カラマツ落葉病の研究—I

病原菌 *Mycosphaerella larici-leptolepis* sp. nov. の生活史

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カラマツの落葉病（葉ふるい病）がはじめて世人の注目をひいたのは大正13年（1924年）9月長野県岩村田付近においてである。つづいて昭和4年（1929年）福島県福島営林署管内のカラマツ林に広大な面積にわたる同一病害が発生し（山田 1931），翌5年（1930年）北島君三が現地調査を行い，本病の病徴および被害状況を報告，病原菌を新種とみとめ，発見者矢野宗幹，窪田円平両氏にちなみ，これを *Phoma Yano-Kubotae* sp. nov. *1 と命名したのは昭和6年のことであつた（北島 1931，1933）。

その後たえて久しく本病に関する実験的研究報告に接しないのであるが，またまた著者の一人伊藤は昭和24年(1949年) 9月，長野県岩村田営林署管内塩野付近のカラマツ植栽地においてこの激害を観察し，ここの資料によつて二，三の実験を行つているうちに，本病の病原菌，伝染経路などについて北島の所説にいささか疑義をもつた。しかし，この疑問を解決しないうちに東京を離れて転勤したのであるが，新任地釜渕分場(山形県最上郡真室川町大字釜渕)構内において以前から注意していた本病の被害林を手近かにひかえる便宜にめぐまれ，昭和28年（1953年）以降，佐藤，太田とともに疑点の究明に努力した結果，予想の正当であつたことが実証され，本病病原菌の生活史，伝染経路などについていささか新知見を加えることができた。

本病は，近年ますますその被害面積を拡大し，甲信越地方，東北地方および北海道などに広く分布し，被害は増大の一途をたどつており，いまやカラマツ造林地にとつて最大な障害の一に数えられる状態にある（井上 1953，伊藤 1953，小野 1956）。しかるに，本病については北島（1931）の報告以外にいまだみるべきものはなく未解決の多くの分野が残されている。著者らは本病について各方面の試験を計画し一部進捗中であるが，とりあえず本病病原菌の生活史と伝染経路に主点をおき，なお病原菌の性質について明らかにした実験結果の一部を公表することにした。

すでに北島（1931）によつて指摘されているように，本病によく似た病害が，かつてヨーロッパで欧州カラマツに発生し，この病原菌は *Sphaerella laricina* R. HARTIG (1895) と命名された。著者らがわが国において見いだした菌はこれと異なるものと考えられるので，新たに *Mycosphaerella larici-leptolepis* sp. nov. と命名することにした。

著者らが本病の研究に着手以来，たえず激励と助言をいただいている保護部長今関六也氏に深く謝意を表するとともに，原図作成に助力された保護部小林享夫技官，藤島淳三技官，中川道夫技官および釜渕分

*1 分類学的通説にしたがえば，本菌は *Phyllosticta* 属として取りあつかうべきである。

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場小野茂夫技官に心から御礼を申しあげる。

病　　徴

7月上旬頃初期の病徴が認められる。最初針葉の表面に微細な褐色小斑点が形成され，これは大きさを拡大し，なおその縁辺付近は少しく褪色して暈帯状を呈する。一針葉上の病斑数は普通 5～7 個であるが，時としては20個以上を数えることもあり，病斑は融合してその幅 1 *mm*以上に達することもまれではない。9月上旬頃病斑上に微細な小黒点が認められるのであるが，これは病原菌の spermogonium である (Plate 1)。

被害林分を遠望すると赤褐色を呈し，山火あるいは晩霜にでもあつたように見え，8月下旬頃からはなはだしい落葉がおこる。被害樹の下方の枝葉は上方のものにくらべて常に被害が大で，連年本病におかされる枝は枯死することがある。これによつて，被害樹はただちに枯死することはないが，早期落葉による生長のそ害の甚大なことは容易に考えられるところである (Plate 2)。

なお本病は成木のみならず，3年生ぐらいの苗木にも発生する。

本 菌 の 生 活 史

北島 (1931) は *Phoma Yano-Kubotae* の“柄子殻”内に形成される“柄胞子”によつて本病は伝播するものと考えたらしいが，これについて実験的にたしかめてはいない。ところでこの形態的特徴，病害の進展状況などから病葉上に形成される“柄子殻”と“柄胞子”は病原菌のスペルモゴニウム時代 (spermogonium stage) で，かんじんの伝染源となる胞子型は越冬した前年の罹病葉上に形成されるのではあるまいかと予想した。

昭和29～31年 (1954～1956年) の3カ年にわたり，山形県釜渕の材料によつて釜渕および秋田市で行つた実験結果は，まさにこの予想をうらがきするものであつた。すなわち，北島(l. c.)のいう“柄胞子”は，いろいろな実験にもかかわらず，まつたくその発芽を認めることができず，形態および形成方法からみて spermatia として取りあつかうべきである。spermatia は9月中旬ごろから認められ，落葉後もひきつづきこれが形成され翌年2月中旬頃まで存在する (Text-fig. 1)。これ以後 spermogonia は空虚になり spermatia は消失する。5月中～下旬頃，越冬落葉に *Mycosphaerella* に属する子嚢菌が完熟し，7月中～下旬まで子嚢胞子の飛散をみとめる (Text-fig. 1)。子嚢胞子の完熟時期は環境条件によつてかなり左右されるものらしく，昭和30年 (1955年) にはほかの2カ年にくらべてやや早かつたのは，3～4月の気温が高かつたからではないかと考えられる (Text-fig. 2)。

子嚢胞子はきわめて容易に発芽し，これによる接種試験および子嚢胞子からの培養による接種試験はいずれも明らかに陽性の結果がえられた。着葉の期間中も，また落葉後もほかの分生子時代は認められなかつた。

上に述べたことがらから，従来“柄子殻”時代とされてきたものは，実は spermogonium 時代で，これは直接本病の伝播に関与するものではなく，伝染源は越冬病落葉に形成される *Mycosphaerella* 属菌の子嚢胞子であることが判明したのである。

本菌の発芽

A. Spermatia

いろいろな液体培養基（2％ブドウ糖液，2％蔗糖液）および寒天培養基（馬鈴薯寒天，2％ブドウ糖寒天，蒸溜水寒天）を使用して数回の発芽試験を行つたが，発芽はまつたくみとめられなかつた。

B. 子嚢胞子

a．各種の培養液と発芽　カラマツ針葉煎汁，2％ブドウ糖加用カラマツ針葉煎汁，2％ブドウ糖液および蒸溜水を使用して発芽試験を行つた。発芽はきわめて容易で胞子各細胞からおのおの1本ずつの発芽管を伸長した（Text-fig. 3; Text-fig. 4, C; Plate 4, A, B)。6時間後の発芽率は2％ブドウ糖液および蒸溜水においてほかの培養液よりも高かつたが，20時間後ではどの培養液でも大差がなかつた(Table 1)。

b．発芽におよぼす温度の影響　本菌の子嚢胞子は 17～30°C で発芽し，最適温度は 25°C (Table 2)。

c．発芽におよぼす湿度の影響　関係湿度 100％および98％において良好な発芽を認め，94％でもわずかに発芽するが92％およびそれ以下ではまつたく発芽しない（Table 3—4)。

