilifera* (Fr.) Winter.
         Perithecium often with outgrowths. *C. Schrenkiana* n. sp.
         Perithecium with glandular hairs. *C. echinella* E. et E.
       §§ Terminal filaments long and slender. *C. capillifera* n. sp.
     ‡ Fringe to ostiolum often supplemented by additional rings beneath. *C. plurinannualata* n. sp.

** Beaked ostiolum only once or twice the height of the perithecium.
Terminal filaments short and thickend. *C. monir* n. sp.
Terminal filaments lengthened and slender. *C. exigua* n. sp.

2. Conidia borne continuously, either singly or in moniliform chains.
Perithecium with conical spines. *C. moniliformis* n. sp.

In the next year MÜNCH (1907) published a famous article on this problem. He devided the blue-staining fungus, which had been known under the name *C. pilifera* (FR.) WINTER, into 5 different species. The summerized characteristics of the 5 species given by him in Naturwissenschaftliche Zeitschrift für Forst- und Landwirtschaft, Jahrgang 5. S. 535—6 are cited as follows:

1. *Ceratostomella pini* n. sp., der wichtige Blaufäulepilz der Kiefer, ist an den sehr kleinen (80 μ dicken), kurzsgechnäbelten Perithecien zu erkennen. Diese entstehen in der Regel unter der Rinde in einem Hohlraum, der durch einige, die Rinde vom Holz abhebende Organe gebildet wird.

Ausserdem wurden drei weitere *Ceratostomella*-Arten gebildet, die sich einander sehr nahe stehen und zu einer „*Pilifera*-Gruppe" vereinigt werden können. Die Arten dieser Gruppe unterscheiden sich lediglich in ihren Nebenfruchtformen, während die Perithecien in allen systematisch verwertbaren Unterscheidungsmerkmalen vollkommen übereinstimmen. Die Perithecien dieser Pilzgruppe sind grösser und länger geschnäbelt als die der vorigen Art. Hierher gehören:

2. *Ceratostomella piceae* n. sp., mit einer Nebenfruchtform, die unter den Fungi imperfecti in der Gattung *Graphium* geführt wird und wahrscheinlich mit *Graphium penicillioides* CORDA identisch ist.

3. *Ceratostomella cana* n. sp., mit einem anderen *Graphium*, das sich vom vorigen hauptsächlich durch grössere Konidien und andere Art der Konidienabschnürung unterscheidet.

4. *Ceratostomella coerulea* n. sp., das kein *Graphium* hervorbringt.

Diese vier *Ceratostomella*-Arten haben ausserdem noch Konidien, die ohne Fruchtkörperbildung am Mycel oder einzelnen Konidienträgern entstehen. Ihre Form und Anordungsweise ist für die vier Species verschieden und ebenfalls als Artcharakteristikum zu verwenden.

Für einen weiteren, zur seitherigen *Ceratostomella pilifera* gehörigen Pilz, dessen Perithecien denen der drei zuletztgenannten sehr ähnlich sind, musste seiner eigentümlichen Nebenfruchtform halber eine eigene Gattung geschaffen werden. Der Pilz wurde als

5. *Endoconiophora coerulescens* n. sp. bezichnet. Seine Konidienform wurde seither als *Chalara Ungeri* SACC. unter den Fungi imperfecti aufgeführt.

In Jugo-Slavia GEORGEVITCH (1926, 1930) described 2 new species of *Ceratostomella* on oak. They are *Ceratostomella Querci* np. and *C. merolinensis* n. sp. Recently C. T. RUMBOLD (1931) described a new species of this genus under the name *Ceratostomella ips* n. sp. on sap wood of *Pinus echinata*, *P. rigida*, *P. reginosa* and *P. palustris*, infested with *Ips* spp. BUISMAN (1932) discovered the complete stage of *Graphium ulmi* SCHWARZ, the causal fungus of the 'Dutch elm

disease' or 'Ulmensterben', and named it *Ceratostomella ulmi* (SCHWARZ) BUISMAN. Besides these some species are known on broad leaved trees and grasses.

The described characteristics of all the above stated species, with one exception of *Ceratostomella ips* RUMBOLD, do not coincide with those of the writers' fungus. The technical description given by C. T. RUMBOLD (1931) in the Journal of Agricultural Research, Vol. 43, p. 870 is as follows:

Ceratostomella ips n. sp. Young colonies with conidia white changing to sepia and black, with perithecia; young hyphae hyaline, 2 μ to 3 μ in diameter, conidia hyaline appear first on hyphae, ovoid to ellipsoidal, range from 3 μ long, 1 μ wide, to 15 μ long, 3 μ wide; young conidiophores single hyaline hyphae bearing conidia in clusters, older conidiophores single brown hyphae bearing at their tops branches, biverticilate, on the tips solitary conidia that collect in a head held together by a mucilaginous substance; perithecia nongeotropic, globose, slightly hirsute, height of base, extreme range 96 μ to 320 μ, sextile range 177 μ to 234 μ, mean 206 μ, width of base, extreme range 55 μ to 301 μ, sextile range 167 to 226 μ, mean 198 μ, length of neck, extreme range 215 μ to 3,860 μ, sextile range 906 μ to 1,620 μ, mean 1,273 μ, the ostiole usually without terminal filaments —those bristles seen measure 27 μ to 45 μ long—sometimes ostiole is not used, the ascospores escaping from the broken base; asci ephemeral, polyhedral, 7 μ to 8 μ by 9 μ to 10.4 μ; ascospores 8, hyaline, cylindrical or slightly curved, 2.9 μ to 4.6 μ long, sextile range 3.3 μ to 4.2 μ, mean 3.8 μ and 1.2 μ to 2.8 μ wide, sextile range 1.9 μ to 2.5 μ, mean 2 μ.

According to the above cited characteristics, *Ceratostomella ips* RUMBOLD resembles to the writers' fungus. Further the writers studied all the available strains of *Ceratostomella* spp. in pure culture, which were kindly supplied to the writers by Professor J. WESTERDIJK and by Dr. C. T. RUMBOLD. The writers' comparative studies of the pure culture of avilable species confirmed them in their belief, which had been secured through the studies in literature. Therefore they have identified the blue-stained fungus with *Ceratostomella ips* RUMBOLD.

5. Host range and distribution.

Ceratostomella ips RUMBOLD has been reported by the original writer on sap wood of *Pinus echinata* infested with *Ips calligraphus* as well as *Ips grandicollus* in N. C., *Pinus rigida* infested with *Ips calligraphus* in N. J., *Pinus resinosa* infested with *Ips* sp. in Pa., and *Pinus palustris* infested with *Ips* sp. in Fla., U. S. A. RUMBOLD has written to the present writers that the fungus under consideration distributed rather wide in the forests of the United States.

In Japan the present writers have found the fungus *Ceratostomella ips* RUMBOLD upon sap wood of "Akamatu", *Pinus densiflora* SIEB. et ZUCC., and "Kuromatu" *Pinus Thunbergii* PERS., in an association with the damage of the bark beetles only in the districts near the coast of the "Inland See" in Hyôgo and Osaka Prefectures. Many blue-stained materials of various coniferous wood, collected

in the Kiso Imperial Houshold Forestry, and in forests in Sagalien and Hokkaido were studied by the writers. From these materials they secured *Ceratostomella* spp., but the greater part of them developed the conidia belonging to *Graphium*, and they could not find *Ceratostomella ips*. Therefore the distribution of *Ceratostomella ips* in this country seems to be not wide, so far the writers are aware.

6. Characteristics of the fungus on culture media.

Ceratostomella ips Rumbold grows pretty well on all the more commonly used media. Cultural experiments of the fungus were undertaken by the writers. In this experiment the following strains were used: (1) Strain No. 442, isolated from sap wood of *Pinus densiflora* Sieb. et Zucc., sent from Himezi, (2) No. 443, from another material of *Pinus densiflora*, (3) No. 581, isolated from *Pinus Thunbergii*, (4) No. 706, from *Pinus echinata* in America.

The culture media used are as follows:

Malt extract agar: Malt extract 30 gr., agar 20 gr., water 1000 cc.

Bouillon agar: Pepton 10 gr., meat extract 10 gr., NaCl 5 gr., agar 20 gr., water 1000 cc.

Potato agar: Potato (200 gr.), cane sugar 20 gr., agar 20 gr., water 1000 cc.

Onion agar: Onion decoction (prepared from onion 500 gr. and water 500 cc.) 100 cc., soja 50 cc., cane sugar 50 gr., agar 20 gr., water 850 cc.

Apricot agar: Dried apricot (20 gr.), agar 20 gr., water 1000 cc.

Rice straw agar: Dried rice straw (100 gr.), agar 20 gr., water 1000 cc.

Uschinsky's agar: NaCl 5 gr., $CaCl_2$ 0.1 gr., $MgSO_4$ 0.2 gr., K_2HPO_4 1 gr., asparagin 3.4 gr., ammonium lactate 10 gr., glycerin 40 cc., agar 20 gr., water 1000 cc.

Cohn's agar: KH_2PO_4 5 gr., $MgSO_4$ 5 gr., ammonium tartarate (neutral) 10 gr., KCl 0.5 gr., agar 20 gr., water 1000 cc.

Currie's agar: NH_4NO_3 2.5 gr., KH_2PO_4 1.0 gr., $MgSO_4$ 0.25 gr., cane sugar 150 gr., agar 20 gr., water 1000 cc.

Richards' agar: KNO_3 10 gr., KH_2PO_4 5 gr., $MgSO_4$ 2.5 gr., cane sugar 50 gr., agar 20 gr., water 1000 cc.

Sterilized blocks of pine wood, oak wood, watermelon and potato tubers.

In this experiment more important culture characteristics only were recorded, as shown tabularly in Table VIII and IX. Table VIII gives the results after 7 days' culture at 27°C., in which (1) radial growth of colonies, determined by their diameter, (2) formation of aerial mycelium and (3) of conidia, (4) characteristics of margins and (5) coloration of colonies are recorded. The radial growth of colonies is shown by their diameter, but sometimes by the number of plus signs. Throughout the table, the more the plus signs mean the better the growth of colonies or the formation of aerial mycelium, conidia and perithecia respectively. Table IX shows the results after 4 weeks' culture at the same temperature, in which records of the perithecium formation are also given.

Table VIII.

Summerized Characteristics of *Ceratostomella ips* Rumbold on Culture Media after 7 Days' Incubation at 27°C.

