広葉樹の斑点性病害に関する研究—IV

クルミの新病害　白黴葉枯病

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昭和25年（1950）秋，著者らは山梨，山形両県下において，いわゆる“frosty mildew”症状を呈するオニグルミの病害を発見した。その後これは東京都，埼玉県および宮城県にも見い出され，なおオニグルミのみならずヒメグルミにも被害を与えていることがわかつた。これまでのところ，そう大きな被害はないが，病状はかなりはげしいものであるから，将来クルミの重要病害にかぞえられる可能性は充分にある。

本病は不完全菌類の *Cercosporella* によるものであるが，くわしい研究の結果，病落葉上に見い出される子嚢菌 *Sphaerulina* と，この *Cercosporella* 菌の同根関係が立証され，*Sphaerulina* 菌は *Cercosporella* 菌の完全時代であることがわかつた。

本報文はこの新病害について，主として病原菌の生理，生活史および寄生性に重点をおいて行つた諸実験結果をのべたもので，なお本菌は未記載のものと考えられるので，これを *Sphaerulina juglandis* sp. nov. と命名することにした。

本研究を実施するにあたり有益な御助言と激励をいただいた，保護部長今関六也氏および原図作成に助力された中川道夫氏に心から感謝の意を表する。

病徴および標徴

初期の病徴は7月上旬に認められ，夏の終りから秋にかけて被害は特に顕著にあらわれる。これによつて病樹は枯死することはないが，罹病葉は早期落葉するので生長は阻害され，これは特に苗木において著しい。

はじめ葉の表面に褐色小斑としてあらわれ，ついで円形～楕円形となり，漸次拡大して灰褐～淡灰褐色不整斑となり，往々にして病斑周縁には水浸状の褪色帯を生ずる。病斑初期においては，葉の裏面にはほとんど変化が認められない。

これらの病斑表面は直ちに白粉を撒いたような状態になり，なお時として病斑は癒合して巨大になり，葉の大部分が結霜状の白粉でおおわれることがある。はげしく侵された罹病葉はまきちじれて早期に落葉する（Plate 1）。

病原菌の越冬

昭和25および26年10月に，山形県最上郡及位村釜渕で採集したオニグルミの病葉を東京にはこび，戸外

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において越冬させて，病原菌の経過消長をしらべた。

12月上旬においてすでに，病落葉上の分生胞子のほとんど大部分は脱落消失し，翌年の1～3月には spermatia を充満する spermogonium が多量に形成される。spermogonium の多くは子座内に生じ，また病斑以外の部分にも形成される。

子嚢殻は11月中旬ころその始原体が認められるが翌年1月ころになるといつそう明瞭になり，子嚢胞子は6月中旬にいたつて成熟する。この形態は *Sphaerulina* 属の特徴をよくそなえている。本病の第一次伝染はこれら子嚢胞子によるものと考えられる。

病原菌の生理的性質

1. 分　　離

分生胞子と子嚢胞子から本菌の純粋培養をえた。すなわち分生胞子の懸濁液に2％硫酸銅液を滴加し，これを2％ブドウ糖寒天にぬり，胞子の発芽をまつて単個培養を行つた。つぎに子嚢胞子からの場合は，越冬病落葉の小片をペトリ皿のふたの内面につり，これから2％ブドウ糖寒天上に子嚢胞子を落下させ，その後数時間を経て発芽子嚢胞子から単個培養をえた。

2. 培養上の性質

菌叢の発達　単一分生胞子から出発した菌叢の発育はきわめて遅々としており，25°C，1カ月後にその直径はやつと 10*mm* に達するぐらいである。菌叢はやや隆起して凹凸があり，淡黄色を呈してちようど酵母あるいはバクテリアの菌叢によくにている。顕微鏡検査によつて，この菌叢は分生胞子の集塊であることがわかつた (Plate 2, A, b).

2カ月後には，菌叢は暗緑色になり，その表面はうすい白色の気中菌糸によつておおわれる (Plate 2, A, a)。菌叢表面の気中菌糸は細くて 2μ の幅を有し，多数の隔膜をもち無色で，この部分には分生胞子がみとめられない。これに反して，菌叢内部は多量の緑褐色厚膜胞子様体からなり (Text-figs 1, 2)，また分生胞子および spermogonium も介在する。

子嚢胞子の単個培養の特徴は分生胞子からの場合と多くの点においてよく一致する。

各種寒天培養基上における菌叢の発育　分生胞子および子嚢胞子の単個培養から，菌叢の小片をいろいろな寒天培養基に移植して菌叢の発育状態をしらべた。供試培養基は，馬鈴薯寒天，2％ブドウ糖寒天，醤油寒天，RICHARDS 氏寒天，CZAPEK 氏寒天，WAKSMAN 氏寒天，ブイオン寒天，クルミ葉煎汁寒天およびアスパラギン寒天の9種とした。

その結果は，馬鈴薯寒天，醤油寒天，RICHARDS 氏寒天，CZAPEK 氏寒天，WAKSMAN 氏寒天およびアスパラギン寒天において良好な発育をしたが，これに反して2％ブドウ糖寒天，ブイオン寒天およびクルミ葉煎汁寒天では発育不良であつた。なお，*Cercosporella* 時代の分生胞子からの菌株と *Sphaerulina* 時代の子嚢胞子からの菌株の間には差が認められなかつた (Table 1～Table 2) (Plate 2, B)。

菌叢の発育におよぼす温度の影響　ペトリ皿法により馬鈴薯寒天を使用して実験を行つた。本菌は10～28°Cでよく発育し，最適温度は 25°C，最低，最高はそれぞれ 1°C および 28°C である (Table 3～Table 4) (Plate 5, A)。

菌叢の発育におよぼす水素イオン濃度の影響　ペトリ皿法，馬鈴薯寒天によつてしらべた結果によると水素イオン濃度は本菌の発育に対して顕著な影響をおよぼさないが，pH 5.6～6 が最適のようである

(Table 5) (Plate 5, B)。

3. 培養基上における分生胞子の形成

発芽にあたつて分生胞子の各細胞は著しく膨大し，胞子両端細胞および中間細胞から発芽管を伸長させる。発芽管が生長するとまもなく隔膜を生じかつ分岐し，やがて発芽管の基部細胞は膨大して，ここから分生胞子を新生する。新生胞子はまた上記経過をたどつて発芽し，ついで分生胞子を形成するという順序をくりかえして多量の分生胞子の形成がみられ，なお古い細胞は暗色化して厚膜細胞になるわけである。したがつて菌叢は比較的永い間分生胞子と厚膜胞子様体からのみできている状態を呈する(Text-figs. 3, 4, 5)。

各種培養基上における分生胞子の形成　上記9種の寒天培養基上における分生胞子の形成程度を比較すると，これは菌叢の発育程度にほぼ等しい。2%ブドウ糖寒天をのぞけばいずれの培養基においても分生胞子の形成は，はなはだ良好である (Table 6)。

分生胞子の形成におよぼす温度の影響　馬鈴薯寒天を使用して行つた実験結果は 1～28°C において分生胞子が形成され，15～28°C で特に良好である (Table 7)。

分生胞子の形成におよぼす水素イオン濃度の影響　水素イオン濃度は分生胞子の形成に大きな影響をおよぼすことはなく，pH 4.2～7.6 においてよく形成されるが，pH 3.4 では形成不良である (Table 5)。