寒天培養基上における本菌菌糸の発育

a．各種培養基上の菌叢　馬鈴薯寒天，醤油寒天，WAKSMAN 氏寒天，CZAPEK 氏寒天，RICHARDS 氏寒天およびブイオン寒天の6種に本菌を培養した。本菌の菌糸は馬鈴薯寒天において最も良好な発育をし，ブイオン寒天では不良，ほかの培養基ではこれらの中間を示した（Table 5)。なお，培養基上に分生胞子型の形成はまつたく認められなかつた。

b．菌糸の発育におよぼす温度の影響　醤油寒天を使用し，ペトリ皿法によつてしらべた結果は次のとおりである。すなわち，本菌の菌糸は 20°～25°C おいて良好な発育をするが，35°C ではまつたく発育しない（Table 6)。

c．菌糸の発育におよぼす水素イオン濃度の影響　馬鈴薯寒天を使用しペトリ皿法によつてしらべた結果，pH 2.7～pH 9.0 の間においては菌糸の発育にいちじるしい影響をおよぼさなかつた（Table 7; Plate 4, C)。

本菌の病原性

本菌の病原性を確かめるために，昭和29年（1955年）および同30年の2カ年，釜渕分場において接種試験を行つた。

a．カラマツに対する接種試験　1～3 年生実生苗およびさし木苗に接種を行つた。接種源として子嚢胞子からの単個培養と，子嚢胞子を形成している越冬病落葉を使用した。その結果はいずれの場合も比較対照区を除いて，すべてに発病を認め spermogonia の形成を確認した。なお，苗令の高いものは低いものにくらべて罹病程度はいちじるしく大で，また潜伏期は約1カ月であつた（Table 9)。

b．各種針葉樹に対する接種試験　トドマツ，ヒノキ，スギ，カラマツ，欧州トウヒ，トウヒ，アカマツおよびクロマツの苗に対して，越冬病針葉上の子嚢胞子を接種源として接種を行つた。その結果カラマツだけは明らかに発病したが，ほかの樹種ではまつたく陰性におわつた（Table 9)。

本菌の形態と分類

すでに述べたように，本病の病原菌として北島(1931)が記載した *Phoma Yano-Kubotae* は著者らの研究によつて本病原菌の spermogonium 時代である。沢田(1950)が斑葉病菌として新たに名づけた*Phyllosticta Laricis* は，その記載からみて明らかに *Phoma Yano-Kubotae* と同一と見なしてよいものと考えられる。

カラマツ属 (*Larix*) に寄生する *Mycosphaerella* (*Sphaerella*) は2種記録されている。その一は HARTIG (1895) によつて欧州カラマツ (*L. decidua*) 上に発見命名された *Sphaerella laricina* であり，その二は沢田 (l.c.) による *Mycosphaerella cryptomeriae* SHIRAI et HARA である。

S. laricina R. HARTIG は本菌とにているが，子嚢がいちじるしく小さいことと分生子褥と棍棒状の分生胞子時代を有すること，および spermogonia の形成状態からみてこれらを同一種として取りあつかうことはできない (Table 10)。次に *M. cryptomeriae* は原 (1918) によつてスギに記載されたもので形態，培養上の性質からみて (伊藤・渋川・小林 1952) 本菌とは明らかに別種である。そのほかの針葉樹に記載された *Mycosphaerella* (*Sphaerella*) 属菌類の中に本菌に該当するものは見いだされない。それで著者らは本菌を未記載のものと認め *Mycosphaerella larici-leptolepis* sp. nov. と命名することにした。

本菌の和文記載は次のとおりである (Text-fig. 4; Plate 3)。

Mycosphaerella larici-leptolepis sp. nov.

Syn. *Phoma Yano-Kubotae* KITAJIMA (1931) (nom. nud.)

Phyllosticta Laricis SAWADA (1950)

子嚢殻は葉の両面に孤生あるいは群生，黒色，球形，はじめ埋没しのちに突出，孔口小さくやや乳頭状，大きさ 88～156×84～142μ。子嚢は棍棒状円筒形，大きさ 44～99×7～12μ，8胞子を含み，側糸を欠く。子嚢胞子は不規則に2列にならび，舟型，2胞より成り各細胞の大きさ不等，隔膜部でくびれ，無色，大きさ 11～18×3～5μ。

spermogonia は多くは葉の表面に生じ，球形，黒色，大きさ 83～165×74～143μ，spermatia は細菌状，単胞，無色，大きさ 3～5×0.5～1μ。

子嚢殻はカラマツの越冬落葉上に形成され，また spermogonia は生葉および落葉上に認められる。

付　図　説　明

Plate 1.　カラマツ落葉病

右は罹病針葉の拡大図で，病斑上の小黒点は病原菌の spermogonia

Plate 2.　落葉病にかかつたカラマツ

A，C，昭和24年10月5日撮影 (山形県釜渕)

B，昭和25年9月24日撮影 (同上)

Plate 3.

A，病原菌 *Mycosphaerella larici-leptolepis* sp. nov. の spermogonium　×310

B，同上　×450

C，*M. larici-leptolepis* の子嚢殻　×310

D，同上　×450

E，*M. larici-leptolepis* の子嚢および子嚢胞子　a．子嚢；s，子嚢胞子　×800

Plate 4.

A，*M. larici-leptolepis* の子嚢胞子の発芽　×250

B，同上　×500

C，*M. larici-leptolepis* の菌糸の発育におよぼす pH の影響

a，pH 2；b，pH 2.2；c，pH 2.7；d，pH 3.6；e，pH 4.0；f，pH 4.6；g，pH 5.0；h，pH 5.3；i，pH 6.5；j，pH 7.0；k，pH 7.6；l，pH 8.2；m，pH 9,0；n，pH 10.4；o，pH 10.8

Studies on the Needle Cast of Japanese Larch-I. Life history of the causal fungus, *Mycosphaerella larici-leptolepis* sp. nov.

Kazuo ITÔ, Kunihiko SATÔ and Noboru ÔTA

Introduction

The first authentic record of this disease in Japanese larch (*Larix leptolepis* (SIEB. et ZUCC.) GORDON., *L. kaempheri* SARG.) plantations was made by M. YANO in Nagano Prefecture in 1924. Nothing more appears to have been written concerning this disease until 1931 when KITAJIMA (1931)[10] reported that he had found it in Fukushima Prefecture the previous year. In the same year, YAMADA (1931)[19] noted that the larch stands in Fukushima Prefecture, about 1,000 *ha* in area, were found to be severely affected by this disease.

The disease has become increasingly prevalent during the past decade, and the destructive effects of repeated heavy attacks are very apparent. The occurrence of numerous affected trees in extensive stands often presents an alarming appearance. This disease is thus a matter of concern to the forest managers.