Culture Media used	No. of Strain tested	Radial Growth of Colonies	Formation of Aerial Mycelium	Characteristics of Margin of Colonies *	Coloration of Colonies		Formation of Conidia	Remarks
					Color Name	Degree		
		mm.						
Malt Extract Agar (3%)	No. 442	64	−	R. T.	Dark olive	++	++	
	" 443	66	−	R. T.	"	++	++	
	" 581	90	−	R. T.	"	++	++	
	" 706	62	−	Rr. To.	Clove brown	++	++	
Bouillon Agar	No. 442	28	−	I. T.	Milk-white	−	++	
	" 443	46	−	I. T.	"	−	++	
	" 581	44	−	I. T.	"	−	++	
	" 706	29	−	I. T.	"	−	++	
Potato Decoction Agar	No. 442	72	+	R. C.	Olivaceous black (1)	++	+	
	" 443	88	+	R. C.	"	++	++	
	" 581	60	−	R. C.	"	++	++	
	" 706	80	−	R. C.	Dark grayish olive	++	++	
Onion Decoction Soja Agar	No. 442	78	+	R. C.	Hair brown	++	+	
	" 443	89	−	R. C.	Chaetura black	++	+	
	" 581	73	−	R. C.	"	++	+	
	" 706	83	−	R. C.	"	++	+	
Apricot Agar	No. 442	48	+	R. C.	Clove brown	++	+	
	" 443	91	++	R. C.	Olive brown	++	++	
	" 581	61	+	R. C.	Clove brown	++	++	
	" 706	89	++	R. C.	Dark olive	++	++	
Rice Straw Decoction Agar	No. 442	36	+	R. Ct.	Olivaceous black (1)	++	++	
	" 443	42	+	R. Ct.	"	++	++	
	" 581	45	+	R. Ct.	"	++	++	
	" 706	34	−	R. Ct.	Dark grayish olive	++	+	
Sterilized Block of Pine Wood	No. 442	++	−	.	Grayish olive	++	+	
	" 443	++	−	.	"	++	+	
	" 581	++	−	.	"	++	+	
	" 706	++	−	.	Light grayish olive	++	+	

Culture Media used	No. of Strain tested	Radial Growth of Colonies	Formation of Aerial Mycelium	Characteristics of Margin of Colonies *	Coloration of Colonies		Formation of Conidia	Remarks
					Color Name	Degree		
		mm.						
Sterilized Block of Oak Wood	No. 442	###	##	.	Olivaceous black	+	+	
	" 443	###	##	.	"	+	+	
	" 581	###	##	.	"	+	+	
	" 706	###	+	.	Dark grayish olive	+	+	
Steamed Water Melon	No. 442	20	+	.	Colorless	−	±	
	" 443	47	+	.	"	−	+	
	" 581	27	+	.	"	−	+	
	" 706	25	+	.	"	−	###	
Steamed Potato	No. 442	13	+	.	Colorless	−	+	
	" 443	16	+	.	"	−	+	
	" 581	10	+	.	"	−	##	
	" 706	9	+	.	"	−	##	
USCHINSKY's Agar	No. 442	24	−	Ii. T.	Colorless	−	##	Conidia only in Cephalosporium-like clusters.
	" 443	13	−	Ii. T.	"	−	###	"
	" 581	18	−	Ii. T.	"	−	###	"
	" 706	8	−	Ii. T.	"	−	###	"
COHN's Agar	No. 442	18	−	Ii. Tt.	Colorless	−	+	Conidia only in Cephalosporium-like clusters.
	" 443	10	−	Ii. Tt.	Smoke gray	±	+	"
	" 581	15	−	Ii. Tt.	"	±	+	"
	" 706	10	−	Ii. Tt.	Colorless	+	+	"
CURRIE's Agar	No. 442	±	−	I. Tt.	Colorless	−	−	
	" 443	±	−	I. Tt.	"	−	−	
	" 581	±	−	I. Tt.	"	−	−	
	" 706	±	−	I. Tt.	"	−	−	
RICHARDS' Agar	No. 442	+	−	Ii. T.	Colorless	−	+	
	" 443	+	−	Ii. T.	"	−	+	
	" 581	+	−	Ii. T.	"	−	+	
	" 706	+	−	I. T.	"	−	+	

* In this columm **R** means that the margin of colonies is of regular circle, **Rr** entirely regular, **I** irregular and **Ii** more irregular. On the otherhand **T** represents the margin of colonies thin, **Tt** very thin, **C** compact, **Cc** extremely compact and **Ct** the transition of the both.

Table IX.

Summerized Characteristics of *Ceratostomella ips* Rumbold on Culture Media after 4 Weeks' Incubation at 27°C.

Culture Media used	No. of Strain tested	Formation of Aerial Mycelium	Coloration of Colonies		Formation of Conidia	Formation of Perithecia		Remarks
			Color Name	Degree		Submerg.	Superf.	
Malt Extract Agar (3%)	No. 442	−	Blackish brown (3)	++	+++	±	±	
	〃 443	−	〃	+++	+++	++	+	
	〃 581	−	〃	+++	+++	++	+	
	〃 706	−	Clove brown	++	+++	+++	±	
Bouillon Agar	No. 442	−	Milk-white	−	++	−	−	
	〃 443	−	〃	−	++	−	−	
	〃 581	−	〃	−	++	−	−	
	〃 706	−	〃	−	++	−	−	
Potato Decoction Agar	No. 442	+	Chaetura black	++	++++	−	++	
	〃 443	+	〃	+++	++++	+	++	Conidia sporodochial
	〃 581	−	〃	++	++++	−	+	Conidia pionnotal
	〃 706	−	〃	+++	+++	−	−	
Onion Decoction Soja Agar	No. 442	+	Hair brown	++	+++	−	−	
	〃 443	+	Chaetura black	++	+++	−	−	Conidia sporodochial
	〃 581	−	Dark olive Buff	++	++++	−	−	Conidia pionnotal
	〃 706	−	〃	++	++++	−	−	〃 〃
Apricot Agar	No. 442	+	Blackish brown (3)	+++	++	−	−	
	〃 443	++	〃	+++	++	±	+	Conidia sporodochial
	〃 581	+	〃	+++	+++	−	+	
	〃 706	++	〃	+++	++	−	−	
Rice Straw Decoction Agar	No. 442	+	Blackish brown (3)	+++	++	−	++	
	〃 443	+	〃	+++	++	+	++	
	〃 581	+	〃	+++	+++	−	+	
	〃 706	−	〃	++	++	−	−	
Sterilized Blocks of Pine Wood	No. 442	−	Blackish brown (3)	++	++	++	++	
	〃 443	−	〃	++	++	++	+++	
	〃 581	−	〃	++	++	++	+++	
	〃 706	−	〃	+	++	++	++	

Culture Media used	No. of Strain tested	Formation of Aerial Mycelium	Coloration of Colonies		Formation of Conidia	Formation of Perithecia		Remarks
			Color Name	Degree		Submerg.	Superf.	
Sterilized Blocks of Oak Wood	No. 442	++	Blackish brown (3)	+++	++		++	
	" 443	++	"	+++	++		+++	
	" 581	++	"	+++	++		+++	
	" 706	+	"	+++	++		++	
Steamed Potato	No. 442	−	Deep olive Buff	+	+	−	−	
	" 443	−	"	+	+	−	−	
	" 581	−	"	+	++	−	−	
	" 706	−	"	+	+	−	−	
Uschinsky's Agar	No. 442	−	Colorless	−	+++	−	−	
	" 443	−	"	−	+++	−	−	
	" 581	−	"	−	+++	−	−	
	" 706	−	"	−	+++	−	−	
Cohn's Agar	No. 442	−	Colorless	−	+	−	−	
	" 443	−	"	−	+	−	−	
	" 581	−	"	−	+	−	−	
	" 706	−	"	−	+	−	−	
Currie's Agar	No. 442	−	Colorless	−	+	−	−	
	" 443	−	"	−	+	−	−	
	" 581	−	"	−	+	−	−	
	" 706	−	"	−	+	−	−	
Richards' Agar	No. 442	−	Colorless	−	+	−	−	
	" 443	−	"	−	+	−	−	
	" 581	−	"	−	+	−	−	
	" 706	−	"	−	+	−	−	

7. Effect of temperature upon the growth of the fungus.

Thermal relations to the growth of species of *Ceratostomataceae*, the blue-staining fungi, have been objects of investigations by previous authors. MÜNCH (1908) states, that *Endoconiophora coerulescens* commences the growth at a lower temperature than the other species of *Ceratostomella*, and at 7°C. a slow growth may be observed on gelatine. He considers the optimum temperature to be lie above 20—25°C for species of *Ceratostomataceae* studied.

HOWARD (1922) states that the optimum temperature as a rule presumably lies at 27—29°C. Experiments of RAGERBERG, LUNDBERG and MELIN (1927) show that *Endoconiophora coerulescens* and *Leptographium Lundbergii* seem to have a fairly restricted optimum on malt agar (5% LIEBIG's malt extract), the former at 22.5°C.: the latter at 25°C. As to the minimum temperature, the growth is shown at 3—4°C. upon malt agar. The maximum temperature lies above 27°C.

Concerning the thermal relations to the growth of the species under consideration there exists no record. So the present writers have studied the effects of temperature upon the mycelial growth, the conidium formation and other culture characteristics.

(1) METHODS.

Strains of the writers' fungus were grown on a malt extract agar, which contained 3% malt extract and 2% agar. Fifteen cc. of the medium were tubed in each test tube, and sterilized in an autoclave at a pressure of 15 pounds for 20 minutes. After sterilization they were poured into Petri dishes, 9 cm. in diameter. On the center of the plates a circular, 2 mm., agar bit, in which the mycelium of the fungus was growing, was inoculated. These plates were kept in the incubators set at 6°, 8°, 15°, 20°, 24°, 27°, 29°, 31°, 33° and 35°C. respectively. Throughout the course of this experiment special cares were paid to keep the indicated temperature as constant as possible. Triplicate Petri dishes were used for each set of temperatures studied.

(2) EFFECT OF TEMPERATURE ON THE VEGETATIVE GROWTH.

i. Effect on the radial growth of colonies.

To determine the effect of temperature on the horizontal, radial growth, the diameters of colonies grown on the above said plates were measured in two rectangular directions every other day. The results of the first experiment are given in Table X.

According to the results of this experiment, the strains No. 443, that was isolated from *Pinus densiflora*, No. 581, from *Pinus Thunbergii*, and No. 706, from the American *Pinus echinata*, all show somewhat similar characteristics in their thermal relations. The optimum temperature for the growth of the colonies of these three strains seems to be about 29°C., although the growth of the strain No. 443 after 7 days' incubation is better at 27°C. than at 29°C., but the growth

at 27°C. is generally worse than at 29°C., but much better than at 31°C. At about 6°, 8° and 35°C. they show no growth even after 7 days' incubation.

Table X.

Diameter of Colonies of *Ceratostomella ips* Rumbold, Grown on 3% Malt Extract Agar at Various Temperatures.

(Results of the First Experiment.)

Temperature, C.	Strain No. 443			Strain No. 581			Strain No. 706		
	Diameter of Colonies after			Diameter of Colonies after			Diameter of Colonies after		
	3 days	5 〃	7 〃	3 days	5 〃	7 〃	3 days	5 〃	7 〃
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
6°	0	0	0	0	0	0	0	0	0
8°	0	0	0	0	0	0	0	0	0
15°	0	13.8	22.3	0	12.0	21.2	0	11.3	22.3
20°	11.1	24.0	39.3	11.1	25.3	40.4	10.8	27.5	44.0
24°	15.0	28.3	46.0	14.7	27.0	46.6	13.0	30.3	49.8
27°	16.2	32.1	52.6	16.2	34.5	57.8	15.3	39.2	59.3
29°	18.8	32.1	51.3	18.1	37.6	62.3	18.7	42.0	66.5
31°	16.4	28.0	49.0	10.0	24.5	39.3	14.0	37.0	60.2
33°	7.0	16.8	26.0	8.8	17.7	32.6	5.5	21.0	41.5
35°	0	0	0	0	0	0	0	0	0

Two more experiments were repeated in the same manner. Average of results of these three are presented in following table:

Table XI.

Diameter of Colonies of *Ceratostomella ips* Rumbold, Grown on 3% Malt Extract Agar at Various Temperatures.

(Average of the Results of triplicated Experiments.)