4. 分生胞子の発芽

分生胞子は2%ブドウ糖寒天 25°Cで数時間内に発芽する。胞子の各細胞はまずいちじるしく膨大するためあたかも念珠状になり，やがて両端細胞および中間細胞から発芽管を出す。しかしすべての細胞から発芽管を突出することはまれで1胞子から3～4本の場合がもつとも多い (Text-fig. 3)。

各種培養基上における分生胞子の発芽　2%ブドウ糖寒天，2%蒸溜水寒天およびクルミ葉煎汁寒天上における発芽状態を 25°Cの定温でしらべた。供試菌株は分生胞子から単個培養したものと子嚢胞子からの2つとし，いずれも馬鈴薯寒天上に形成された胞子を使用した。結果はいずれの寒天培養基上でも大差なく良好な発芽をなし，発芽率は 80～90%をかぞえた (Table 8)。

分生胞子の発芽と経過時間との関係　上と同一の方法によつてしらべた結果，分生胞子は 2～4 時間で発芽開始し，24時間後には発芽率90%以上に達した (Table 9)。

分生胞子の発芽におよぼす温度の影響　2%蒸溜水寒天および2%砂糖寒天を使用して実験を行つたところ，8～35°Cで発芽し，特に 20～28°Cで発芽は良好であつた (Table 11～Table 12)。

分生胞子の発芽におよぼす水素イオン濃度の影響　Van Tieghem cell 法，蒸溜水によつてしらべた結果は，発芽は水素イオン濃度に影響されることはすくなく，pH 2～9.6 の広い範囲にわたつてみとめられたが pH 6 付近を最適とするもののようである (Table 13)。

分生胞子の発芽におよぼす関係湿度の影響　塩類の過飽和水溶液を使用する方法によつて各段階に空気湿度を調節した。実験結果は関係湿度 100%区においてよく発芽したが94%では発芽するものきわめてすくなく，また92%以下での発芽はまつたく認められなかつた (Table 14)。

5. 子嚢胞子の発芽

子嚢胞子は条件がよければ数時間で発芽を開始し，14時間後には約 100%の発芽率を示す。なお，発芽の状態は分生胞子の場合ときわめてよくにている (Text-fig. 6)。

子嚢胞子の発芽におよぼす温度の影響　越冬病葉の小片をペトリ皿蓋の内面につつて子嚢胞子を2%蒸溜水寒天に落下させたのち，所定の定温器に移入し24時間後に各温度における発芽状態をしらべた。子嚢

胞子発芽の最適温度は 22°C と 25°C の間にあり，3°C 付近を最低温とするもののようで，分生胞子が発芽した 30°C および 35°C においては子嚢胞子の発芽はみられなかつた (Table 15)。

子嚢胞子の発芽におよぼす水素イオン濃度の影響　2～7%蒸溜水寒天を使用して行つた実験によると，胞子の発芽におよぼす水素イオン濃度の作用はいちじるしいものではなく，pH 6.2 付近で他にくらべてやや良好な程度であつた (Table 16)。

本菌の病原性

本菌の病原性を明らかにするために，数回にわたつて人工接種試験を行つた。

1. クルミ類に対する接種試験

昭和26年 (1951) 春～秋に，オニグルミ，ヒメグルミおよびシナノグルミの3種について接種試験を実施した。

実験—1　6月18日に，*Cercosporella* 時代の分生胞子から分離した菌株をもちい，噴霧接種法によつた。供試3樹種とも明らかな病徴を示し，潜伏期はオニグルミおよびヒメグルミでは18～21日，シナノグルミではやや長く21～25日であつた (Table 17) (Plate 2, C, D)。

実験—2　*Cercosporella* 時代の分生胞子から分離した菌株と *Sphaerulina* 時代の子嚢胞子からの菌株の2つを用い，上と同一方法によつて9月15日に実施した。結果はいずれの樹種にも明らかな病斑が形成され，病斑上には *Cercosporella* の分生胞子および分生子梗がおびただしく認められ，なお供試両菌株の間に病原性の差はまつたくなかつた。この実験においてもシナノグルミでの潜伏期はオニグルミ，ヒメグルミに比べてやや長い結果を示した (Table 18) (Plate 3)。

2. いろいろな樹種に対する接種試験

本菌の寄主範囲をたしかめるために，クルミ類をも含めて，15科，19属，28種の樹木に対して接種試験を行つた。本実験は昭和27年 (1952) と翌28年 (1953) の2回，噴霧接種法によつて実施した。供試樹種は次のとおりである。

イチヨウ，モニリヘラヤマナラシ，シモニドロ (テリハドロ)，オニグルミ，ヒメグルミ，シナノグルミ，ペカン，ヤシャブシ，ハンノキ，マテバシイ，アカガシ，クヌギ，カシワ，シラカシ，コナラ，アベマキ，ケヤキ，コウゾ，ハンテンボク，コブシ，モミジバスズカケ，シロハギ，ニセアカシア，ウルシ，マサキ，ツバキ，アオキ，トネリコ。

実験結果をみると，本菌はクルミ類だけに病原性を示し，他のすべての樹種を侵す能力はまつたくないことがわかつた (Table 19～Table 20)。

本菌の形態と分類

これまで述べて来た実験結果からみて，生葉上の *Cercosporella* と落葉上の *Sphaerulina* の同根関係に疑をはさむ余地はまつたくない。それで *Sphaerulina* は *Cerecosporella* の完全時代であり，またこれは明らかに本病の病原菌であるという結論がみちびかれるわけである。

次には本菌の各世代における形態学的特徴を述べ，さらにその分類学的所見にふれることにする。

1. Cercosporella 時代

子座は主として寄主の気孔内に生じ，これを構成する菌糸はほとんど無色，子座の直径は 55～71μ。分

生子梗は子座から束状に突出して，直立あるいはわずかに曲り，短くて単胞，無色，その大きさ 32～68×3～5μ。分生胞子は通常彎曲あるいは，さらに巻き，まれに真直，棍棒状を呈して無色，1～10 の隔膜を有し両端円く，大きさ 32～68×3～5μ (Table 21, Text-fig. 7)。

これまでクルミ属に記載された *Cercosporella* 菌はまつたく見い出されず，近縁なものとしては *Cercospora juglandis* KELL. et SAW. がある。しかし，これは分生子梗および分生胞子の色，形，大きさからみて，本菌とはまつたく別種である。

各種の樹木に記載された *Cercosporella* 菌は次のとおり多数ある。すなわち *C. Evonymis* ERIKSS.—*Euonymus*, *C. pirina* ELL. et EV.—*Pirus*, *C. prolificans* (ELL. et EV.) SACC.—*Sambucus*, *C. ulmicola* v. HÖHNEL—*Ulmus*, *C. Aceris* DEARN. et BARTH.—*Acer*, *C. Alni* DEARN. et BARTH.—*Alnns*, *C. Mori* PECK—*Morus*, *C. maculans* (BERENG.) WOLF＝*Mycosphaerella Mori* (FUCKEL) WOLF—*Morus*, *C. arachnoidea* WOLF＝*Mycosphaerella arachnoidea* WOLF—*Morus*, *C. rubi* (WINTER) PLAKIDAS—*Rubus*, *C. persica* SACC.＝*Mycosphaerella persica* HIGGINS et WOLF—*Prunus*, *C. Caryigena* (ELL. et EV.)＝*Mycosphaerella caryigena* DESM. et COLE—*Carya*, *C. theae* PETCH＝*Calonectria theae* LOOS—*Thea*.