A short note describing briefly the disease and the causal fungus was made by KITAJIMA (1931)[10]. He named the fungus *Phoma Yano-Kubotae* nov. sp. in honor of Messrs. M. YANO and E. KUBOTA who were the first actually to see the disease, but no further studies of this disease have been made in the meantime so far as the authors know.

From the evidences mentioned above, it was considered that there were many phases of the subject still requiring further extensive investigation, and that clarification of the biology and life history of the fungus was needed in order to develop control measures and to forecast more accurately the occurrence and severity of attack. The present work was undertaken in 1949 to study (1) the life history, (2) cultural characteristics, (3) spore production and dissemination, (4) pathogenicity of the causal organism, (5) pathological physiology of the host, and (6) control measures. This article is the first report of these investigations and deals with the biology and life cycle of the causal fungus, with particular emphasis on the source of primary inoculum. Hereafter, on other phases of the disease, the progress of the study will be reported in the series under the same title. A portion of this paper has already been published in preliminary reports (ITÔ 1953[8], ITÔ, SATÔ & ÔTA 1956[9]).

The authors take this opportunity to express their heartiest thanks to Mr. Rokuya IMAZEKI, Chief of the Forest Protection Division of the Government Forest Experiment Station, under whom some of this work was conducted, for many valuable suggestions. They are also indebted to Messrs. Takao KOBAYASHI, Zyunzô FUJISHIMA, Michio NAKAGAWA and Shigeo ONO for their assistance in preparing the illustrations.

Symptoms and damage

The earliest indication of the disease occurs usually during the first week in July.

Generally, scattered spots on the needle are first infected. They are first minut brown and surrounded by a faint yellow halo. As the disease progresses, these spots increase in size and coalesce to attain a width of 1 *mm* or more. Lesions are counted 5 to 7, rarely 20, a needle. The discolored needles bear small black fruit bodies on the upper surface of the dead area that prove to be spermogonia of the causal fungus. From a distance the infected trees give the appearance of having been scorched by fire or injured by late frost.

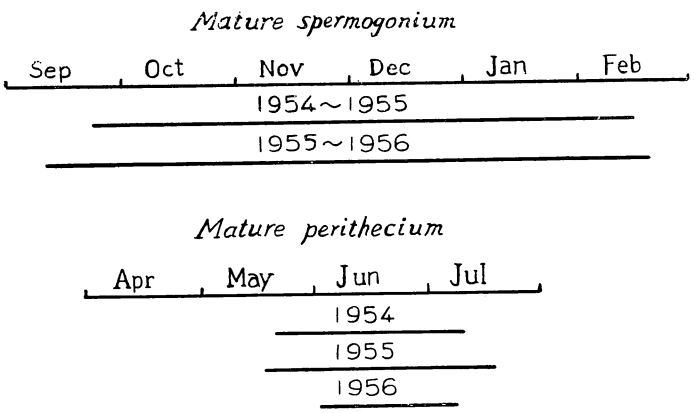
The characteristic symptom of the disease is a browning of the needles over all or part of the crown. This browning is most conspicuous in the summer and autumn and gives the impression that the tree is dying. Later the diseased needles drop off, leaving the trees with all or portions of their crowns thin and the remaining needles confined to tufts at the end of the branches. Commonly, more needles in the lower crown of a diseased tree are blighted than in the upper crown, and small trees with their crowns close to the ground are often completely diseased. Repeated serious defoliations may bring about a considerable decrease in growth increment, and frequenlt result in the death of shoots and twigs (Plates 1, 2).

In mixed hardwood-larch plantations, the damage of this disease is said to be usually very slight or not observed at all. And although it is in plantations that trees are most seriously affected, seedlings and saplings are also subject to the needle cast.

A detailed and extensive survey has never been conducted, but the disease is probably widespread throughout most of Japan, especially in the Kô-shin-etsu, Tôhoku and Hokkaidô districts (INOUYE 1953[5], ITÔ 1953[8], ONO 1956[13]).

Life history of the fungus

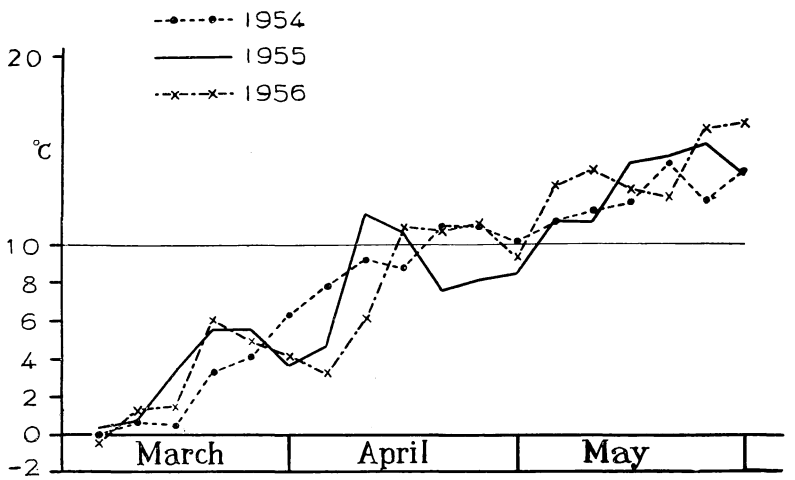
The causal organism of the disease *Phoma Yano-Kubotae* sp. nov. was described by KITAJIMA (1931)[10]. He thought that the disease was probably infected by the "pycnospores" formed in the "pycnidia", though he had not made any experimental trial. From the morphological characteristics and the progress of the disease, the senior author, however, presumed that the "pycnidia" and "pycnospores" existent on green needles



Text-fig. 1 Mature spermogonium and perithecium formation of *Mycosphaerella larici-leptolepis* sp. nov. at Kamabuchi and Akita.

might be the spermogonial stage and the ascigerous stage constituting the source of inoculum probably formed in over-wintered fallen needles affected the previous year. This assumption was soon verified by the authors' life historical studies carried out at Kamabuchi, Yamagata Prefecture and Akita city for three successive years (1954～1956).

In the middle of September, spermogonia discharging spermatia were present on the affected needles. In the latter part of November, numerous diseased needles which had been collected at Kamabuchi were placed outdoors at Akita city in flooress wire cages, so that the needles would be in contact with the soil. Examinations of the stored material were made at intervals of about two weeks to trace the development of the fungus during the winter and the following seasons.



Text-fig. 2　Mean air temperature of each half decade at Akita (from the records observed at Akita Weather Station).

The spermogonia were actively discharging spermatia when observed at intervals between November and February of the following year, and all of the spermogonia became empty in March (Plate 3, A, B). Repeated attempts to germinate the spermatia in various media have been unsuccessful.

Perithecia began to develop during the late autumn and winter, but did not sufficiently differentiate to be recognized as perithecial primordia until early March. Ascospores matured in late May or mid-June; matured spores were at first obtained in late May in 1954, in mid- May in 1955, and in early June in 1956, respectively (Text-fig. 1). The diversity in the time of the fungous maturity may be probably attributed to the differences of the environmental conditions in these years, especially to the temperature (Text-fig. 2). The ascospores continued to produce within decaying needles left lying on the ground unitl mid-July.