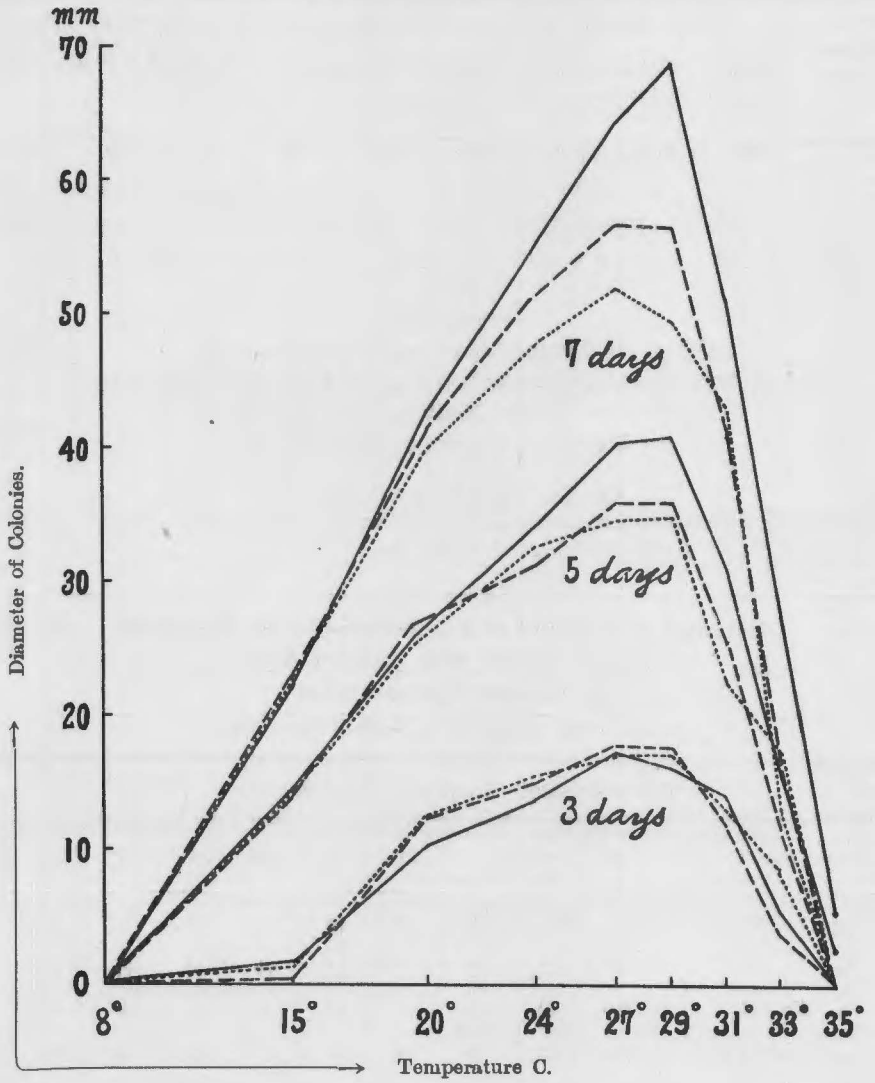
Temperature, C.	Strain No. 443			Strain No. 581			Strain No. 706		
	Diameter of Colonies after			Diameter of Colonies after			Diameter of Colonies after		
	3 days	5 〃	7 〃	3 days	5 〃	7 〃	3 days	5 〃	7 〃
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
6°	0	0	0	0	0	0	0	0	0
8°	0	0	0	0	0	0	0	0	0
15°	1.2	14.3	22.7	+	14.0	22.9	1.6	14.6	22.1
20°	12.2	25.4	40.0	12.1	26.9	41.4	10.2	25.9	42.9
24°	15.3	32.6	47.8	15.0	31.2	51.3	13.8	34.2	55.2
27°	17.2	34.5	51.8	17.9	35.8	56.5	17.3	40.3	64.2
29°	17.2	34.8	49.3	17.9	35.7	56.3	16.1	40.9	68.6
31°	13.2	22.8	42.7	11.9	25.3	40.8	14.0	31.0	51.0
33°	8.8	17.4	13.0	4	11.7	16.3	5.5	15.9	27.4
35°	0	0	+	0	0	0	0	2.8	5.5

These figures in the above table are shown graphically in Fig. I.

Fig. I.

Showing Diameter of Colonies of *Ceratostomella ips* Rumbold after 3, 5 and 7 Days' Culture on Malt Extract Agar at Various Temperatures.

(Average of the Results of Tripliate Experiments.)



..... Growth of the strain No. 443, isolated from *Pinus densiflora* Sieb. et Zucc.
– – – – – „ „ „ „ No. 581, „ „ *Pinus Thunbergii* Pebs.
——— „ „ „ „ No. 706, „ „ *Pinus echinata* in America.

According to the results given in Table XI and Figure I, the three strains of the *Ceratostomella ips* RUMBOLD studied show a pretty good similarity in the temperature relation to the mycelial growth. At 6°C. they do not grow at all. At 8°C. they scarcely commence it after 12 days' incubation. Then the growth becomes better with the rise of culture temperature. The strain No. 443 shows the best growth at 27°C. while No. 581 at 27° and 29°C., and No. 706 at 29°C. At 31°C. it is much worse than that of 27° and 29° and sometimes even than that at 24°C. The maximum temperature for the mycelial development of the three strains seems to be about 35°C. Although they showed small colonies at 35°C., it seemed to be due to the fact that the temperature of the incubator set to be 35°C. might fluctuate and was not constant in a very strict sense.

ii. Effect on the formation of aerial mycelium.

The thermal relations to the growth of the strains under consideration were tested chiefly on malt extract agar. Upon this medium, however, these strains developed a very few aerial mycelium as shown in the following table:

Table XII.

Effect of Temperature on the Formation of Aerial Mycelium of *Ceratostomella ips* Rumbold after 7 Days' Incubation on 3% Malt Extract Agar and on Sterilized Blocks of Oak Wood.

On 3% Malt Extract Agar.

Temperature, C.	8°	15°	20°	24°	27°	29°	31°	33°	35°
Strain No. 443	No	−	−	−	−	−	−	−	No
" No. 581	No	−	−	−	−	−	−	−	No
" No. 706	No	−	−	−	+	−	−	−	No

On Sterilized Blocks of Oak Wood.

Temperature, C.	8°	15°	20°	24°	27°	29°	31°	33°	35°
Strain No. 442	No	+	+	++	++	+	+	+	No
" No. 443	No	±	+++	+++	++	++	++	++	No
" No. 581	No	++	+++	+++	++	+	+	+	No
" No. 706	No	±	+	++	+	+	+	+	No

Remarks: In this table + sign means the formation of aerial mycelium, − no formation, No shows no growth of colonies.

On sterilized blocks of oak wood these strains develops aerial mycelium pretty well. Therefore a test was made on this point. Oak timber blocks of

about 30×5×5 mm. were tubed, added about 3 cc. of tap water and then sterilized in an autoclave under a pressure of 15 pounds for 30 minutes. After inoculation these tubes were kept at various degrees of temperature. Result after 7 days' incubation is also given in Table XII.

iii. Effect on the coloration of colonies.

The colors of the colonies of these strains after 7 days' incubation on malt extract agar are given in Table XIII. They vary according to the different parts of the colonies, viz., darkest at the center and lighter towards the margin. In the table the color tones of the darkest part of a colony are given. For the description of the color name the RIDGWAY's Color Standard is followed.

Table XIII.

Effect of Temperature on the Coloration of Colonies of *Ceratostomella ips* Rumbold after 7 Days' Incubation on 3% Malt Extract Agar.

Temperature, C.	Strain No. 443			
	Experiment I		Experiment II	
8°	No growth		No growth	
15°	Light grayish olive	+	Light grayish olive	+
20°	Grayish olive	++	Grayish olive	++
24°	〃	++	〃	++
27°	〃	++	〃	++
29°	〃	++	〃	++
31°	Light grayish olive	+	Light grayish olive	+
33°	〃		〃	
35°	No growth		No growth	

Temperature, C.	Strain No. 581			
	Experiment I		Experiment II	
8°	No growth		No growth	
15°	Light grayish olive	+	Smoke gray	+
20°	Grayish olive	++	Grayish olive	++
24°	〃	++	〃	++
27°	〃	++	〃	++
29°	〃	++	〃	++
31°	〃	++	〃	++
33°	〃	+	〃	+
35°	No growth		No growth	

Temperature, C.	Strain No. 706	
	Experiment I	Experiment II
8°	No growth	No growth
15°	Colorless	Colorless
20°	Vinaceous buff +	Vinaceous buff +
24°	〃 +	〃 +
27°	〃 +	Deep olive buff +
29°	〃 ++	〃 ++
31°	〃 ++	〃 ++
33°	〃 +	〃 +
35°	No growth	No growth

Remarks: In this table, number of plus signs shows the degree of coloration.

The strains No. 443 and No. 581 produce grayish olive colonies at a moderate temperature between 20° and 29°C. But near the limit temperature for growth the colonies are lighter or colorless. The American strain No. 706 shows somewhat different colors on 3% malt extract agar in relation to temperature. The color of the colonies produced at a moderate temperature of 20° to 31°C. is vinaceous buff or deep olive buff.

iv. Effect on the shape of colonies.

The shape of colonies produced on malt extract agar varies according to the culture temperature. Especially the margin of colonies are subject to variation. So they are recorded after 7 days' incubation on the said medium at various

Table XIV.

Margins of Colonies of *Ceratostomella ips* Rumbold, Grown on 3% Malt Extract Agar at Various Temperatures after 7 Days' Incubation.

Temperature, C.	Strain No. 443	Strain No. 581	Strain No. 706
8°	No growth	No growth	No growth
15°	Regular, thin	Regular, thin	Regular, somewhat compact
20°	〃 〃	〃 , somewhat compact	〃 〃
24°	〃 〃	〃 , thin	〃 〃
27°	〃 〃	〃 〃	〃 〃
29°	〃 〃	〃 〃	〃 〃
31°	〃 , somewhat compact	〃 〃	〃 〃
33°	〃 , compact	〃 , somewhat compact	〃 〃
35°	No growth	No growth	No growth

temperatures. The result is given in Table XIV. As shown in the table, colonies of the strains studied are regular circle at all temperatures studied. Margins of the colonies of the strains No. 443 and No. 581 developed at moderate or lower temperatures are thin, but those at high temperatures are somewhat compact. The colonies of the strain No. 706 are always somewhat compact without regard to temperature.

(3) Effect of temperature on the reproductive growth.

i. Effect on the perithecium formation.

Almost all the strains of *Ceratostomella ips* Rumbold tested by the writers, produced the perithecia on sterilized blocks of pine or oak wood. Tests were made as to the thermal relation to the perithecium formation of this fungus. The result is given in Tables XV and XVI. As shown in the tables, the perithecial formation of this fungus on sterilized blocks of pine and oak wood seems to be the best at about 27° and 29°C. The strain No. 443 produces the perithecia even at 24°C. at which the other strains (No. 442, 581 and 706) do not or scarcely produce them.

Table XV.

Effect of Temperature on the Formation of Perithecia of *Ceratostomella ips* Rumbold after 10 Days' Incubation on a Sterilized Block of Pine Wood.

Strain	Experiment	Temperature C.								
		8°	15°	20°	24°	27°	29°	31°	33°	35°
No. 443	Exp. 1	No	−	−	+	++	+++	++	−	No
	Exp. 2	No	−	−	++	+++	++	+	−	No
No. 581	Fxp. 1	No	−	−	−	+	+	−	−	No
	Exp. 2	No	−	−	−	+	+	−	−	No
No. 706	Exp. 1	No	−	−	−	+	+	−	−	No
	Exp. 2	No	−	−	−	+	++	−	−	No

Remarks: In this table + sign means the formation of perithecia, the more + signs the better formation. − sign shows no formation of perithecia. No means no growth of colonies.

Table XVI.

Effect of Temperature on the Formation of Perithecia of *Ceratostomella ips* Rumbold after 10 Days' Incubation on a Sterilized Block of Oak Wood.

Temperature, C.	8°	15°	20°	24°	27°	29°	31°	33°	35°
Strain No. 442	No	−	−	−	+	+	−	−	No
" No. 443	No	−	−	+	+++	+++	+	+	No
" No. 581	No	−	−	+	+++	+++	++	++	No
" No. 706	No	−	−	+	+	+	+	+	No

Remarks: In this table + sign means the formation of perithecia the more + signs the better formation. − sign shows no formation of perithecia. No means no growth of colonies.

ii. Effect on the conidium formation.

All the strains of this fungus studied, develop the conidia vigorously. Thermal relations to the conidia formation were also tested. Two kinds of conidia are produced on the malt extract agar, viz., submerged and superficial. In the present tests chiefly the submerged conidia formation was studied. Results after 7 days' incubation are given in the following table:

Table XVII.

Effect of Temperature on the Conidia Formation of *Ceratostomella ips* Rumbold after 7 Days' Incubation on 3% Malt Extract Agar.