上にあげたもののうちクルミ科に属するペカンに寄生する *C. caryigena* は形態的に著者の菌と差が認められ，なお著者らの菌はペカンを侵さないこと，および *C. caryigena* の完全時代は *Mycosphaerella* であることからしておのおのまつたく別種である。

そのほかいずれの菌も形態および病原性において本菌と一致しない。

2. Spermogonium 時代

病落葉の裏面に形成される spermogonium は直径 65～113μ，無数の spermatia を含む。spermatia はまつたく発芽しない (Table 22) (Plate 4, A, B)。

3. Sphaerulina 時代

子嚢殻は主として越冬落葉裏面に形成され，その初期には spermogonium との区別が困難である。

子嚢殻は孤生または群生，球形，小乳頭状，大きさ 99～118×71～99μ，子嚢は棍棒状円筒形，端部円く，側糸を欠き，8個の子嚢胞子を含み，大きさ 43～56×8～9μ。子嚢胞子は 2～3 列に並び，円筒状紡錘形でわずかに彎曲し，隔膜においてくびれることなく，3～7の隔膜を有し，無色，大きさ 24～35×3～4μ (Table 23) (Plate 4, C～G; Text-fig. 8)。

著者らがしらべた範囲内ではクルミ属に寄生する *Sphaerulina* に関する記載は見当らない。従来木本性植物に記載された *Sphaerulina* 属菌類をあげると次のとおりである (Table 24)。

S. myriadea (DC.) SACC.—*Quercus* および *Fagus*, *S. serograpta* (DURR. et MONT.) SACC.—*Quercus*, *S. fraxinea* SACC. et SPEG.—*Fraxinus*, *S. intermixta* (B. et BR.) SACC.—*Rubus*, *S. camelliae* PASS.—*Camellia*, *S. phellogena* D. SACC.—*Acer*, *S. tilliaris* FAUTR. et LAMB.—*Tilia*, *S. Pruni* MCALP.—*Prunus*, *S. Aucubae* SHIRAI et HARA—*Aucuba*, *S. Rubimoriforiae* HARA—*Rubus*, *S. Fuji* HARA—*Kraunhia*, *S. Euptelaceae* HARA—*Euptella*, *S. Magnoliae-Kobusii* HARA—*Magnolia*, *S. Rubi* DEMAREE et WILCOX—*Rubus*.

これらのうち形態的にみて本菌に類似しているのは *S. fraxinea* であるが，本菌はトネリコ (*Fraxinus*) に対して病原性を示さないことから *S. fraxinea* とは別種とみるべきである。その他の菌もまた明らか

に本菌とことなるもので，これにがい当する菌を見い出すことができない。それで本菌を未記載のものと考えて *Sphaerulina juglandis* K. ITÔ et T. KOBAYASHI, sp. nov. と命名することにした。

摘　　要

本報文はクルミの新病害白徽葉枯病の，主として病原菌の形態，生理，生活史および病原性について行つた諸実験結果を述べたものである。

本病は不完全菌類の *Cercosporella* によるものであるが，越冬病落葉に見い出される子嚢菌 *Sphaerulina* はこの完全時代であることが立証された。そして，これは未記載の菌と考えられるので，新たに *Sphaerulina juglandis* K. ITÔ et T. KOBAYASHI sp. nov. と命名し，正規の記載を行つた。

本菌はオニグルミ，ヒメグルミに見い出されたが，人工接種試験の結果は，シナノグルミにも明らかな病原性をあらわすことがわかつた。潜伏期はオニグルミおよびヒメグルミでは 14～17日，シナノグルミではやや長く17～27日であつた。広汎な接種試験を行つたが，本菌はクルミ以外の樹種をまつたく侵さなかつた。

本病によつて病樹は枯死することはないが，早期落葉は生長をはなはだしく阻害するようで，これは特に苗木の場合にいちじるしい。現在までのところほとんど注目されていないが，将来クルミの重要病害として重視されるであろう。

図 版 説 明

Plate 1

A—D.　*Sphaerulina juglandis* sp. nov. によるオニグルミの白徽葉枯病×1

Plate 2

A—B.　寒天培養基上における *S. juglandis* sp. nov. の菌叢

C.　オニグルミに対する人工接種試験結果

D.　ヒメグルミに対する人工接種試験結果

Plate 3　人工接種試験結果

A, B,　オニグルミ；C,　ヒメグルミ；D,　シナノグルミ

Plate 4

A—B.　*S. juglandis* のスペルモゴニウム

C—G.　*S. juglandis* の子嚢殻

Plate 5

A.　*S. juglandis* 菌糸の発育におよぼす温度の影響

B.　*S. juglandis* 菌糸の発育におよぼす pH の影響

Notes on Some Leaf-Spot Diseases of Broadleaved Trees-IV.*
A new species of *Sphaerulina* causing frosty mildew of walnut trees.

Kazuo ITÔ and Takao KOBAYASHI

Introduction

In the autumn of 1950, several occurrences of a walnut foliage disease showing frosty mildew in appearances first arrested the authors' attention in Yamanashi and Yamagata Prefectures. Since that time the same disease has also been observed in other districts of Japan. The disease has not been of great severity but may become a problem in the future.

The microscopic examination revealed that the disease was caused by a species of *Cercosporella*. In the course of investigations the authors obtained an ascomycete belonging to the genus *Sphaerulina* as the perfect stage of the *Cercosporella*.

The present work was undertaken to study this previously undescribed disease of walnut trees with particular emphasis on the life history and parasitism of the pathogen. After making a thorough study of the fungus and searching relevant literature for an organism either identical with or similar to it, the authors have come to the conclusion that it is a new species of *Sphaerulina*, and propose giving it the name *Sphaerulina juglandis* sp. nov. A brief note on the preliminarly study was published by the authors (ITÔ & KOBAYASHI 1950)[7].

Here the authors wish to express their indebtedness to Mr. Rokuya IMAZEKI, Chief of the Forest Protection Division, of the Government Forest Experiment Station, under whom this investigation has been carried out, for suggestions and encouragement during its progress. And thanks are also extended to Mr. Michio NAKAGAWA for his kindness in preparing the illustrations.

Symptoms and signs

Early symptoms of this disease generally appear as small brown spots on the upper surface of leaves during the first week in July. At this stage, there is little if any apparent discoloration when affected leaves are viewed from the under leaf surface.

As the disease progresses, these spots become grayish brown to light grayish brown, round or oblong, 2～10 *mm* in diameter, and surrounded by a broad watersoaked margin.

This disease is characterized by the presence of an effuse, white, powdery coating on the lesion as if it is frosted.

In severe infections, the lesions commonly coalesce to form large necrotic areas, and finally kill part or almost all of the leaf. The dead areas may become so large that the leaves look as if they had been damaged from drought (Plate 1).

Serious damage occurs in late summer to mid-autumn, and the disease causes severe defoliation with a consequent marked retardation in growth.

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* The third paper under this general title was published in Bull. Gov. For. Exp. Sta., **92**, 65～80, 1956.