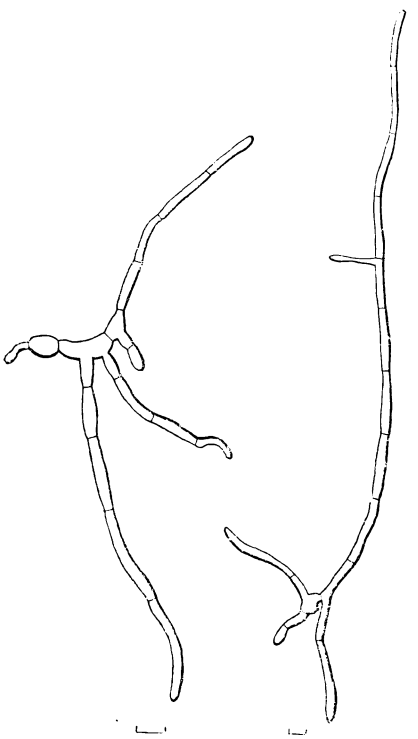
The ascospores germinate very readily in a few hours and the evidence points to the fact that they furnish the inoculation of the disease.

Morphological features of the fungus in the ascigerous stage agree well with the description for the genus *Mycosphaerella* (Plate 3, C, D).

Germination of spermatia and ascospores

A. Spermatia

Germination of the spermatia was tested by the VAN TIEGHEM cell method using 2 per cent dextrose and 2 per cent sucrose solutions, but no germinating spermatia were obtained in either solution. Another experiment was undertaken on the following agar media: Potato sucrose agar*1, 2 per cent dextrose agar*2 and water agar*3. No germination occurred also in this experiment. In all of the repeated experiments the authors have failed to successfully germinate the spermatia of the fungus.



Text-fig. 3 Germinating ascospores of *M. larici-leptolepis* sp. nov. (—=10μ).

B. Ascospores

The ascospore of the fungus germinated within several hours after liberation from an ascus. Usually a germ-tube grew from each cell of the spore (Plate 4, A, B; Text-fig. 3).

Germinaton in several nutrient solutions

Ascospores were collected on the over-wintered fallen needles on June 6, 1955 and germination tests were made by the VAN TIEGHEM cell method using the following solutions: Larch needle decoction*4, larch needle decoctioion plus 2 per cent dextrose solution*5, and distilled water. Results of the experiment at 25C° are summarized in table 1.

As shown in table 1, germination percentage at the end of six hours is larger in 2 per cent dextrose soution and distilled water than in the other solutions, but, at the end of 20 hours, there were no remarkable differences in germination percentage among the nutrient solutions tested.

Effect of temperatures on germination

Drop of the spore suspension were placed on sterile slide glasses in Petri dishes, keeping them in moist condition, and then all the spores were incubated at different temperatures. Results of the experiment at the end of each of 6 and 20 hours are given respectively in table 2.

Table 2 shows that germination of ascospores occurs at the temperatures ranging from 17° to 30°C with an optimum at 25°C. At 10° and 35°C, germination did not take place in these experimental periods.

Effect of relative humidities on germination The authors made an investigation

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*1 Distilled water 1 *l*, potato 200*g*, sucrose 20*g*, agar-agar 20*g*.

*2 Distilled water 1 *l*, dextrose 20*g*, agar-agar 20*g*.

*3 Disitilled water 1 *l*, agar-agar 20*g*.

*4 Distilled water 1 *l*, larch needles 20*g*.

*5 Distilled water 1 *l*, larch needles 20 *g*, dextrose 20*g*.

Table 1. Germination of ascospores in several nutrient solutions.

Germination / Nutrient solution	After 6 hours		After 20 hours	
	Germination percentage (%)	Max. length of germ-tube (μ)	Germination percentage (%)	Max. length of germ-tube (μ)
Larch needle decoction	11	12	77	62
Larch needle decoction plus 2 % dextrose	4	4	79	85
2 % dextrose solution	28	16	94	132
Distilled water	22	22	77	101

Table 2. Effect of temperaturs on germination of ascospores.

Germination / Temperature (°C)	After 6 hours		After 20 hours	
	Germination percentage (%)	Max. length of germ-tube (μ)	Germination percentage (%)	Max. length of germ-tube (μ)
6 ± 2	0	—	0	—
10 ± 2	0	—	0	—
17 ± 1	13	4	71	47
20 ± 1	72	21	79	69
25 ± 1	79	23	94	70
28 ± 1	53	18	60	69
30 ± 1	11	7	26	66
35 ± 1	0	—	0	—

Table 3. Effect of relative humidities on germination of ascospores—I.
(After 20 hours)

Salt in over-saturated solution	Relative humidity (%)	Germination percentage (%)	Max. length of germ-tube (μ)
H_2O	100	77	92
K_2SO_4	98	56	77
KNO_3	94	7	5
K_2HPO_4	92	0	—
KCl	87	0	—
KBr	84	0	—

Table 4. Effect of relative humidities on germination of ascospores—II.

Salt in over-saturated solution	Relative humidity (%)	After 6 hours		After 20 hours	
		Germination percentage (%)	Max. length of germ-tube (μ)	Germination percentage (%)	Max. length of germ-tube (μ)
H_2O	100	35	19	98	101
K_2SO_4	98	37	19	79	101
KNO_3	94	0	—	+	+
K_2HPO_4	92	0	—	0	—
KCl	87	0	—	0	—
KBr	84	0	—	0	—

of the effect of relative air humidity upon germination of the ascospores by the method reported in the previous paper (ITÔ & HOSAKA 1957[7]). Resultes of the repeated experiments are presented in tables 3—4.

From tables 3—4, it is clear that the germination is generally favoured at a saturated atmosphere and at 98 per cent relative humidity, respectively, and the ascospores geminated slightly in 94 per cent humidity, while those kept at 92 per cent humidity and below 92 per cent show no signs of germination.

Mycelial growth on agar media

Isolation and culture Single-ascospore isolations were obtained by a modification of the YOSHII's method (YOSHII 1933[20], ITÔ & HOSAKA 1952[7]). Generally, the mycelial growth on agar media was not vigorous. Conidial forms have never been found in culture on media.

Mycelial colonies on various agar media The isolate of the fungus was cultured on potato agar plates, and for the inocula the margin of the mycelial colonises was cut with a sterile needle into small pieces and then these were transplanted to following agar media: Potato sucrose agar*1, SAITÔ's soy agar*2, WAKSMAN's solution agar*3, Czapek's solution agar*4, RICHARDS' solution agar*5, and bouillon agar*6.

Macroscopic appearances of the mycelial colonies after about a month at 25°C are briefly noted in table 5.

Effect of temperatures on mycelial growth The relation of temperature to the growth of the mycelium was examined by the Petri dish method using SAITÔ's soy agar.