Strain	Experiment	Temperature, C.								
		8°	15°	20°	24°	27°	29°	31°	33°	35°
No. 443	Exp. 1	No	+	+	++	+++	+++	+++	No	No
	Exp. 2	No	+	+	++	+++	++	++	+	No
No. 581	Exp. 1	No	+	+	++	+++	+++	+++	No	No
	Exp. 2	No	+	++	++	+++	++	+++	+++	No
No. 706	Exp. 1	No	−	+	+	+	+	+	+	No
	Exp. 2	No	+	+	+	+	+	+	+	No

Remarks: In this table + sign means the formation of conidia, − sign no formation. No means no growth of colonies.

The table shows that the conidium formation takes place at almost all the temperature, at which these strains grow. Especially the good conidium formation

was observed at 27° to 31°C. Only in the strain No. 706, it seems to show no great difference in regard to temperature.

(4) Thermal death points.

Thermal death points of *Ceratostomella ips* were determined by the writers. Conidia developed on 3% malt extract agar were scraped off into water, and the conidia suspension were poured through a sterilized gauze to remove any traces of conidiophores, mycelium and medium, that may be loosend during the process. Two cc. of the conidia suspension, thus prepared, were tubed into sterilized test tubes of 1.5 cm. in diameter. These tubes were inserted into hot water in a thermostat regulated to a certain constant temperature. After 5, 10, 15 and 20 minutes' exposure, two platinum loopfuls were transferred from the tubes to malt extract solution. The results after 5 and 10 days' incubation were recorded. They are presented in Table XVIII.

Table XVIII.

Relations of Periods and Thermal Death Points of Conidia of *Ceratostomella ips* Rumbold.

Strain	Periods of Exposure	Control	Temperature C. tested					
			46°	48°	50°	52°	54°	56°
No. 442	5 Minutes	+	+	+	+	−	−	−
	10 〃	+	+	+	−	−	−	−
	15 〃	+	+	+	−	−	−	−
	20 〃	+	+	+	−	−	−	−
No. 443	5 Minutes	+	+	+	+	+	−	−
	10 〃	+	+	+	+	−	−	−
	15 〃	+	+	+	+	−	−	−
	20 〃	+	+	+	+	−	−	−
No. 581	5 Minutes	+	+	+	+	+	−	−
	10 〃	+	+	+	+	−	−	−
	15 〃	+	+	+	+	−	−	−
	20 〃	+	+	+	+	−	−	−
No. 706	5 Minutes	+	+	+	+	−	−	−
	10 〃	+	+	+	+	−	−	−
	15 〃	+	+	+	+	−	−	−
	20 〃	+	+	+	−	−	−	−

Remarks: In this table − sign means the conidia was killed by the exposure, and + not killed.

As shown in Table XVIII, the conidia of the strain No. 442 lose the vitality by immersion into hot water at 50°C. for 10 minutes. The thermal death points of the strain Nos. 443, 581 and 706 are a little higher than those of the above said strain. They lose the vitality at 52°C. for 10 minutes.

8. Relation of nutrient-concentration and culture-age to the growth.

The writers' fungus grows fairly well on malt extract agar medium. They employed usually the medium containing 3% malt extract and 2% agar. The ingredients of the malt extract (commercial) used by them are as follows: 76.72% maltose, 1.76% dextrose, 1.03% protein, 0.05% fat, 1.95% ash and 18.49% water. Tests were made to know the relations of the concentration of malt extract in agar media to the fungus growth. Fifteen cc. of 2% agar medium containing respectively 1%, 5%, 10% and 20% of the malt extract were tubed, sterilized and poured into a Petri-dish. On the center of the medium, thus prepared, the inoculum was transferred. Three of these inoculated plates were incubated at 15°, 24°, and 29°C. respectively. To know the daily growth of colonies, their diameter was measured every day. The results are given in the following tables:

Table XIX.

Mycelial Growth of *Ceratostomella ips* Rumbold on the Agar Media Containing Malt Extract in Various Concentrations at 15°C.

Concentration		1%	5%	10%	20%
Diameter of Colonies after	2 days	4.0	4.0	+	
	3 "	8.1	7.4	+	±
	4 "	13.4	11.0	5.2	±
	5 "	18.3	14.5	8.4	±
	6 "	22.9	18.1	11.2	±
	7 "	26.6	21.4	14.0	±
	8 "	31.4	24.9	16.4	+
	9 "	35.2	28.7	19.2	+
	10 "	40.9	33.9	22.2	+
	11 "	44.5	38.2	25.6	6.0
	12 "	48.1	41.7	29.1	4.0

Concentration		1%	5%	10%	20%
Daily Growth of Colonies on the	3. day	4.1	3.4		
	4. "	5.3	3.6	3.2	
	5. "	4.9	3.5	3.2	—
	6. "	4.6	3.6	2.8	—
	7. "	3.7	3.3	2.8	—
	8. "	4.8	3.5	2.4	—
	9. "	3.8	3.8	2.8	—
	10. "	5.7	5.2	3.0	—
	11. "	3.6	4.3	3.4	—
	12. "	3.6	3.5	3.5	—
	Average	4.4	3.8	3.0	—

Table XX.

Mycelial Growth of *Ceratostomella ips* Rumbold on the Agar Media Containing Malt Extract in Various Concentrations at 24°C

Concentration		1%	5%	10%	20%
Diameter of Colonies after	2 days	8.9	7.3	+	—
	3 〃	15.1	12.8	7.2	—
	4 〃	22.4	18.6	12.5	±
	5 〃	28.2	24.9	18.2	+
	6 〃	35.8	31.1	26.1	+
	7 〃	43.9	37.9	32.6	+
	8 〃	51.9	45.3	39.4	+
	9 〃	59.4	53.6	44.5	+
	10 〃	65.6	62.9	49.4	+
	11 〃	72.8	70.5	55.2	+
	12 〃	80.1	76.7	61.7	+

Concentration		1%	5%	10%	20%
Daily Growth of Colonies on the	3. day	6.2	5.5	5.2	—
	4. 〃	7.3	5.8	5.3	—
	5. 〃	5.8	6.3	5.7	—
	6. 〃	7.6	6.2	7.9	—
	7. 〃	8.1	6.8	6.5	—
	8. 〃	8.0	7.4	6.8	—
	9. 〃	7.5	8.3	5.1	—
	10. 〃	6.2	9.3	4.9	—
	11. 〃	7.2	7.6	5.8	—
	12. 〃	7.3	6.2	6.5	—
	Average	7.1	6.9	6.0	—

Table XXI.

Mycelial Growth of *Ceratostomella ips* Rumbold on the Agar Media Containing Malt Extract in Various Concentrations at 29°C.

Concentration		1%	5%	10%	20%
Diameter of Colonies after	2 days	4.7	7.2	—	—
	3 〃	10.5	12.2	—	—
	4 〃	17.1	17.4	5.8	—
	5 〃	26.2	23.8	9.7	±
	6 〃	34.4	30.9	14.4	±
	7 〃	41.9	37.8	19.5	±
	8 〃	48.6	42.8	25.3	+
	9 〃	55.9	48.7	30.0	+
	10 〃	63.1	55.7	36.8	+
	11 〃	70.2	65.6	42.5	+
	12 〃	78.0	74.8	48.5	+

Concentration		1%	5%	10%	20%
Daily Growth of Colonies on the	3. day	5.8	5.0		
	4. 〃	6.6	5.2	3.6	
	5. 〃	9.1	6.4	3.9	
	6. 〃	8.2	7.1	4.7	
	7. 〃	7.5	6.9	5.1	
	8. 〃	6.7	5.0	5.8	
	9. 〃	7.3	5.9	4.7	
	10. 〃	7.2	7.0	6.8	
	11. 〃	7.1	9.9	5.7	
	12. 〃	7.8	9.2	6.0	
	Average	7.1	6.6	5.2	

According to the above given results, the daily growth of the colonies varies somewhat greatly. The variation in the daily measurements of a strain on a certain concentration of the medium, however, does not show a definite tendency. So the variation seems to be attributable to experimental erross as well as to fluctuation of the temperatures tested, which might take place during the experiments.

In regard to the concentration of the malt extract in agar medium, the 1% medium is the best for the plane growth of the colonies measured by the diameter, if their thickness or the formation of aerial mycelium is neglected. As to the diameter of the colonies, the 5% medium is next to the 1% medium, then 10% medium. On the agar medium containing 20% of the malt extract the present fungus shows scarcely traces of mycelial growth, even after 12 days' incubation. The colonies of this fungus grown on malt extract agar show generally olive color. Tones and degrees of the coloration of the colonies vary according to the concentration of malt extract in medium and the temperature incubated. Results of tests upon these relations are given in Table XXII.

Table XXII.

Coloration of Colonies of *Ceratostomella ips* Rumbold Developed on the Agar Media, Containing Malt Extract in Various Concentrations, at Various Temperatures.

Concentration of Malt Extract	15°C.	24°C.	29°C.
1%	Tilleul buff	Grayish olive	Pale olive buff
5%	Buffy brown	Deep olive	Deep olive buff
10%	Olive brown	〃	Dark olive buff
20%	Colorless	Colorless	Colorless

As shown in Table XXII the coloration of colonies is the darkest on the 10% medium within the range of the concentration studied. On the 20% medium the colonies are colorless, and on 1% medium they are light color.

Table XXIII.

Margin of Colonies of *Ceratostomella ips* Rumbold Developed on the Agar Media, Containing Malt Extract in Various Concentrations, at Various Temperatures.

Concentration of Malt Extract	15°C.	24°C.	29°C.
1%	Rough, thin	Regular, thin	Regular, thin
5%	Regular, somewhat compact	〃 , somewhat compact	〃 , somewhat compact
10%	〃 〃	〃 , compact	〃 , compact
20%	〃 〃	〃 〃	〃 〃

Marginal features of the colonies also vary according to concentrations of the nutrients. On medium of week nutrition, the margin is thin and rough, while on strong medium it is regular and compact, as shown in Table XXIII.

9. Effect of free oxygen on the growth of the fungus.

(1) EFFECT OF FREE OXYGEN ON THE MYCELIAL GROWTH.

Effect of free oxygen on the growth of this fungus was tested with the pyrogallic acid method after BUCHNER. Glass tubes of 100 cc. in the capacity were used. To the tube 1 gr. of pyrogallol and 10 cc. of 10% water solution of caustic potash were added, then a culture tube inoculated with the fungus were immediately introduced into it; and the tube was stoppered with gum and sealed with melted paraffin. Thus prepared tubes were kept in an incubator set at 25—26°C. The culture media used were boiled beforehand carefully to expell free oxygen, which might dissolve in the medium. As the controls, test tube cultures were kept in the similar tubes as above, but added with tap water and not pyrogallol and potash solution. The results are presented in the following table:

Table XXIV.

Effect of Free Oxygen on the Mycelial Growth of *Ceratostomella ips* Rumbold.

Strain	After 3 days		After 7 days	
	Without free Oxygen	Control	Without free Oxygen	Control
No. 442	No growth	Good growth, colonies 11 mm. in diameter	No growth	Colonies cover all the surface of the medium
No. 443	No growth	Good growth, colonies 8 mm. in diameter	No growth	Ditto
No. 581	No growth	Good growth, colonies 13 mm. in diameter	No growth	Ditto

As shown in the foregoing table, after three days' incubation at 25—26°C., good growth of the fungus was observed on culture medium in the control tubes. On the contrary, there aroses no growth in the tubes containing pyrogallol and potash solution. Even after 7 days' incubation no trace of the growth was observed in the anaerobic cultures.

(2) EFFECT OF FREE OXYGEN ON THE CONIDIUM GERMINATION.