Overwintering of the fungus

In the latter part of October, 1950 and also 1951, numerous diseased leaves of *Juglans Sieboldiana* which had been collected in Yamagata Prefecture and sent to Tokyo were placed in wire baskets and left in the open tied to the branch of a small tree in order to trace the development of the pathogen during the winter. Examinations of the stored material were made at various times during the winter and spring.

In early December, almost all of the conidia of the *Cercosporella* which had remained on the lesions of the fallen leaves disappeared. The spermogonia were actively discharging spermatia, when observed at intervals between January and March of the following year. The spermogonia developed within the subepidermal stromata. These stromata were observed bearing conidiophore bases on their exposed surface in some cases and in other cases not. The spermogonia were often found on the area extending beyond the limits occupied by the lesions. Repeated attempts to germinate the spermatia in various nutrient solution have been unsuccessful.

Perithecia began their formation in middle November, but did not become sufficiently differentiated to be recognized as perithecial primordia until January. The ascospores matured in the middle of June and evidence pointed to the fact that they furnished the chief primary inoculation of the disease. Morphological features of the fungus in the ascigerous stage agreed well with the description for *Sphaerulina* SACC.

Physiological characters of the fungus

1. Isolation of the fungus

The fungus has been isolated in pure culture from both conidia and ascospores.

Mono-conidial isolations were obtained by streaking water suspensions of spores on 2 per cent glucose agar in Petri dishes, adding a drop of 2 per cent aqueous solution of copper sulphate and transferring germinating single conidium to potato sucrose agar in tubes (YOSHII 1933[22], ITÔ & HOSAKA 1950[6]).

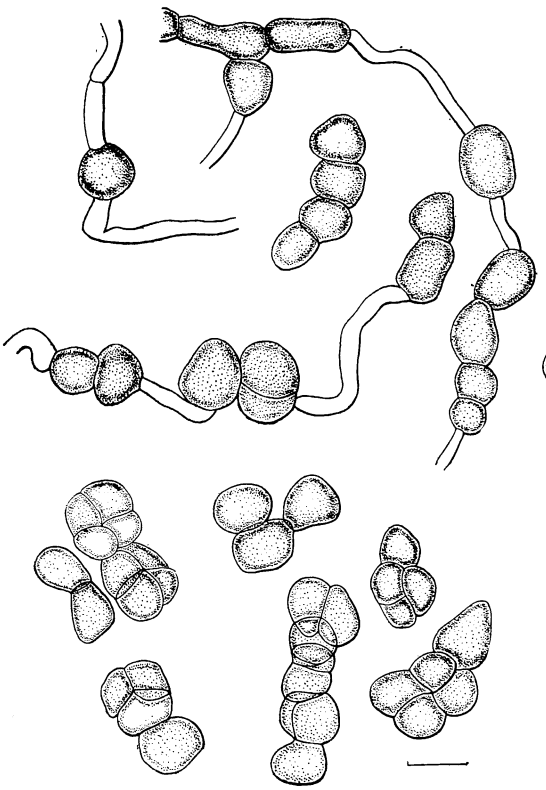
Single ascospore isolates of the fungus were made by attaching pieces of the overwintered fallen leaf to the inside of the cover of a Petri dish containing acidified 2 per cent glucose agar, so that ascospores could be ejected onto the agar. Germinating ascospores were isolated singly and cultured on potato glucose agar in tubes.

2. Characters in culture

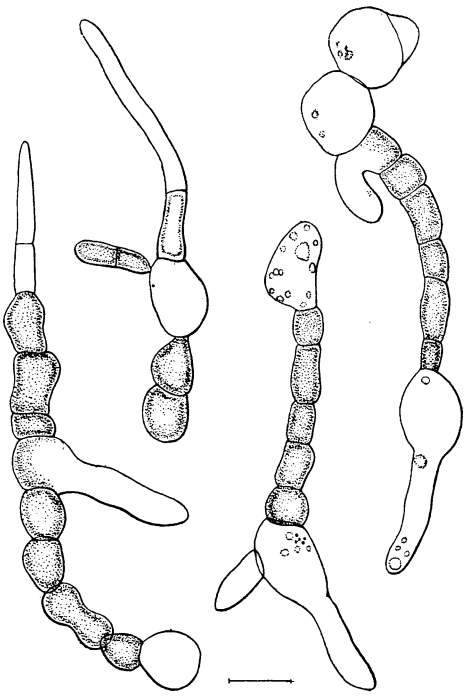
Development of the fungus colonies started from single spore The colonies started from single conidium, grow very slowly and become about 10 *mm* in diameter at the end of 1 month's culture at 25°C. In the macroscopic appearances these colonies are elevated, rugged, and light yellow, resembling those of yeasts or bacteria. Under the microscope these colonies are found to be conidial masses.

Colonies cultured for about 2 months at 25°C are dark green in color and covered sparsely with white aerial mycelium. Aerial hyphae in superficial parts are slender, 2μ in width, many septate, hyaline and lack in conidium, while inner parts of the colonies are made of numerous chlamydospore-like bodies, greenish brown in color, and conidia, accompanying spermogonia filled with spermatia (Plate 2, A, B ; Text-figs. 1,2).

Characteristics of the colonies started from single ascospore are much accordant with those from single conidium in many respects.



Text-fig. 1　Chlamydospore-like bodies of *Sphaerulina juglandis* sp. nov. formed on 2 per cent glucose agar (—=10μ).



Text-fig. 2　Germination of chlamydospore-like bodies of *Sphaerulina juglandis* sp. nov. on 2 per cent sucrose agar (—=10μ).

Fungus growth on various agar media　The isolates from both conidium and ascospore were cultured on potato sucrose agar plates respectively, and the small bits of colonies were used as inocula. The fungus was cultured on the following agar media: potato sucrose agar *1, 2 per cent glucose agar*2, SAITO's soy agar*3, RICHARDS' solution agar*4, CZAPEK's solution agar*5, WAKSMAN's solution agar*6, bouillon agar*7, walnut leaf decoction agar*8, and asparagine agar*9.

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*1 Distilled water 1 *l*, potato 200*g*, sucrose 20*g*, agar-agar 25*g*.
*2 Distilled water 1 *l*, glucose 20*g*, agar-agar 25*g*.
*3 Distilled water 850cc, onion decoction 100 cc, Japanese soy 50cc, sucrose 50*g*, agar-agar 25*g*.
*4 Distilled water 1 *l*, KNO_3 10*g*, KH_2PO_4 5*g*, $MgSO_4 \cdot 7H_2O$ 2.5*g*, sucrose 50*g*, agar-agar 25*g*.
*5 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*, sucrose 30*g*, $FeSO_4$ 0.01*g*, agar-agar 25*g*.
*6 Distilled water 1 *l*, glucose 10*g*, peptone 5*g*, KH_2PO_4 1*g*, $MgSO_4 \cdot 7H_2O$ 0.5*g*, agar-agar 25*g*. (pH 5.6)
*7 Distilled water 1 *l*, peptone 10*g*, meat extract 10*g*, NaCl 5*g*, agar-agar 25*g*.
*8 Distilled water 1 *l*, walnut leaves 100*g*, sucrose 20*g*, agar-agar 25*g*.
*9 Distilled water 1 *l*, K_2HPO_4 5*g*, asparagine 2.5*g*, $MgSO_4 \cdot 7H_2O$ 0.2*g*, sucrose 10*g*, agar-agar 25*g*.