*1 Distilled water 1 *l*, potato 200*g*, sucrose 20*g*, agar-agar 20*g*.

*2 Distilled water 850 *cc*, onion decoction 100*cc*, Japanese soy 50*cc*, sucrose 50*g*, agar-agar 20*g*.

*3 Distilled water 1 *l*, peptone 5*g*, KH_2PO_4 1*g*, $MgSO_4 \cdot 7H_2O$ 0.05*g*, dextrose 10*g*, agar-agar 20*g*.

*4 Distilled water 1 *l*, $MgSO_4 \cdot 7H_2O$ 0.5*g*, K_2HPO_4 1*g*, KCl 0.5*g*, $NaNO_3$ 2*g*, $FeSO_4$ 0.01*g*, sucrose 30*g*, agar-agar 20*g*.

*5 Distilled water 1 *l*, KNO_3 10*g*, KH_2PO_4 5*g*, $MgSO_4 \cdot 7H_2O$ 2.5*g*, $FeCl_2$ trace, sucrose 50*g*, agar-agar 20*g*.

*6 Distilled water 1 *l*, peptone 10*g*, meat extract 10*g*, NaCl 5*g*, agar-agar 20*g*.

Table 5. Macroscopic appearances of mycelial colonies on various agar media.

Agar medium	Degree of mycelial growth	Color of mycelial colony
Potato sucrose agar	++++	Olive-Gray
SAITO's soy agar	+++	Light Olive-Gray
WAKSMAN's sol. agar	+++	Deep Olive-Gray
CZAPEK's sol. agar	++	Olive Light-Gray
RICHARDS' sol. agar	++	Smoke Gray
Bouillon agar	+	Pale Olive-Buff

Table 6. Effect of temperatures on mycelial growth.

Temperature (°C)	5 ± 2	15 ± 3	20 ± 1	25 ± 1	30 ± 1	35 ± 1
Diameter of colony (*mm*)	3	7	15	11	6	0

Results obtained at the end of three weeks are presented in table 6.

From table 6, it can be seen that the mycelium grows favorably at 20—25°C, but no growth is observed at 35°C.

Effect of H-ion concentrations on mycelial growth The relation of H-ion concentrations to the mycelial growth was tested with potato sucrose agar in Petri dishes. By addition of certain amounts of normal HCl or NaOH solutions, the H-ion concentration of agar medium after sterilization was varied as follows: pH 2, 2.7, 3.6, 4.0, 4.6, 5.0, 5.3, 6.5, 7.0, 7.6, 8.2, and 9.0. Effects of pH value on the mycelial growth were determined by taking the averaged diameters of the colonies at the end of 20 days at 25°C. Results of the experiment are given in table 7 (Plate 4, C).

Table 7. Effect of H-ion concentrations on mycelial growth.

pH	Diameter of colony (mm)
2.0	0
2.7	10
3.6	12
4.0	12
4.6	12
5.0	12
5.3	12
6.5	11
7.0	11
7.6	10
8.2	9
9.0	9

As shown in table 7, the influence of H-ion concentration is not remarkable in the media with exponents ranging from 2.7 to 9.0, so far as examined by such a simple method.

Pathogenicity of the fungus

In order to make clear the pathogenicity of the fungus to Japanese larch and some other conifers, inoculation experiments were performed at Kamabuchi, Yamagata Prefecture in 1955 and 1956.

Inoculation experiment to Japanese larch Potted seedlings and cuttings of various ages were prepared for the inoculation test. The 4-week-old fungus culture

which had been derived from the mono-ascosporous isolate and cultured on potato sucrose agar was used as the inoculum. The fungus colonies from the slants were first broken in sterile distilled water and then filtered through double sheets of cotton cloth.

On June 7, 1955, the needles of the plants were inoculated by atomizing with the fungous suspension on the surfaces of the needles. Then, these plants were placed in a humidity chamber and held at 100 per cent relative humidity for 48 hours. The check plants were sprayed with sterile water instead of the fungous suspension.

Careful observations were continued for more than three months after inoculation. On the inoculated needles the first indication of the disease appeared on July 8. The disease progressed gradually, and the conspicuous symptoms showing spermogonial formation and defoliation were brought about in early September. It may be said that the older the seedlings were, the severer was the damage caused. In check plants, no sign of the disease was observed on any of the plants even after three months. Results of the experiment examined on September 2 are summarized in table 8.

Table 8. Inoculation experiment to Japanese larch.

Plant No.	Plant	Treatment	Degree of infection	Spermogonium formation
1 ～ 16	0-1-seedling	Inoculated	+	+
17 ～ 20		Control	−	−
21 ～ 28	1-2-seedling	Inoculated	++	+
29 ～ 32		Control	−	−
33 ～ 38	2-3-seedling	Inoculated	+++	+
39 ～ 42		Control	−	−
43 ～ 46	Cutting*1)	Inoculated	+	+
47 ～ 48		Control	−	−

Note: *1)....The plants were propagated from cuttings made in 1952 and had grown 2 years in the nursery.

On the same date as the experiment mentioned above, another inocultion test was undertaken to the potted 3-year-old seedlings with the over-wintered diseased needles as the inoculum. The first sign of the disease appeared distinctly about a month after inoculation, and severe infection occurred at the end of two months.

The evidences presented herein show that the fungus is doubtlessly pathogenic to Japanese larch, and the incubation period of this disease is about a month.

Inoculation experiment to various conifers Pathogenicity of the fungus to various coniferous seedlings was tested by using the over-wintered diseased needles as the inoculum. Plant species used in this experiment were as follows: *Abies Mayriana, Chamaecyparis obtusa, Cryptomeria japonica, Larix leptolepis, Picea excelsa, P. hondoensis, Pinus densiflora* and *P. Thunbergii*. In the spring of 1956, fifteen seedlings of each of all these species were planted at a distance of 20 *cm* apart in the nursery at Kamabuchi. On May 8, the over-wintered fallen needles which had been gathered from the larch plantation affected by the disease the previous year were placed among the seedling in a thickness of 1 *cm*. Results of the experiment obtained in early September are briefly summarized in table 9.

Table 9.　Pathogenicity of the fungus to various conifers.

Plant species	Age of plant tested	Pathogenicity
Abies Mayriana	2-4-seedling	−
Chamaecyparis obtusa	3-4- 〃	−
Cryptomeria japonica	2-3- 〃	−
Larix leptolepis	2-3- 〃	+
Picea excelsa	2-4- 〃	−
Picea hondoensis	2-4- 〃	−
Pinus densiflora	2-3- 〃	−
Pinus Thunbergii	2-3- 〃	−

As shown in table 9, no positive results were obtained in any of the plant species tested except the larch. Therefore, it seems clear that the fungus is selectively pathogenic toward the genus *Larix*.