Effect of free oxygen on the germination of the conidia was tested also with the BUCHNER's method. In this experiment, however, the method was somewhat modified. Weighing tubes of the capacity of about 25 cc. were used. They were packed in paper and sterilized in an autoclave. On the underside of the glass

stopper of the sterilized weighing tube a piece of thin layer of 3% malt agar, about 5 mm. square in size, was laid aseptically. On that layer thus prepared a small drop of conidia suspension of the fungus was put. When the drop was nearly dry, the glass stopper was fitted into the tubes, which was at the moment filled with 0.3 gr. of pyrogallol and 3 cc. of 10% solution of caustic potash. The tubes were then sealed with melted paraffin and kept at 25—26°C. Similarly prepared tubes with the addition of tap water in the place of pyrogallol and alkaline solution were used for control. After 2 days' incubation the agar layers, on which the conidia were sown, were transferred from the glass stoppers to slide glasses and covered with cover glasses. The germination of the conidia was studied under a microscope. The result is given in Table XXV:

Table XXV.

Effect of Free Oxygen on the Germination of Conidia of *Ceratostomella ips* Rumbold.

Strain	Culture without free Oxygen	Control
No. 442	No germination, but exceptionally traces of germination were observed	Vigorous conidium-germination was observed. Small colonies were produced with conidium formation
No. 443	No germination	Ditto
No. 581	No germination, but traces of germination were rarely observed	Ditto
No. 706	No germination	Ditto

As shown in the foregoing table, no conidia germinate under an anaerobic condition even after 2 days' incubation at 25—26°C. Meanwhile they germinate profusely and produce abundant conidia in the control tubes.

V. Summary.

1) The present paper is the first report on the sap stains of wood in Japan and deals with a blue-staining fungus of pine wood, which causes the death of the living pine trees on the roots, at least, promotes the death in forests of western Japan.

2) The blue-staining fungus under consideration has been identified by the present writers with *Ceratostomella ips* Rumbold, which was recently described in America on sap wood of *Pinus* spp. infested with *Ips* spp.

3) In Japan the fungus attacks the sap wood of "Akamatu" (*Pinus densiflora* Sieb. et Zucc.) and "Kuromatu" (*Pinus Thunbergii* Pers.), chiefly in association with the damage of bark beetles, which seem to belong *Ips* sp.

4) The blue-staining fungus was studied by the writers chiefly morphologically and physiologically. The results are given in detail.

5) The hyphae of the present fungus grow at first radially through parenchymatous cells of medullary rays from the cortex toward the heart wood. Meanwhile their branches proceed from medullary rays into resin ducts as well as tracheids and run through the tissues in the longitudinal direction ; they grow also through bordered pits of tracheids in the tangentilal direction of trees.

6) The conidia are produced chiefly in *Cephalosporium*-like-clusters, and not on *Graphium*-like-bundles of conidiophores. The perithecium is spherical and provided with a long beak, on which no fringe-like-appendages are observed The ascospores are cylindrical and with truncated ends.

7) The fungus are readily cultured on almost all kinds of media. More important cultural characteristics are given in tabular form.

8) The minimum temperature for the mycelial growth lies at about 6—8°C., the optimum 27—29°C. and the maximum 35°C. The conidia are produced at almost all the temperatures, at which the fungus grow, but best at 27—31°C. The perithecium formation on sterilized blocks of pine and oak wood seems to be best at 27—29°C.

9) Thermal death points of the conidium are about 52°C. for ten minutes' exposure.

10) Without free oxygen the conidium germination as well as the mycelial growth do not take place.

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Y. Nisikado and K. Yamauti:

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

Studies on *Ceratostomella ips* Rumbold, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE XLVI.

Explanation of Plate XLVI.

Fig. 1. Transverse section of wood of "Kuromatu" (*Pinus Thunbergii* PARL.), infected by the blue-staining fungus. Showing the blue stain of sap wood along medullary rays radially. (×1/3).

Fig. 2. Transverse section of wood of "Akamatu" (*Pinus densiflora* SIEB. et ZUCC.), infected by the blue-staining fungus. (×2/3).

Fig. 3. A perithecium of *Ceratostomella ips* RUMBOLD, produced on a 3% "Miduame" agar. The "Miduame" is a sort of jelly produced from rice. (×200).

Fig. 4. A perithecium of *Ceratostomella ips* RUMBOLD, produced on a sterilized block of pine wood after 10 days' culture at 27°C. (×100).

TAFEL XLVI.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Y. NISIKADO and K. YAMAUTI:

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

Studies on *Ceratostomella ips* RUMBOLD, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE XLVII.

Explanation of Plate XLVII.

Fig. 1. A perithecium of the blue-staining fungus, *Ceratostomella ips* RUMBOLD, produced on a sterilized block of pine wood after 10 days' culture at 27°C. (×100).

Fig. 2. A perithecium of *Ceratostomella ips*, produced on timber of the host, *Pinus densiflora*, in nature. (×120).

Fig. 3. Microtome section of a perithecium of *Ceratostomella ips* developed on a sterilized block of pine wood in a test tube. Stained with methylen blue and eosin after the RIDGEWAY's method. In the cavity, irregularly arranged, mature ascospores are shown. (×550).

Fig. 4. A test tube culture of *Ceratostomella ips* showing perithecia growing in the middle of the agar. This picture is taken and kindly sent by Dr. C. T. RUMBOLD to the writers. (×4).

TAFEL XLVII.

Fig. 1.

Fig. 2.

Fig. 3.

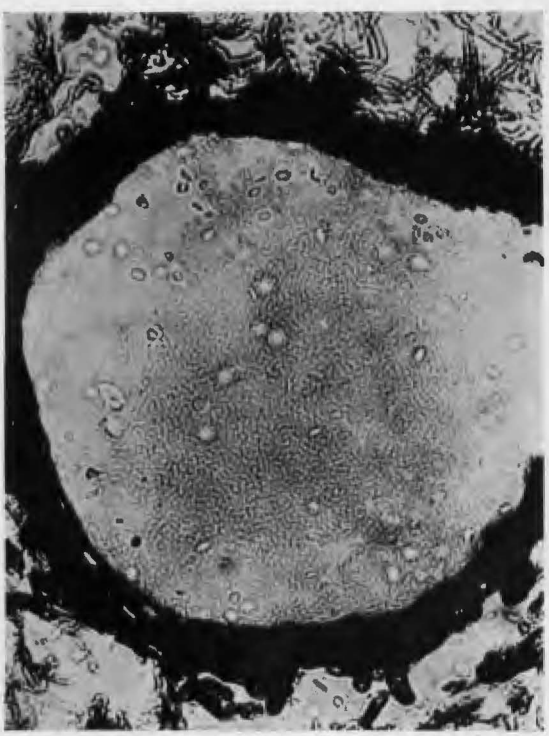
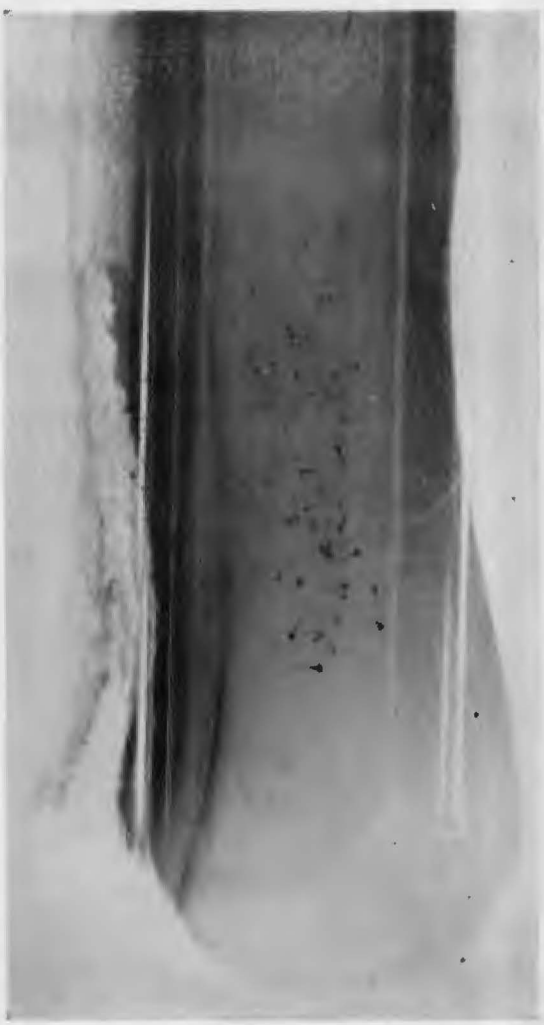


Fig. 4.



Y. Nisikado and K. Yamauti:

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

Studies on *Ceratostomella ips* Rumbold, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE III.

Explanation of Plate XLVIII.

Fig. 1. Conidia and conidiophores of *Ceratostomella ips* RUMBOLD, produced on malt extract agar. Showing a conidiophore ramifying profusely and producing conidia very abundantly. (×230).

Fig. 2. A conidiophore of *Ceratostomella ips*, produced on malt extract agar after 10 days' culture at 27°C. To show the branching of conidiophores clearly, a conidiophore, from which the conidia are detached off, is photographed. (×750).

Fig. 3. Conidia of *Ceratostomella ips*, produced on malt extract agar. They contain one, rarely two, glanular, light-refreeting bodies. (×2200).

Fig. 4. Ascospores of *Ceratostomella ips*, produced on a sterilized block of pine wood. They are cylindrical and the ends are truncated. Therefore they seem to be rectangular in the photomicrograph. They contain also one, rarely two, glanular bodies. (×2200).

TAFEL XLVIII.

Fig. 1.

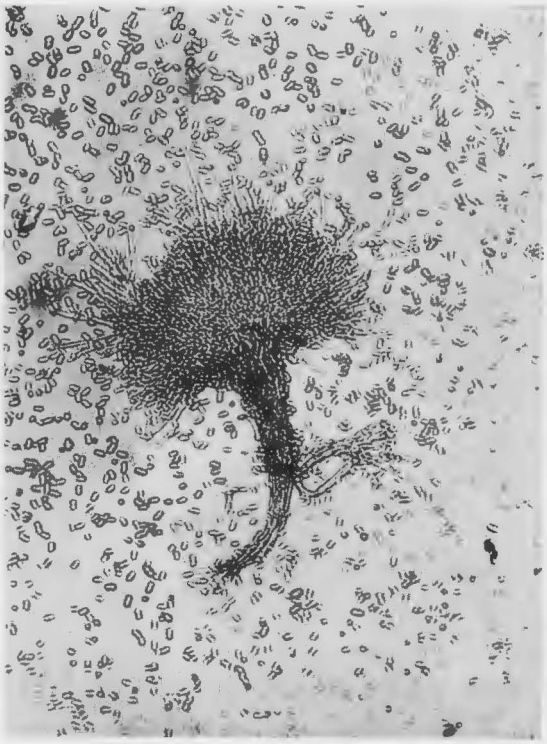


Fig. 2.

Fig. 3.