In macroscopic appearances of the colonies on various agar media, there were no differences between the isolate from conidium and that from ascospore.

Diameters of colonies originated from both conidium and ascospore on agar media noted above were measured at various intervals. Results of the measurement for the fungus colonies kept at 25°C are presented in tables 1—2.

Table 1. Mycelial growth of *S. juglandis* on various agar media—1.

Agar medium	Isolate	Diameter of mycelial colony (*mm*)					
		Period lapsed (day)					
		7	15	29	45	85	127
Potato sucrose agar	*Cercosporella*	6	22	35	39	45	47
	Sphaerulina	6	18	35	39	45	45
2 % glucose agar	*Cercosporella*	3	6	10	15	23	29
	Sphaerulina	2	5	8	12	20	26
SAITO's soy agar	*Cercosporella*	3	12	19	33	39	45
	Sphaerulina	3	14	20	29	39	41
RICHARDS' sol. agar	*Cercosporella*	2	9	13	21	34	40
	Sphaerulina	2	8	10	18	32	37
CZAPEK's sol. agar	*Cercosporella*	3	11	16	24	32	41
	Sphaerulina	2	9	15	23	34	40
WAKSMAN's sol. agar	*Cercosporella*	3	9	21	28	35	38
	Sphaerulina	3	10	23	31	38	40
Bouillon agar	*Cercosporella*	3	7	9	11	11	12
	Sphaerulina	2	7	7	11	11	12
Walnut decoct. agar	*Cercosporella*	—	5	11	16	24	27
	Sphaerulina	—	—	—	—	—	—
Asparagine agar	*Cercosporella*	4	13	29	42	43	43
	Sphaerulina	3	13	31	40	42	42

Table 2. Mycelial growth of *S. juglandis* on various agar media—2.

Agar medium	Isolate	Diameter of colony (*mm*)				
		Period lapsed (day)				
		12	27	46	81	123
Potato sucrose agar	*Cercosporella*	10	15	20	26	38
	Sphaerulina	11	16	21	29	36
2 % glucose agar	*Cercosporella*	5	6	11	21	32
	Sphaerulina	4	7	13	19	28
SAITO's soy agar	*Cercosporella*	8	17	27	37	49
	Sphaerulina	8	17	26	36	45
RICHARDS' sol. agar	*Cercosporella*	6	11	21	29	35
	Sphaerulina	6	10	17	27	38

Table 2. Mycelial growth of *S. juglandis* on various agar media—2. (Continued)

Agar media	Isolate	Diameter of colony (*mm*) Reriod lapsed (day) 12	27	46	81	123
CZAPEK's sol. agar	*Cercosporella*	5	10	18	26	38
	Sphaerulina	6	8	15	23	35
WAKSMAN's sol. agar	*Cercosporella*	8	13	26	37	41
	Sphaerulina	7	14	25	34	37
Bouillon agar	*Cercosporella*	6	7	9	9	9
	Sphaerulina	4	6	10	10	10
Walnut decoct. agar	*Cercosporella*	8	10	16	22	27
	Sphaerulina	+	10	14	18	23
Asparagine agar	*Cercosporella*	7	11	17	25	36
	Sphaerulina	7	12	20	28	38

It is apparent from data in tables 1—2 that the fungus grows well on potato sucrose agar, SAITO's soy agar, RICHARDS' solution agar, CZAPEK's solution agar, WAKSMAN's solution agar and asparagine agar, while sparsely on the other agar media.

Effect of temperatures on the fungus growth The relation of temperature to the growth of the fungus was tested by plate culture method using potato sucrose agar. For inocula, bits of the colonies originated from each of conidium and ascospore were transplanted to the centre of each plate. Diameter of the colonies at each temperature measured and averaged after the experimental periods are given in tables 3—4.

Table 3. Effect of temperatures on mycelial growth of *S. jnglandis*—1. (on potato sucrose agar).

Temperature (°C)	Isolate	Diameter of colony (*mm*) Peried lapsed (day) 7	19	38	64
1	*Cercosporella*	—	7	9	10
	Sphaerulina	—	7	9	11
4 ~ 6	*Cercosporella*	4	9	13	16
	Sphaerulina	4	9	12	15
9 ~ 11	*Cercosporella*	4	11	15	20
	Sphaerulina	5	11	15	19
15	*Cercosporella*	9	13	21	31
	Sphaerulina	9	14	20	28
20	*Cercosporella*	9	20	26	38
	Sphaerulina	8	16	23	35
25	*Cercosporella*	10	16	26	37
	Sphaerulina	10	17	25	34
28	*Cercosporella*	4	8	14	16
	Sphaerulina	3	8	12	16

Table 3. Effect of temperature on mycelial growth of *S. juglandis*—1, (Continued)

Temperature (°C)	Isolate	Diameter of colony (*mm*) Period lapsed (day) 7	19	38	64
30	*Cercosporella*	—	—	—	—
	Sphaerulina	—	—	—	—
35	*Cercosporella*	—	—	—	—
	Sphaerulina	—	—	—	—
40	*Cercosporella*	—	—	—	—
	Sphaerulina	—	—	—	—

Table 4. Effet of temperatures on mycelial growth of *S. juglandis*—2. (on potato sucrose agar).

Temperature (°C)	Isolate	Diam. of colony (*mm*) Period lapsed (day) 9	27
−2 ～ −1	*Cercosporella*	—	—
	Sphaerulina	—	—
1	*Cercosporella*	—	+
	Sphaerulina	—	+
4 ～ 5	*Cercosporella*	4	8
	Sphaerulina	4	8
11 ～ 13	*Cercosporella*	6	11
	Sphaerulina	6	10
16 ～ 18	*Cercosporella*	6	15
	Sphaerulina	5	14
20	*Cercosporella*	12	30
	Sphaerulina	8	29
25	*Cercosporella*	10	26
	Sphaerulina	11	27
28	*Cercosporella*	7	10
	Sphaerulina	8	13
30	*Cercosporella*	—	—
	Sphaerulina	—	—

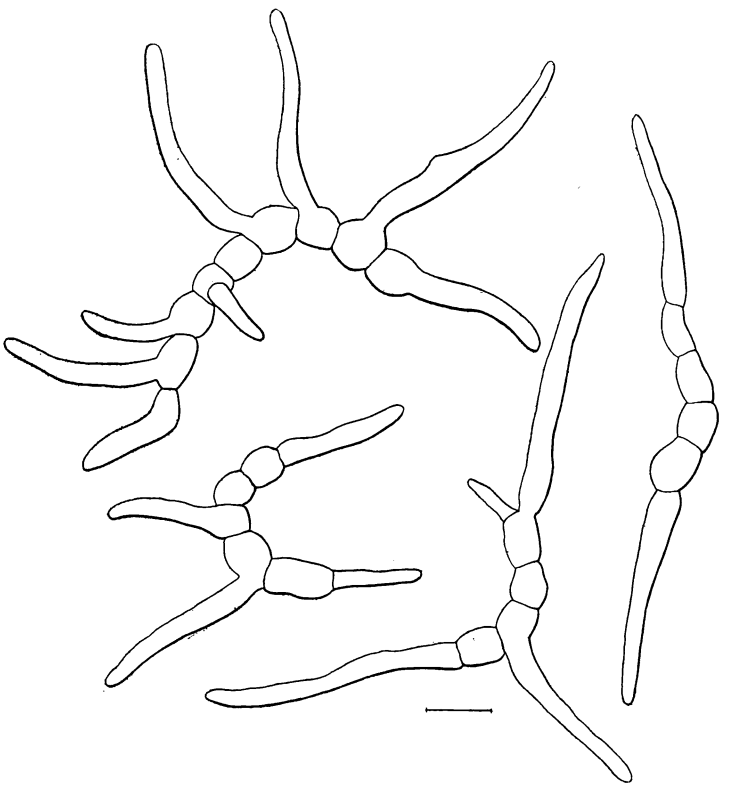
As shown in tables 3—4 the fungus grows favorably at the temperatures ranging from 10 to 28°C with an optimum at 25°C, and the maximum and minimum temperatures for the growth are 1°C and 28°C, respectively (Plate 5, A).