Morphology and taxonomy of the fungus

Morphology

Spermogonia are formed on both green and fallen needles throughout the autumn and winter. They arise meristogenously by the branching of a hypha and the fusion of these branches. Then, by a rapid division of cells, a spherical stromatic mass is formed. The cells at the center of this mass, which are to become the spermatium mother cells, become larger and stain deeper than those of the periphery. The spermatia are liberated through a sterigma-like process, after which the walls of the old mother cells disintegrate into a mucilaginous matrix which invests the spermatia. This process continues centrifugally so that a mature spermogonium consists of a globose cavity filled with spermatia held together in a mucilaginous matrix of degenerating mother-cell walls. The spermogonium wall consists of several cell layers of a brown, thick-wall element, 28～41μ in width. The spermogonia measure 83～165×74～143μ. The mature spermogonia are filled with a great number of hyaline, rod-shaped spermatia, 3～5×0.5～1μ in size (Plate 3, A, B).

Perithecia are formed abundantly on the fallen needle in contact with the soil. Early in March structures that are interpreted to be perithecical initials are observed. The perithecia become differentiated into an inner pseudoparenchymatous medullary portion, surrounded by an outer layer or rind of a thickness of brownish, thick-walled cells. The medulary tissues disappear as the asci develop. The perithecia develop either singly or in groups, and they at first are embedded within the host tissues, but later they become erumpent. The ascospores mature in the latter part of May at Kamabuchi, Yamagata Prefecture.

Mature perithecia are amphigenous, single or in groups, partially erumpent, globose, slightly papillate and measure 88～156 × 84～142μ. Asci are clavate-cylindrical, measure 49～99×7～12μ and contain eight ascospores. Paraphyses are absent. Ascospores are hyaline, unequally two-celled, constricted at septum, and 11～18 × 3～5μ in size (Plate 3, C, D, E; Text-fig. 4).

Taxonomy

KITAJIMA (1931)[10] found a fungus on the lesion of casting needles of Japanese larch and named it *Phoma Yano-Kubotae* sp. nov. Recently another fungus belonging to the family Phomaceae was described by SAWADA (1950)[17] under the name of *Phyllosticta Laricis* sp. nov. According to the authors' research, *Phyllosticta Laricis* is unquestionably congeneric with *Phoma Yano-Kubotae*. When SAWADA described *Phyllosticis Laricis* sp. nov. he was probably unaware that the same fungus had been described previously by KITAJIMA. Morphological characteristics of *Phoma Yano-Kubotae* as well as *Phyllosticta Laricis* are very accordant with those of the spermogonial stage of the causal organism.

A survey of the mycological literature dealing with *Mycosphaerella* (*Sphaerella**1) on *Larix* reveals the fact that only two species of *Mycosphaerella* have been recorded to occur on species of *Larix*. These are *Sphaerella laricina* R. HARTIG (HARTIG 1895[3], SACCARDO 1900[16]) and *Mycosphaerella cryptomeriae* SHIRAI et HARA (SAWADA 1950)[17]. HARTIG (1896)[4] reported that *S. laricina* described on European larch (*Larix decidua*) attacked also Japanese larch.

Table 10. A comparison of *Sphaerella laricina* with the present authors' fungus.

	Sphaerella laricina R. HARTIG	*Mycosphaerella* of the authors
Perithecium	Die zu Anfang Juni ausgereiften Perithecien sind den Conidienpolstern an. Färbung ähnlich, aber etwas kleiner als diese, d. h. zwischen 0.1 bis 0.15 *mm* gross .... und hier entwickelten sich schon bis zum 15. Mai in einer Anzahl der Perithecien reife Ascosporen. Die keulenförmigen Ascen sind 0.05～0.06*mm* lang, enthalten je 8 anfänglich einzellige, später zweizellige Sporen von 0.015～0.017 *mm* Länge, die farblos und an beiden verjüngten Enden abgerundet sind.	In early March the perithecial initials are formed on the overwintered fallen needles. Mature ascospores are observed in late May. Perithecia measure 88～156 ×84～142*μ*. Asci are clavate-cylindrical and 49～99×7～12*μ* in size. Ascospores are hyaline, unequally two celled, constricted at septum, and measure 11～18×3～5*μ*.
Conidium	....Auf der Aussenseite dieser schwarzen Polster entwickeln sich nun zahllose stabförmigen Conidien von 0.03 *mm* Länge. Sie stehen auf kurzen, an der Spitze farblosen pfriemenförmigen Basidien und sind anfänglich einzellig. Bei der Reife zeigen sie eine und später drei Querwänd, so dass sie demnach vierzellig sind ...In Nährgelatinelösung verbracht,... stabförmigen vierzelligen Conidien auf kleine seitlichen Auswüchsen entwickeln, die auf den Conidienpolstern der Lärchennadeln entstehen.	None
Spermatium (Micro-conidium)	....die zuerst dünn scheibenförmigen Conidienlager, die dann zu pseudo-parenchymatischen schwarzbarun Polsterns ich verdicken und die Epidermis sprengen. Im Inner dieser Polster entstehen Höhlungen, deren Wände mit sehr zarten Basidien besetzt sind. Letztere bilden an der Spitze ausserordentlich kleine Mikroconidien. Diese zellen sind nur 0.003 *mm* lang und 0.001 *mm* breit.	Spermogonia are formed on both green and fallen needles throughout the autumn and winter. They are at first subepidermal, later break through the epidermis and become black. Mature spermogonia measure 83～165×74～143 *μ*. Spermatia are hyaline, rod-shaped and measure 3～5 × 0.5～1 *μ*.

*1 The generic name, *Sphaerella* was formerly used instead of *Mycosphaerella*.

S. laricina is very similar to the authors' fungus in several respects, and accordingly a morphological comparison of the two species will be noted in table 10.

Althouth, as shown in table 10, the morphological characters of *Sphaerella laricina* closely resemble those of the present fungus, the asci of the latter are remarkably larger than those of the former. Furthermore, there are some differencces in the process of the spermogonium formation between the two fungi. The most conspicuous diversity found in these species may be the fact that *S. laricina* produces "stabförmigen Konidien" on both the host and the artificial medium, but, on the contrary, *Mycosphaerella* of the authors has no conidial form. Unfortunately the authors have never made a direct comparison with the type specimen of *S. laricina*, but it is probably different from the fungus under consideration.

Considered from the morphological and cultural characters (ITÔ, SHIBUKAWA & KOBAYASHI 1952)[6], *M. cryptomeriae* which was originally described on *Cryptomeria japonica* by HARA (1918)[2] is clearly distinct from the authors' fungus. None of the following Mycosphaerellae (Sphaerellae) described on coniferous trees by the earlier workers is identical with the authors' fungus: *Sphaerella Taxi* COOKE on *Taxus baccata* (SACCARDO 1882)[14], *S. Taxodi* COOKE on *Taxodium* (SACCARDO 1883)[15], *S. Podocarpi* COOKE on *Podocarpus* (SACCARDO 1883)[15], *Mycosphaerella sequoiae* BONAR on *Sequoia* (BONAR 1942)[1], *M. hypodermellae* WEHMYER on *Pinus* (WEHMYER 1946)[18] and *M. Thujopsidis* SAWADA on *Thujopsis* (SAWADA 1950)[17].