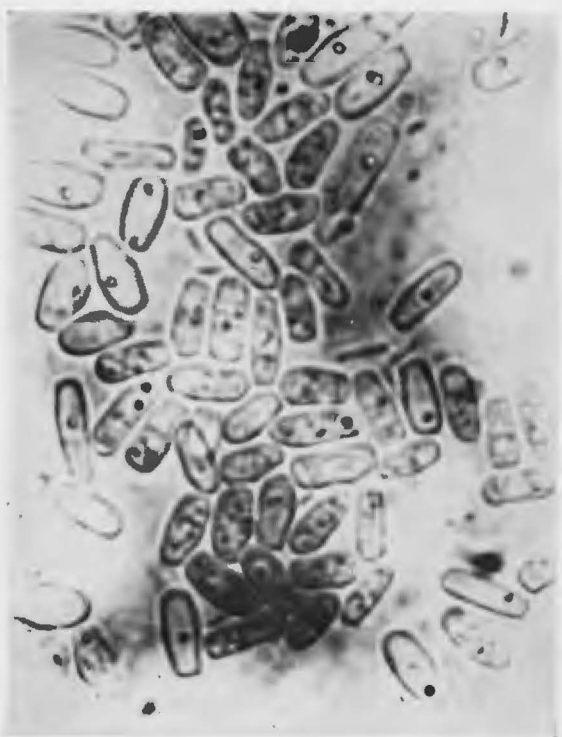
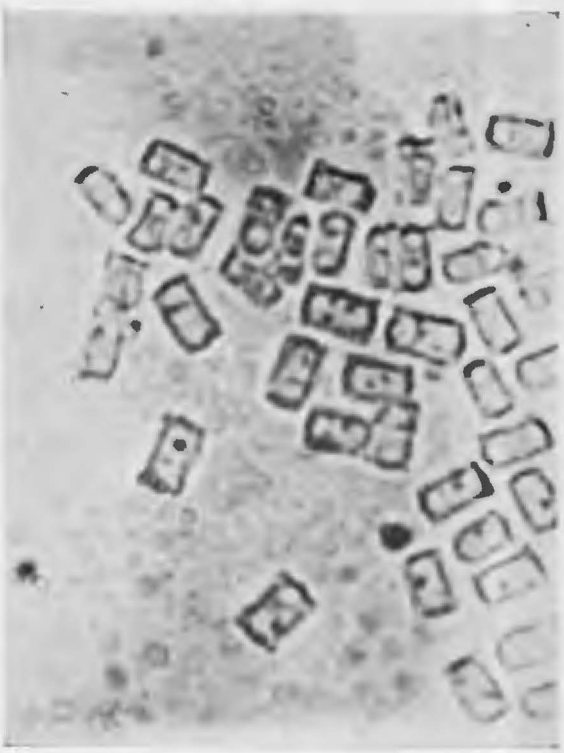


Fig. 4.



Y. NISIKADO and K. YAMAUTI:

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

Studies on *Ceratostomella ips* RUMBOLD, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE IL.

Explanation of Plate XLIX.

Fig. 1. Transverse section of blue stained sap wood of pine (*Pinus densiflora* SIEB. et ZUCC.). Showing the dark hyphae of *Ceratostomella ips* penetrating the parenchymatous cells in the medullary rays, and cross sections of the hyphae in a resin duct and in tracheidal cells. (×150).

Fig. 2. Radial, longitudinal section of a blue-stained sap wood of pine (*Pinus densiflora* SIEB. et ZUCC.). Showing the dark hyphae penetrating radially the medullary rays and longitudinally the tracheids. (×150).

Fig. 3. Tangential, longitudinal section of blue-stained sap wood of pine (*Pinus densiflora* SIEB. et ZUCC.). Showing the dark hyphae penetrating the tracheids and the branches growing into the parenchymatous cells of the medullary rays. (×150).

TAFEL XLIX.

Fig. 1.

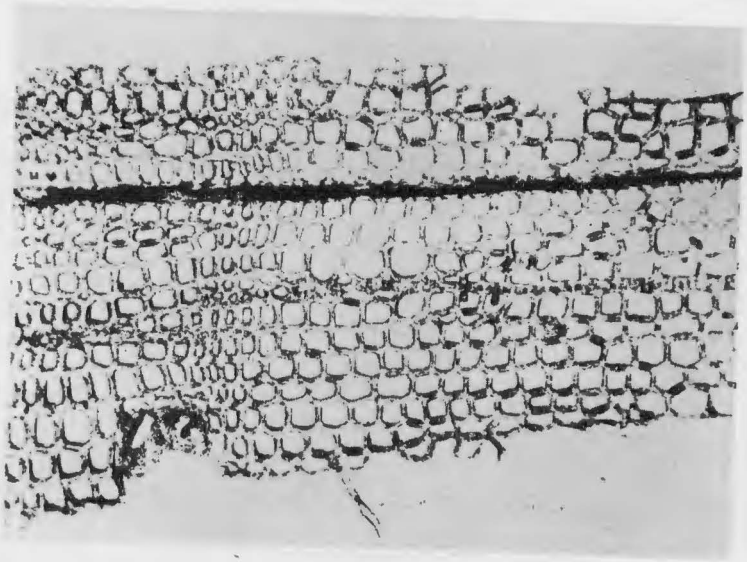


Fig. 2.

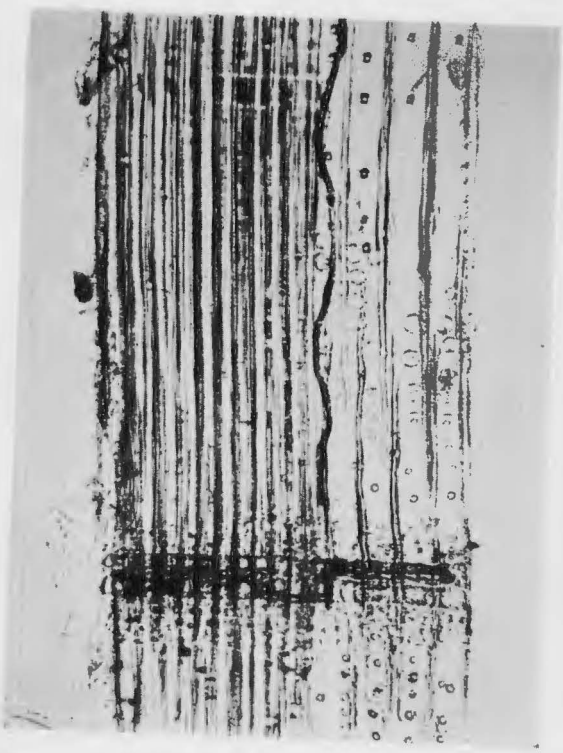


Fig. 3.

Y. Nisikado and K. Yamauti:

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

Studies on *Ceratostomella ips* Rumbold, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE L.

Explanation of Plate L.

Sections of blue-stained sap wood of *Pinus densiflora* SIEB. et ZUCC. infected by *Ceratostomella ips* RUMBOLD. The figures were drawn from water mounted preparations with an aid of a camera lucida under ZEISS K 10× and apochromat 40×; and were reduced to one half of original size. (×250).

Fig. 1 and 2. Transverse section of blue-stained sap wood of pine. Showing the dark hyphae penetrating the cells of medullary rays, and closs sections of the hyphae in tracheidal cells.

Fig. 3 and 4. Radial, longitudinal section of blue-stained sap wood of pine. Showing the dark hyphae penetrating radially medullary rays and longitudinally tracheids.

Fig. 5. Tangential, longitudinal section of blue-stained sap wood of pine. Showing the dark hyphae penetrating tracheids and the branches growing into the parenchymatous cells of medullary rays.

TAFEL L.

Fig. 1.

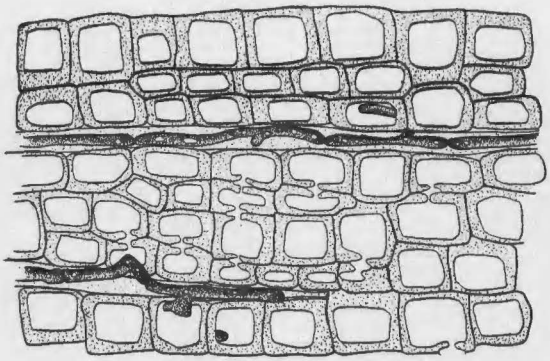


Fig. 2.

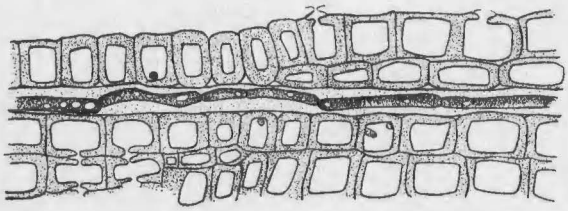


Fig. 3.

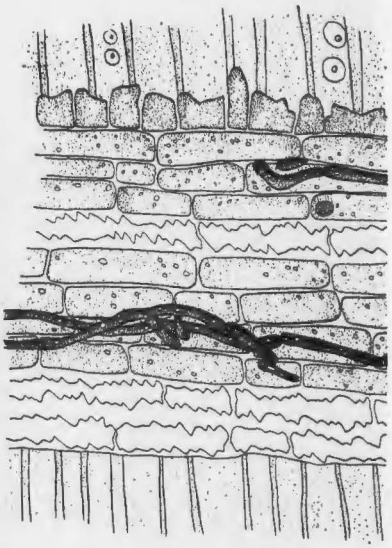


Fig. 4.

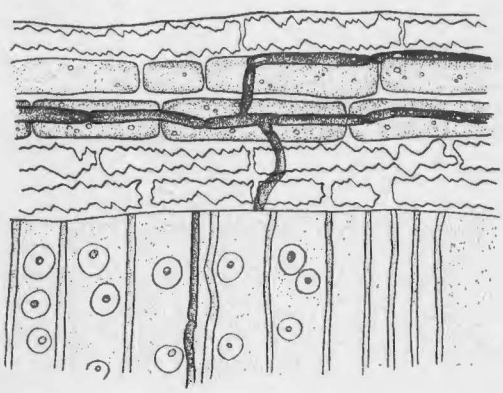
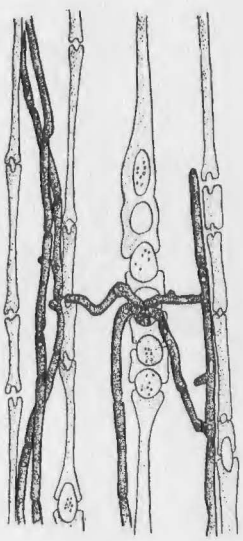


Fig. 5.



Y. NISIKADO and K. YAMAUTI:

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

Studies on *Ceratostomella ips* RUMBOLD, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE LI.

Explanation of Plate LI.

Perithecia of *Ceratostomella ips* RUMBOLD developed on a sterilized block of pine wood (Fig. 1 to 3); and sections of the blue-stained sap wood of *Pinus densiflora* SIEB. et ZUCC. infected by *Ceratostomella ips* (Fig. 4 and 5). These figures were drawn from water mounted preparations with an aid of a camera lucida under ZEISS K 10× and apochromat 40×; and were reduced to one half of original size.

Fig. 1. Basal part of a perithecium, showing the outer appearance. (×250).

Fig. 2. Apical part of the beak of a perithecium, showing the ostiole without any filamentous appendages. This is the most common form of the ostiole. (×500).

Fig. 3. Apical part of the beak of a perithecium, showing the ostiole with some short, frayed cells. (×500).

Fig. 4 and 5. Tangential, longitudinal section of blue-stained sap wood of pine. Showing the dark hyphae penetrating the tracheids and their branches growing into the parenchymatous cells of medullary rays. (×250).

TAFEL LI.

Fig. 1.

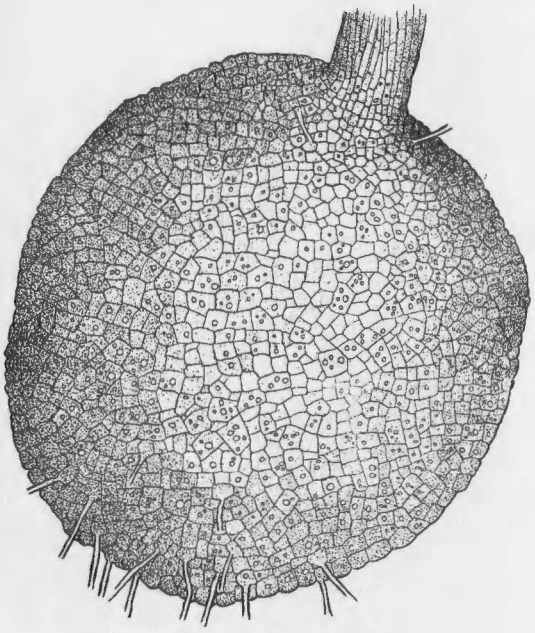


Fig. 2.

Fig. 4.