Effect of H-ion concentrations on the fungus growth A range of pH value was obtained by additions of regulated amounts of HCl or NaOH solution. The relation between H-ion concentration and the fungus growth was tested on potato sucrose agar. The results of the experiment after 11 days at 25°C are presented in table 5.

From the data in table 5, it may be said that influence of H-ion concentration on the growth is not generally remarkable, but the maximum growth lies at the pH values 5.6～6 (Plate 5, B).

Table 5. Effet of H-ion concentrations on mycelial growth and conidial production of *S. juglandis* (after 11 days, at 25°C).

pH	Isolate	Diam. of mycelial colony (*mm*)	Degree of conidial production
3.4	*Cercosporella*	10	++
	Sphaerulina	10	++
4.2	*Cercosporella*	13	+++++
	Sphaerulina	14	+++++
4.6	*Cercosporella*	18	+++++
	Sphaerulina	17	+++++
5.6	*Cercosporella*	23	+++++
	Sphaerulina	19	+++++
6.0	*Cercosporella*	23	+++++
	Sphaerulina	21	+++++
6.6	*Cercosporella*	17	+++++
	Sphaerulina	17	+++++
7.0	*Cercosporella*	16	+++++
	Sphaerulina	15	+++++
7.4	*Cercosporella*	16	+++++
	Sphaerulina	16	+++++
7.6	*Cercosporella*	15	+++++
	Sphaerulina	15	+++++



Text fig. 3　Germinating conidia of *Sphaerulina juglandis* sp. nov. (—=10μ).

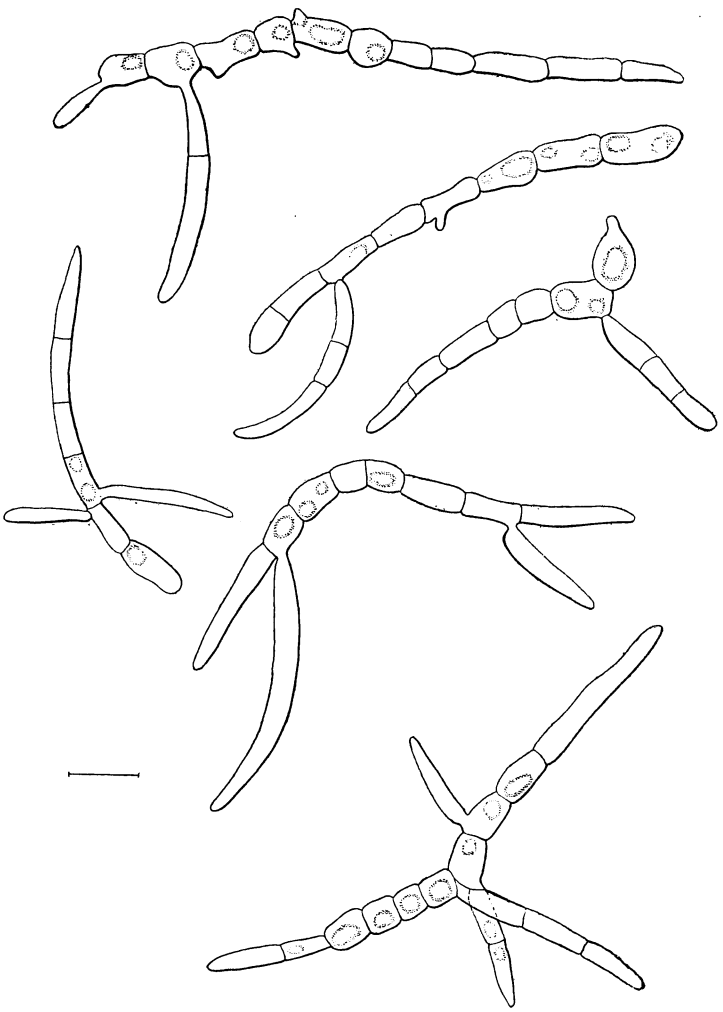
3. Conidial formation on media

Development of the conidial formation starting from single conidium has been traced under the microscope.

In germination each cell of conidium at first swells enormously and a germ tube grows from the free end of each terminal cell as well as from some of the intermediate cells. The germ tubes elongate, soon become septate and branch, and then swell at the older cells, from which conidia are directly produced (Text-figs. 3, 4).

Conidial production on various agar media　Bits of the fungus colonies isolated originally from conidium and ascospore were transferred to nine kinds of agar media and kept at 25°C. Results of the experiment are summarized in table 6.

Table 6 shows that the conidia are abundantly produced on all of the agar media used except 2 per cent glucose agar, on which the conidial production is not favorable (Text-fig. 5).



Text-fig. 4　Conidial formation of *Sphaerulina juglandis* sp. nov. on potato sucrose agar (—=10μ).

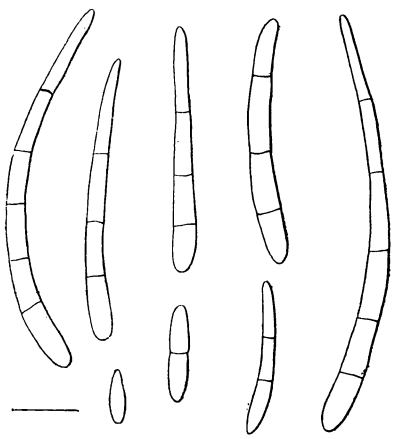
Table 6. Conidial production of *S.juglandis* on various agar media.

Agar medium	Isolate	Degree of conidial production	
		Experiment—1 (after 15 days)	Experiment—2 (after 12 days)
Potato sucrose agar	*Cercosporella Sphaerulina*	+++++ +++++	+++++ +++++
2 % glucose agar	*Cercosporella Sphaerulina*	++ ++	+++ +++
SAITO's soy agar	*Cercosporella Sphaerulina*	+++++ +++++	+++++ +++++
RICHARS' sol. agar	*Cercosporella Sphaerulina*	+++++ +++++	+++++ +++++
CZAPEK's sol. agar	*Cercosporella Sphaerulina*	+++++ +++++	+++++ +++++
WAKSMAN's sol. agar	*Cercosporella Sphaerulina*	+++++ +++++	+++++ +++++
Bouillon agar	*Cercosporella Sphaerulina*	+++++ +++++	+++++ +++++
Walnut leaf decoct. agar	*Cercosporella Sphaerulina*	+++++	+++++
Asparagine agar	*Cercosporella Sphaerulina*	+++++ +++++	+++++ +++++

Conidial production at various temperatures Potato agar plates inoculated with the fungus were placed in incubators regulated at desirable temperatures. Degree of conidial production at each temperature obtained at the end of 19 days is presented in table 7.