Since a search of the literature by the authors has failed to disclose any species like the fungus under consideration, it is regarded as a new species and is here described as follows:

Mycosphaerella larici-leptolepis sp. nov.

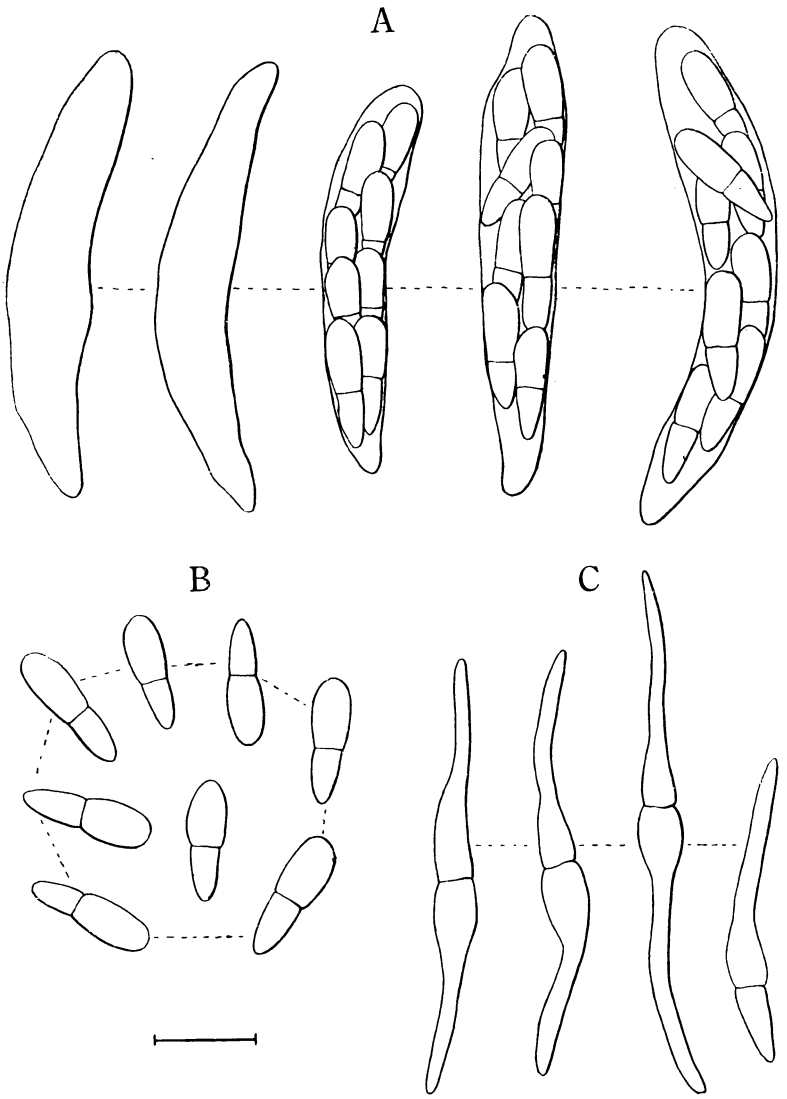
Syn. *Phoma Yano-Kubotae* KITAJIMA (1931) (nom. nud.)
Phyllosticta Laricis SAWADA (1950)

Maculis brunneis; peritheciis amphigenis, saepissime tamen hypophyllis, sparsis vel aggregatis, nigris, globosis, per epidermidem erumpentibus, ostiolo minuto papillulato, 88～156×84～142μ; ascis cylindraceis-clavatis, 44～99×7～12μ, aparaphysatis, octosporis; sporidiis irregulariter 2-stichis, naviculatis, 11～18 × 3～5μ, constricto-1-septatis, loco superiore crassiore, hyalinis.

Hab. in acubus deciduis, Laricis leptolepis (May 22, 1954, Kamabuchi, Yamagata, Japan, by K. SATÔ and N. ÔTA*1).

Spermogoniis in autumno efformantibus, epiphyllis, innatis, punctiformibus, globosis atris, 83～165 × 74～143 μ; spermatiis bacillaribus, hyalinis, 3～5 × 0.5～1 μ.

*1 The type specimen has been deposited in the Herbarium of the Government Forest Experiment Station, Meguro, Tokyo, Japan.



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Text-fig. 4　*M. larici-leptolepis* sp. nov. in perfect stage (—=10μ).
A, asci and ascospores; B, ascospores;
C, germinating ascospores.

Consideration and conclusion

NAMBU (1916)[12] reported a brief note on a foliage disease of dwarf larch trees ("Bonsai") and adopted *Sphaerella laricina* HARTIG as the scientific name of the causal fungus, though he had never made detailed investigations. The first reliable account on the needle cast of Japanese larch was published by KITAJIMA (1931)[10], who named the causal organism *Phoma Yano-Kubotae* sp. nov. The fungus, *Phyllosticta Laricis*, described by SAWADA (1950)[17] as a causal agent of the needle cast of larch is quite accordant with *Phoma Yano-Kubotae*.

As reported already by KITAJIMA (1931[10], 1933[11]) and SAWADA (1. c.), it is evident that the "pycnidia" containing numerous "pycnospores" occur abundantly and universally in affected needles. Therefore, the "pycnidial" stage has long been accepted as the cause of infection and spread of the needle cast. While making a survey of the larch plantations for the disease it was observed that the lesions were always evenly spaced and distributed over the affected tree in a manner expected only where the inoculum is wind-borne. Moreover no instances were found where needles opened after mid-summer were severely diseased. Because of the failure of the so-called pycnospores to germinate or grow in or on various media, and since their characteristic small size and appearance make them unsuitable for wind dissemination, it is concluded that they are not pycnospores but spermatia. Correspondingly, the structures which produce them should be called spermogonia, not pycnidia.

Further investigation of the disease has resulted in the discovery of the perfect stage of the fungus, which is here named *Mycosphaerella larici-leptolepis* sp. nov. The fungus over-winters principally as immature perithecia in the tissue of fallen needles on the ground. Though the time in maturity is somewhat influenced by environmental conditions, the fungus matures in late spring, and ascospores are presented during about two months, from late May to mid-July. The ascospores from the infected needles in the previous season germinated very well in a few hours, and proof of the pathogenicity of the perfect stage of the fungus was obtained in the inoculation experiments initiated early June. From these foregoing facts it may be asserted that the ascospores from over-wintered fallen needles carried by air currents, cause infection of the current season's needles on the trees in nature. The incubation period of the disease has been found to be about a month by inoculation tests, and this is very accordant with the fact that the first symptom of the disease in larch stands usually appears in July.

In the case of *Sphaerella* (*Mycosphaerella*) *laricina*, the causal organism of the needle cast of European larch, there have been two sources of inoculum, ascospore and conidium (HARTIG 1895)[3], but the authors' *Mycosphaerella* lacks the conidial stage in its life history. Since the spermatia have not been observed to germinate, and it is unlikely that they disseminate the fungus, the role of the ascospores in the epidemiology of the disease is the most important.