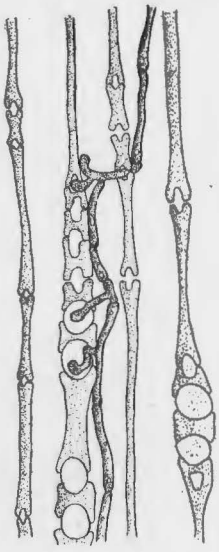


Fig. 5.

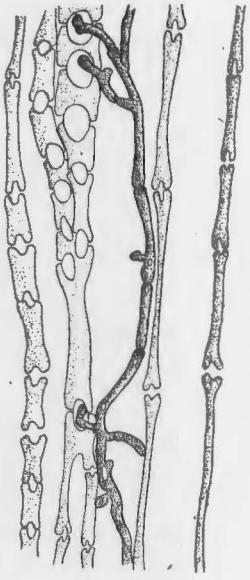


Fig. 3.

Y. NISIKADO and K. YAMAUTI:

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

Studies on *Ceratostomella ips* RUMBOLD, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE LII.

Explanation of Plate LII.

Mycelium of the blue-staining fungus, *Ceratostomella ips* RUMBOLD, developed in pure culture. The figures were drawn from water mounted preparations with an aid of a camera lucida under ZEISS K 10× and oil immersion 60×; and were reduced to two thirds of original size. (×500).

Fig. 1. Mycelium developed on a sterilized block of oak wood. Showing comparatively old, broad and darkened hyphae as well as young, slender, colorless ones.

Fig. 2. Mycelium developed on a "Mizuame" (rice jelly) agar. Showing slender hyphae with budding-like, short celled branches.

TAFEL LII.

Fig. 1.

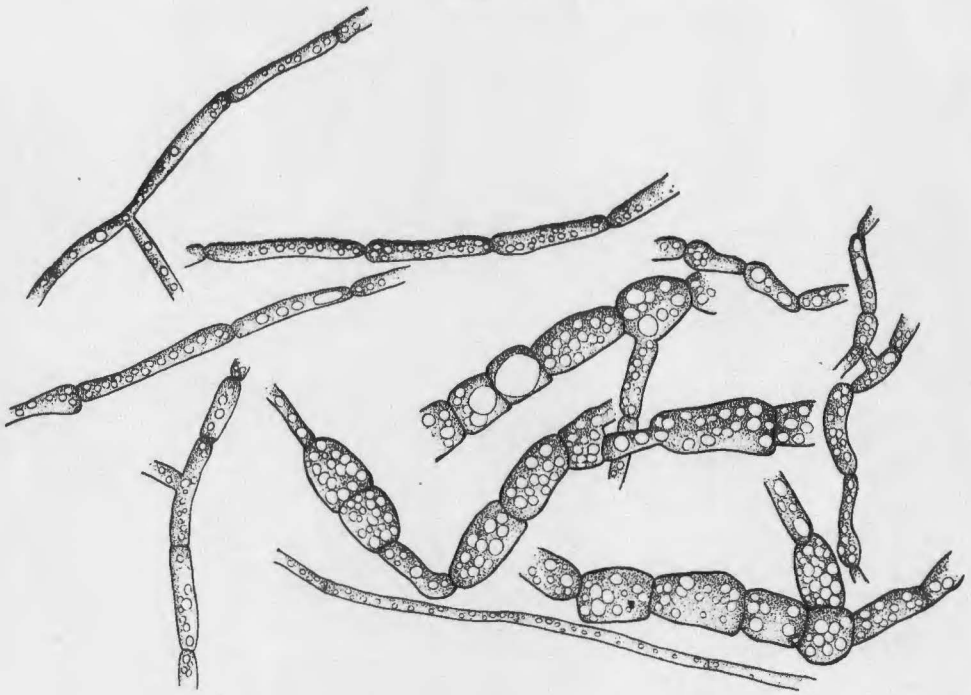
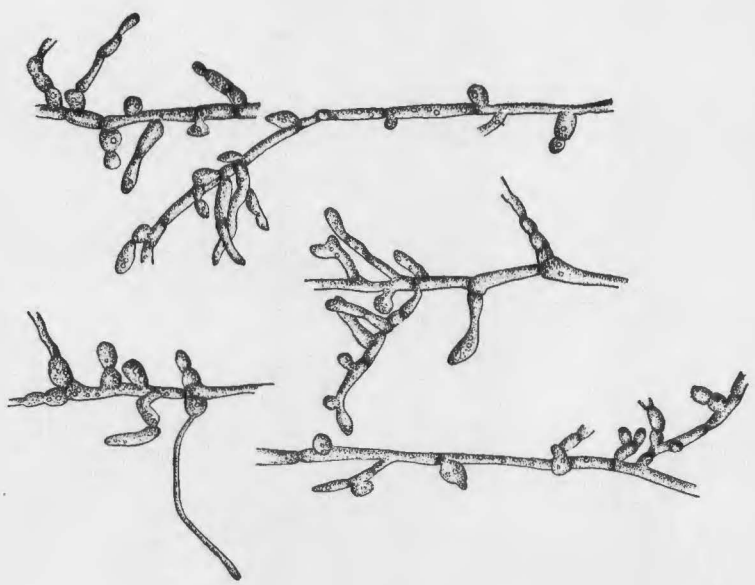


Fig. 2.



Y. Nisikado and K. Yamauti:

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

Studies on *Ceratostomella ips* Rumbold, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE LIII.

Explanation of Ptate LIII.

Conidia and conidiophores of *Ceratostomella ips* RUMBOLD, developed in malt extract agar; and the germination of conidia. The figures were drawn from water mounted preparations with an aid of a camera lucida under ZEISS K 10× and apochromat 40× (Fig. 1) or oil immersion 90×. Fig. 2 was reduced to three quarters of original size.

Fig. 1. Three conidiophores bearing masses of the conidia agglutinated in balls, produced on malt extract agar at 30°C. Two conidiophores in the left developed on a part of the overnight growth of a colony. (×500).

Fig. 2. Germination of conidia in 3% malt extract solution after 24 hours at 24°C. Before the germination conidia swell remarkably, and rarely produce a septum. Germ tubes are produced from one or both ends and sometimes bear secondary conidia. (×750).

TAFEL LIII.

Fig 1.

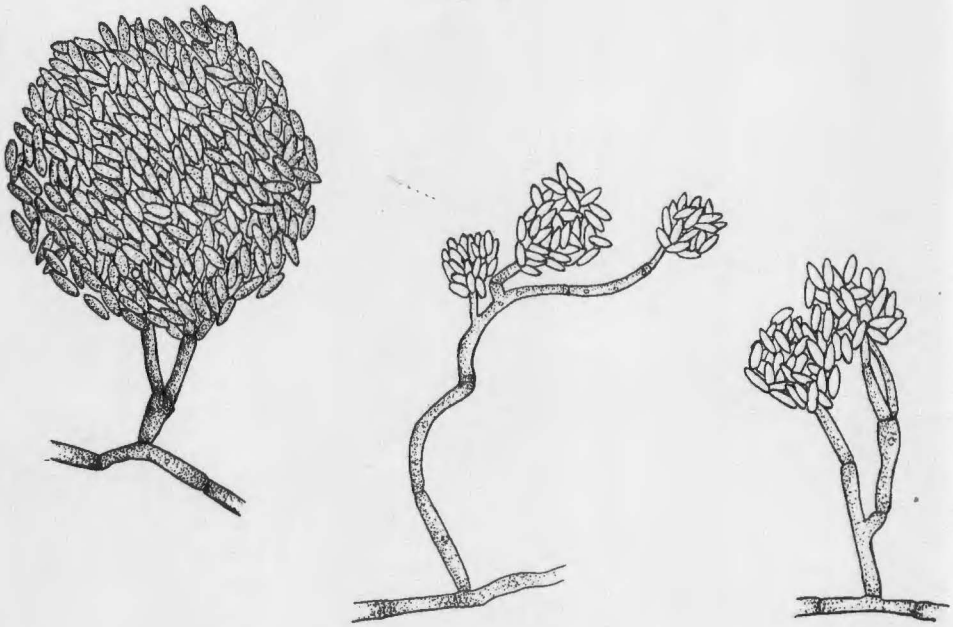
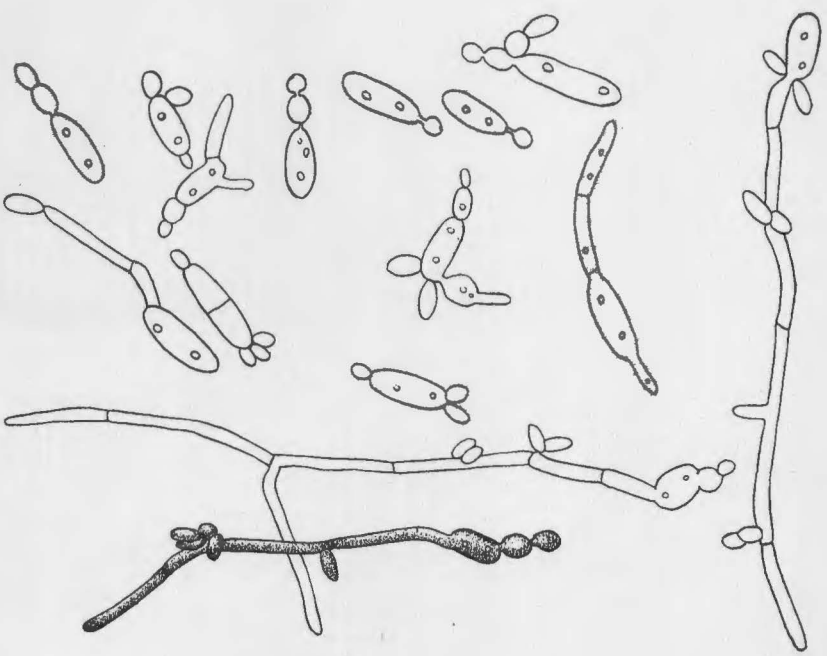


Fig. 2.



Y. Nisikado and K. Yamauti:

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

Studies on *Ceratostomella ips* Rumbold, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE LIV.

Explanation of Plate LIV.

Conidia and conidiophores of *Ceratostomella ips* RUMBOLD, developed on malt extract agar at 30°C. The figures were drawn from water mounted preparations with an aid of a camera lucida under ZEISS K 10× and oil immersion 90×; and were reduced to three quarters of original size. (×750).

Fig. 1 to 4. Conidiophores bearing conidia in *Cephalosporium*-like clusters, developed beneath the surface of medium.

Fig. 5 and 6. Profusely branched conidiophores, which bore large masses of conidia but detached them off, developed on the surface of medium.

TAFEL LIV.

Fig. 1.

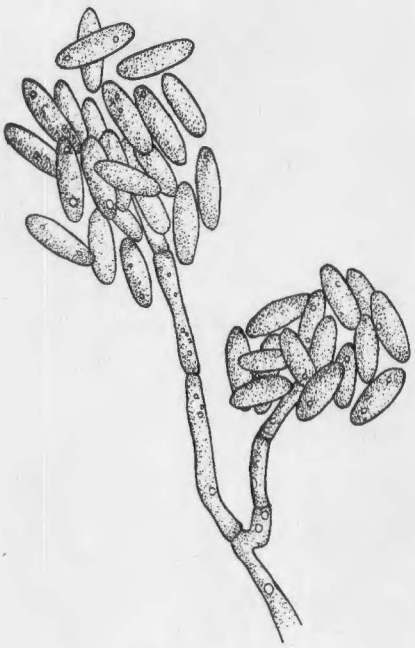


Fig. 4.

Fig. 5.