As shown in table 7, the conidia are produced at the temperatures ranging from 1 to 28°C, very favorably at 15～28°C.

Conidial production at various H-ion concentrations As shown in table 5, the conidial production is not strikingly effected by the change of H-ion concentration within the limits studied, excepting at pH 3.4.



Text-fig. 5 Conidia of *Sphaerulina juglandis* sp. nov. produced on potato sucrose agar(—=10μ).

4. Germination of conidia

On 2 per cent glucose agar conidia of the fungus germinate within several hours at 25°C. In germination the conidia swell at each cell and send out 3 or 4, rarely more, germ tubes.

Germination on several kinds of agar media Conidia of the two isolates which had been produced on potato sucrose agar were used to test the germinability on the following agar media: two per cent glucose agar, 2 per cent plain agar and walnut leaf decoction agar. Besides the readings on germination, the lengths of the

Table 7. Conidial production of *S. juglandis* at various temperatures (after 19 days, on potato sucrose agar).

Temperature (°C)	Isolate	Degree of conidial production
1	*Cercosporella*	++
	Sphaerulina	++
4 ~ 6	*Cercosporella*	+++
	Sphaerulina	+++
9 ~ 11	*Cercosporella*	+++
	Sphaerulina	+++
15	*Cercosporella*	+++++
	Sphaerulina	+++++
20	*Cercosporella*	+++++
	Sphaerulina	+++++
25	*Cercosporella*	+++++
	Sphaerulina	+++++
28	*Cercosporella*	+++++
	Sphaerulina	+++++
30	*Cercosporella*	−
	Sphaerulina	−
35	*Cercosporella*	−
	Sphaerulina	−

Table 8. Germination of *S. juglandis* on various agar-media (after 20 hours at 25°C).

Isolate	Agar medium	Germination percentage (%)	Maximum length of germ-tube (μ)
Cercosporella	2 % glucose ager	80	75
	2 % plain agar	87	137
	walnut decoct. agar*	91	100
Sphaerulina	2 % glucose agar	84	100
	2 % plain agar	84	125
	walnut decoct. agar*	89	113

* Distilled water 1 *l*, walnut leaves 100*g*, sucrose 20*g*, agar-agar 25*g*.

germ tubes were measured. Results obtained after 20 hours at 25°C are given in table 8.

From table 8, it is readily known that the conidia germinate equally well on all agar media tested, counting about 80 to 90 per cent.

Relation between germination and incubation period By the same manner as in the previous experiment, the relation of incubation period to germination of the conidia was examined. Results of the experiment made at 25°C are presented in table 9.

As shown in table 9, the conidia begin to germinate within 2 to 4 hours, and germination is over 90 per cent in 24 hours.

Table 9. Relation between conidial germination of *S. juglandis* and incubation period (at 25°C).

Agar medium	Isolate		Time passed (hour) 2	4	8	12	24
2% plain agar	*Cercosporella*	G P (%)	1	13	94	90	96
		MLG (μ)	—	—	53	75	153
	Sphaerulina	G P (%)	2	15	94	93	96
		MLG (μ)	—	—	50	85	185
2% glucose agar	*Cercosporella*	G P (%)	1	18	87	84	94
		MLG (μ)	—	6	31	78	124
	Sphaerulina	G P (%)	1	15	93	90	95
		MLG (μ)	—	15	35	69	148
Walnut decoction agar	*Cercosporella*	G P (%)	2	6	91	92	95
		MLG (μ)	—	—	31	81	131
	Sphaerulina	G P (%)	2	12	91	96	95
		MLG (μ)	—	—	31	90	131

Notes: GP……Germination percentage; MLG……Maximum length of germ-tube.

Relation between temperature and germination Drops of the conidial suspension were placed on 2 per cent agar in water in Petri dishes and then all spores were incubated at the different temperatures. Besides the readings on germination, the lengths of the germ tubes were measured after 20 hours at various temperatures. Results obtained are given in table 10.

Table 10. Effect of temperatures on germination of conidia of *S. juglandis*—1. (after 20 hrs. on 2% plain agar).

Temperature (°C)	Germination percentage (%)	Maximum length of germ-tube (μ)
0～1	0	—
3	0	—
8	65	38
14	74	88
20	85	125
25	88	163
28	83	138
30	70	100
35	0	—
40	0	—

Another test was undertaken with conidia of isolates which had been originally isolated from conidium and ascospore. Two per cent sucrose agar was used instead of 2 per cent plain agar. Results of the experiment at the end of 20 hours are presented in tables 11 and 12.

From tables 10—12, it may be said the that range of temperature within which germination takes place is from 8 to 35°C, with an optimum between 20 to 28°C.

Relation between H-ion concentration and germination Germination was tested by Van Tieghem cell method using sterile distilled water. A range of pH value was obtained by additions of regulated amounts of HCl or NaOH. Results of the experiment obtained at the end of 20 hours at 25°C are briefly noted in table 13.

From table 13, it is to be inferred that germination of conidia is not strikingly effected by the change of H-ion concentration within the limits studied, but the optimum may be probably obtained at pH 6 or near.

Table 11. Effect of temperature on germination of conidia of *S. juglandis*—2. (after 20 hrs. on 2% sucrose agar).

Temperatue (°C)	Isolate	Germination percentage (%)	Maximum length of germ-tube (μ)
0	*Cercosporella*	0	—
	Sphaerulina	0	—
4	*Cercosporella*	0	—
	Sphaerulina	0	—
12	*Cercosporella*	51	25
	Sphaerulina	48	31
17	*Cercosporella*	74	69
	Sphaerulina	73	63
20	*Cercosporella*	81	113
	Sphaerulina	83	113
25	*Cercosporella*	82	150
	Sphaerulina	83	149
28	*Cercosporella*	81	106
	Sphaerulina	78	100
30	*Cercosporella*	53	19
	Sphaerulina	51	19
35	*Cercosporella*	10	—
	Sphaerulina	8	—
40	*Cercosporella*	0	—
	Sphaerulina	0	—

Table 12. Effect of temperature on germination of conidia of *S. juglandis*—3. (after 20 hrs. on 2% sucrose agar).

Temperature (°C)	Isolate	Germination percentage (%)	Maximum length of germ-tube (μ)
0	*Cercosporella*	0	—
	Sphaerulina	0	—
4	*Cercosporella*	0	—
	Sphaerulina	0	—
9	*Cercosporella*	53	38
	Sphaerulina	60	44
17	*Cercosporella*	59	69
	Sphaerulina	76	75
20	*Cercosporella*	76	81
	Sphaerulina	81	94
25	*Cercosporella*	86	156
	Sphaerulina	88	163
28	*Cercosporella*	77	119
	Sphaerulina	79	119
30	*Cercosporella*	61	69
	Sphaerulina	62	63
35	*Cercosporella*	21	0
	Sphaerulina	22	19
40	*Cercosporella*	0	0
	Sphaerulina	0	0

Table 13. Effect of H-ion concentrations on germination of conidia of *S. juglandis* (after 20 hrs. at 25°C).

	pH								
	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	9.6
Germination percentage (%)	9	11	43	71	76	71	51	50	52
Max. length of germ-tube (μ)	60	60	106	138	188	113	66	88	60

Relation between relative humidity and germination The effect of relative humidity upon the germination of conidia were examined by the procedure reported in the previous paper (ITÔ & HOSAKA 1952[6]). Results of the experiment obtained after 40 hours are presented in tab'e 14.