Recognition of the fact that *Mycosphaerella larici-leptolepis* sp. nov. is a fungus possessing only one infection stage and no conidia, indicates that ascospores produced on the infected needles fallen the previous year constitute only the source of inoculum. This bears directly upon a correct interpretation of the disease cycle of needle cast, and

upon its control.

Summary

The present paper deals with the results of an investigation on the needle cast of Japanese larch with special emphasis on the causal organism.

Phoma Yano-Kubotae described by KITA JIMA (1931)[10] as the pathogen of the disease is the spermogonial stage of the causal fungus. The ascigerous stage of this organism is formed in the tissues of the over-wintered fallen needles attacked the previous year. The fungus was described by the authors as a new species to science under the name of *Mycosphaerella larici-leptolepis* sp. nov.

Mature ascospores are disseminated during late May to mid-July and they constitute the source of inoculum.

Inoculation experiments to several conifers gave results showing that all the conifers except Japanese larch were not attacked by the fungus, and that the incubation period of this disease was about a month.

Furthermore, effects of the environmental factors upon the germination of ascospores and the growth of mycelium were made clear experimentally.

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Explanation of plates

Plate 1

The needle cast of Japanese larch.

Plate 2

Japanese larch trees afffected by the needle cast. At Kamabuchi, Yamagata Prefecture.

A, C, Photographed on Oct. 5, 1954; B, Photographed on Sept. 24, 1955.

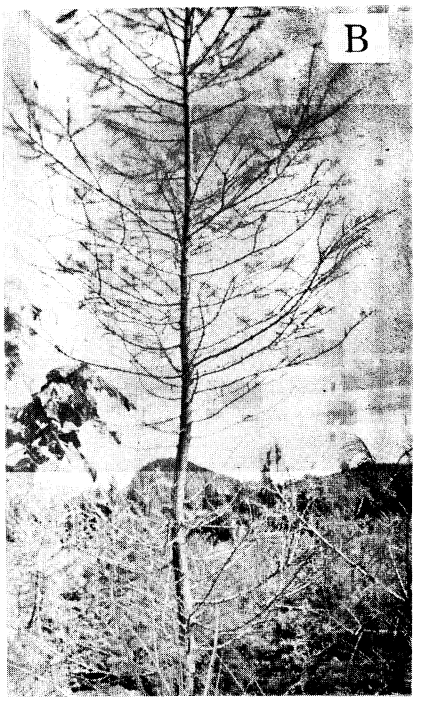
Plate 3

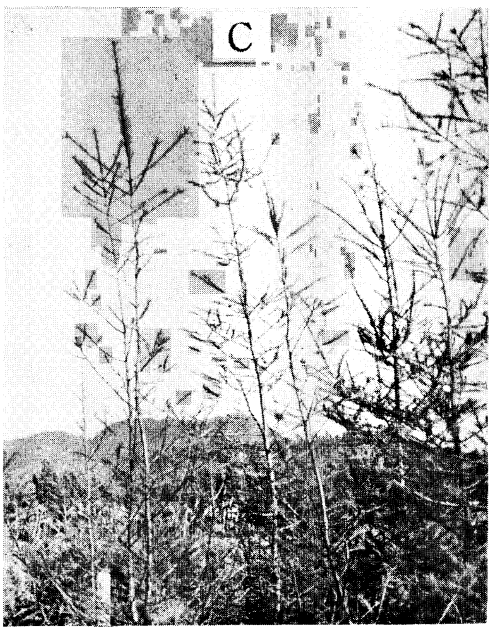
A, Spermogonium of *Mycosphaerella larici-leptolepis* sp. nov. × 310.
B, Ditto. × 450.
C, Perithecium of *M. larici-leptolepis* sp. nov. × 310.
D, Ditto. × 450.
E, Asci and ascospores of *M. larici-leptolepis* sp. nov. × 800.
a, asci; s, ascospores.

Plate 4

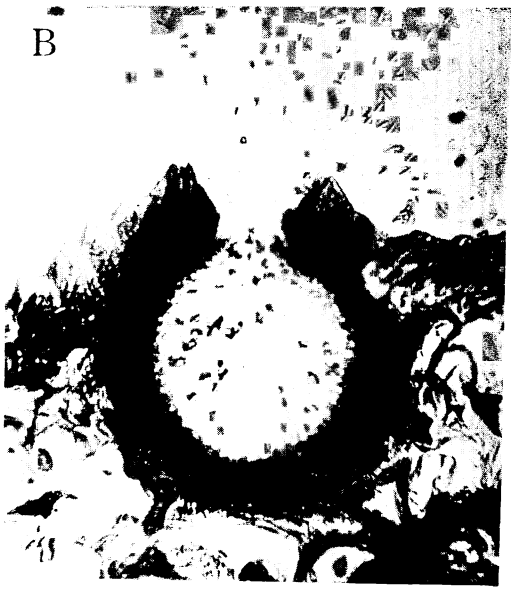
A, Germinating ascospores of *M. larici-leptolepis* sp. nov. × 250.
B, Ditto. × 500.
C, Effect of H-ion concentrations on the mycelial growth of *M. larici-leptolepis* sp. nov.
a, pH 2; b, pH 2.2; c, pH 2.7; d, pH 3.6; e, pH 4; f, pH 4.6; g, pH 5; h, pH 5.3; i, pH 6.5; j, pH 7; k, pH 7.6; l, pH 8.2; m, pH 9; n, pH 10.4; o, pH 10.8.

—Plate 2—






A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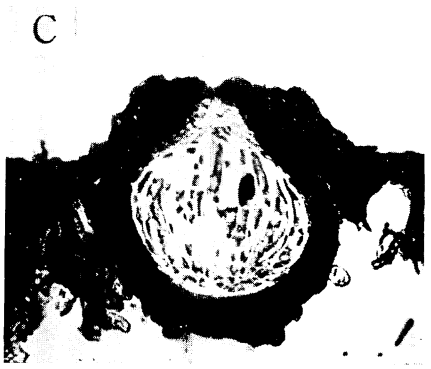
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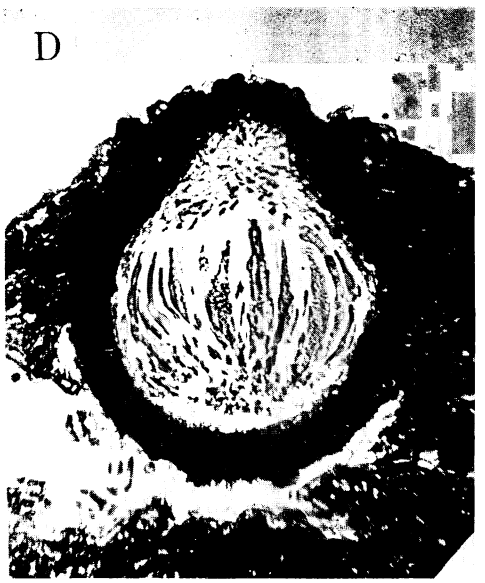
D


E
s
a

—Plate 4—