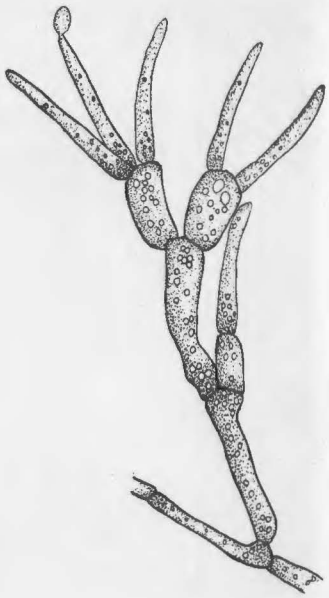
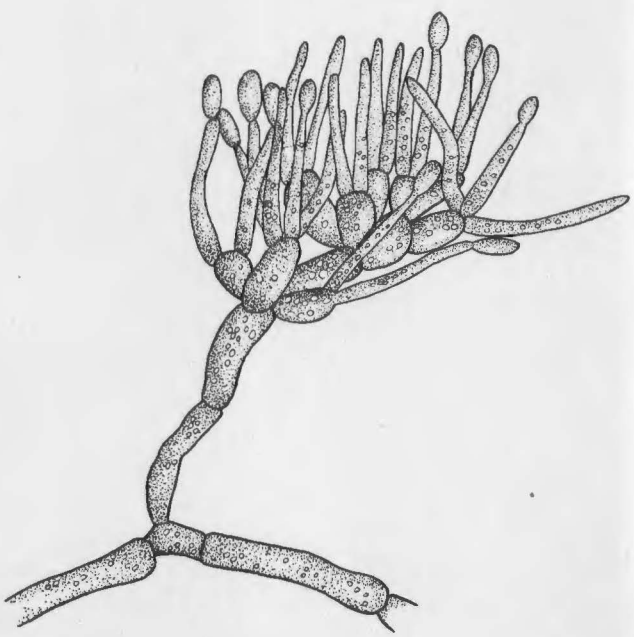


Fig. 2.

Fig. 3.

Fig. 6.



Y. Nisikado and K. Yamauti:

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

Studies on *Ceratostomella ips* Rumbold, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE LV.

Explanation of Plate LV.

Conidia and conidiophores of *Ceratostomella ips* RUMBOLD. The figures were drawn from water mounted preparations with an aid of a camera lucida under ZEISS K 10× or K 20× and oil immersion 90×; and were reduced to three quarters of original size.

Fig. 1 and 2. Conidiophores and conidia developed on malt extract agar at 27°C. after 5 days' incubation. (×750).

Fig. 3. Conidiophores developed on potato dextrose agar at about 27°C. after one month's culture. (×750).

Fig. 4. Conidia developed on potato dextrose agar at about 27°C. after one month's culture. (×1500).

TAFEL LV.

Fig. 1.

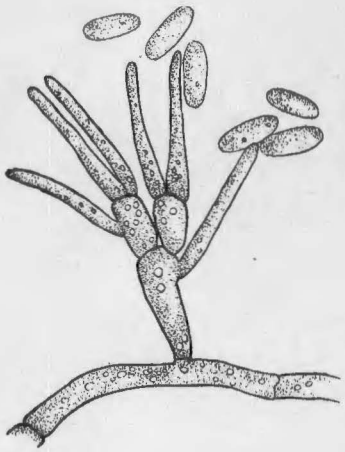


Fig. 2.

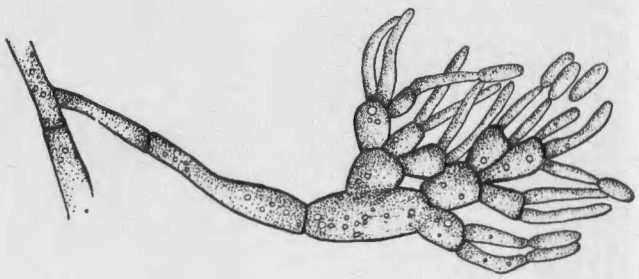


Fig. 4.

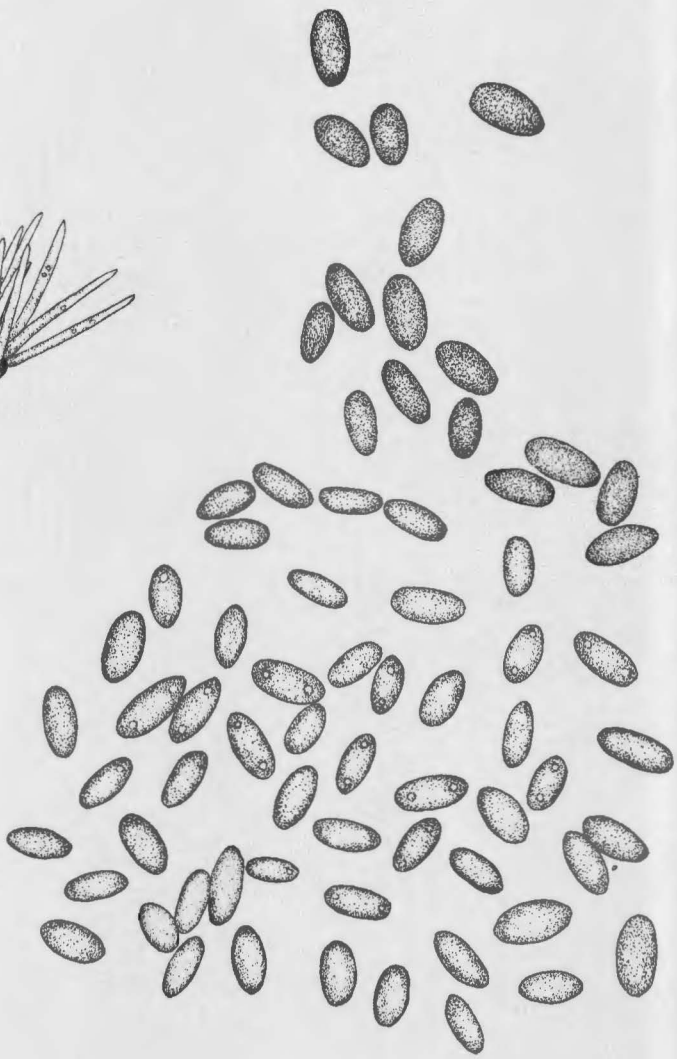


Fig. 3.

Y. Nisikado and K. Yamauti:

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

Studies on *Ceratostomella ips* Rumbold, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE LVI.

Explanation of Plate LVI.

Asci and ascospores of *Ceratostomella ips* RUMBOLD, developed on a sterilized block of pine wood at 27°C. The figures were drawn from water mounted preparations with an aid of a camera lucida under ZEISS K 10× and oil immersion 90×; and were reduced to three quarters of original size. (×750).

Fig. 1 to 3. Asci containing ascospores, discharged from a perithecium into dilute solution of acetic acid. Showing the irregular shape of asci.

Fig. 4. Ascospores from a perithecium, developed on potato dextrose agar at about 27°C.

TAFEL LVI.

Fig. 1.

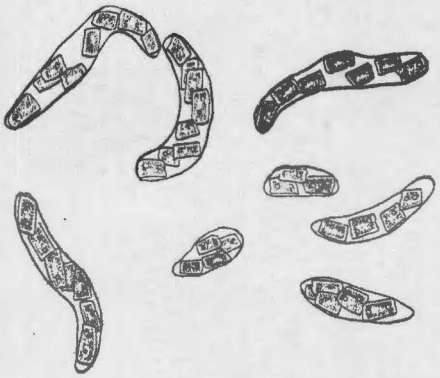
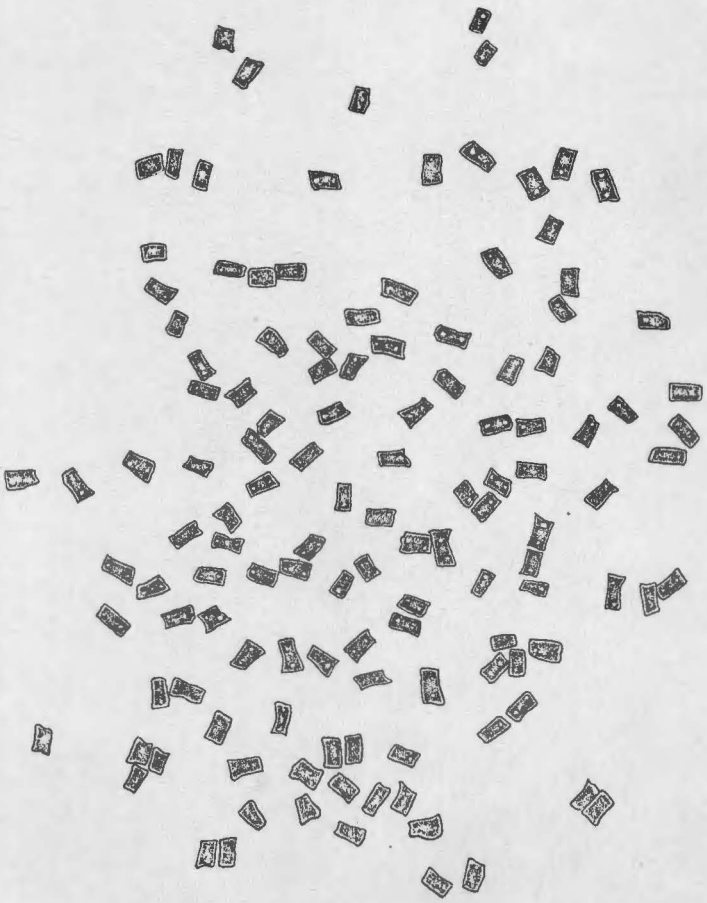


Fig. 2.

Fig. 3.

Fig. 4.



Y. NISIKADO and K. YAMAUTI:

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

Studies on *Ceratostomella ips* RUMBOLD, the Cause of a Blue Stain of Pine Trees in Western Japan.

PLATE LVII.

Explanation of Plate LVII.

Germination of the ascospores of *Ceratostomella ips* RUMBOLD in 3% malt extract solution. The figures were drawn form preparations mounted in malt extract with an aid of a camera lucida under ZEISS K 10× and oil immersion 90× ; and were reduced to three quarters of original size. (×750).

Fig. 1. Germination of the ascospores in malt extract solution at 24°C. after about 30 hours. Before the germination ascospores swell more or less and the ends become round. At the ends of germ-tubes secondary conidia are produced.

Fig. 2. Germination of the ascospores in malt extract solution at 24°C. after 2 days. At the ends and sometimes on the sides of germ-tubes conidia are formed in *Cephalosporium*-like clusters.

TAFEL LVII.

Fig. 1.

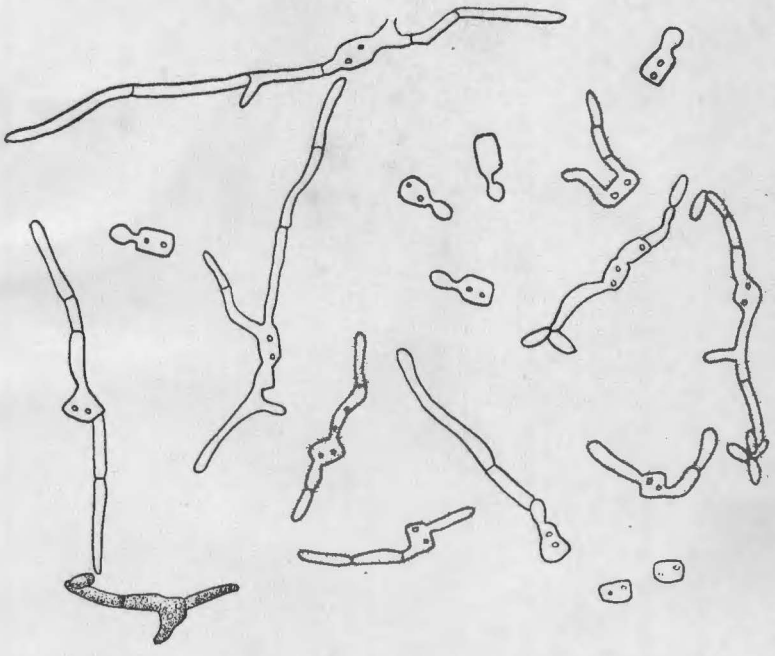


Fig. 2.