Table 14. Effect of relative humidities on germination of conidia of *S. juglandis* (after 40 hrs.).

Relative humidity (%)	Salt in over-saturated aqueous solution	*Cercosporella*-isolate		*Sphaerulina*-isolate	
		Germination percentage (%)	Max. length of germ-tube (μ)	Germination percentage (%)	Max. length of germ-tube (μ)
100	Dist. water	41	100	65	138
98	K_2SO_4	21	38	40	88
94	KNO_3	0	0	1	6
92	K_2HPO_4	0	0	0	0
87	KCl	0	0	0	0

It is evident from the data in table 14, that germination of the conidia is most favorable in a saturated atmosphere, and very sparse in 94 per cent humidity, while conidia kept at 92 per cent humidity and below fail to germinate.

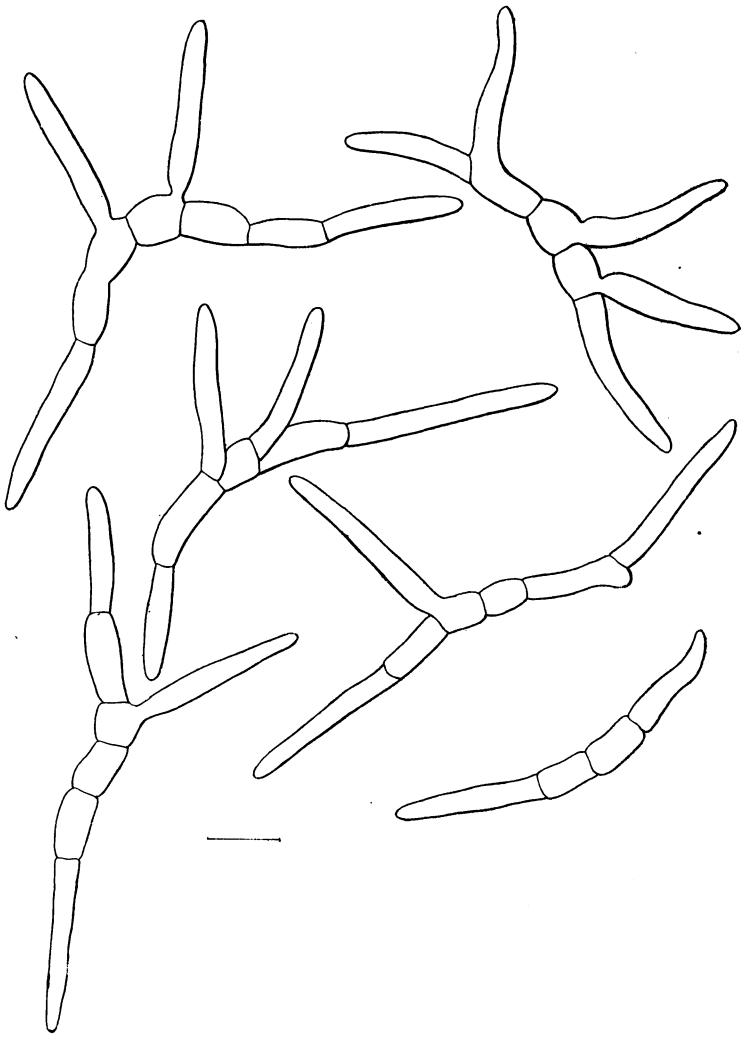
5. Germinatin of ascospore

Ascospores of the fungus germinate in several hours after liberation from an ascus, and the percentage of germination in favorable conditions is 100 per cent or near in 14 hours. Mode of germination in ascospores closely resembles that in conidia (Text-fig. 6).

Relation between temperature and germination Ascospores were obtained by attaching pieces of the overwintered fallen leaf to the inside of the cover of a Petri dish containing 2 per cent plain agar plate, so that ascospores could be ejected onto the agar. The agar plates on which ascospores had been seeded were incubated at 3～5, 13, 18～20, 22, 25 and 30°C, respectively. Germination and length of germ tubes were examined after 24 hours.

The optimum temperature for ascospore germination lay between 22 to 25°C, and the lower limit is somewhat below 3°C. It is noteworthy that ascospores did not germinate at 30°C, while germination of conidia occurred at higher temperatures of 30 and 35°C. Results of the experiment are briefly noted in table 15.

Relation between H-ion concentration and germination Plain agar medium (agar-



Text-fig. 6　Germinating ascospores of *Sphaerulina juglandis* sp. nov. (—=10μ).

Table 15.　Effect of temperatures on the germination of ascospores of *S. juglandis* (on 2% glucose agar, after 24 hours).

	Temperature (°C)					
	3~5	13	18~20	22	25	30
Germination percentage (%)	31	66	100	100	98	0
Maximum length of germ-tube (μ)	6	25	63	213	94	—

agar 2～7 per cent) was titrated with HCl and NaOH to the following pH values; 3, 4, 5, 6, 7, 8 and 9. By the sterilization the pH value of the acidulated agar was slightly raised, while that of the alkalized agar was lowered as follows: 3.6, 4.8, 5.2, 6.8, 7.4, and 7.8, respectively. Ascospores which had been discharged by perithecium in overwintered fallen leaf were seeded on agar plates. Results obtained at the end of 40 hours at 25°C are presented in table 16.

Table 16. Effect of H-ion concentrations on germination of ascospores of *S. juglandis* (after 40 hours at 25°C).

	pH						
	3.6	4.8	5.2	6.2	6.8	7.4	7.8
Germination percentage (%)	80	91	96	94	93	100	79
Maximum length of germ-tube (μ)	56	86	125	175	144	125	106

Table 16 shows that influence of H-ion concentration on ascospore germination is not remarkable, and the optimum may be probably obtained at the exponent near pH 6.2, judging from the germ-tube length.

Pathogenicity of the fungus

In order to make clear the pathogenicity of the fungus, a series of inoculation experiments has been undertaken during the past several years.

1. Inoculation experiments to walnut trees

In the summer of 1951, the healthy seedlings of the following three kinds of walnut trees, very common in Japan, were inoculated under greenhouse conditions: *Juglans Sieboldiana* (Oni-gurumi), *J. Sieboldiana* var. *cordiformis* (Hime-gurumi) and Shinano walnut (Shinano-gurumi *).

Experiment—1. Mono-conidial isolate of the fungus was used as inoculum in this experiment. On June 18, a spore suspension from pure cultures was atomized onto leaves of the plants, and bell jars lined with absorbent cotton containing water were put over the plants to maintain a humid atmosphere. The jars were removed after two days. Check plants were similarly treated except that they were atomized with water instead of spore suspension.

On the leaves of *J. Sieboldiana*, as well as *J. Sieboldiana* var. *cordiformis* spots began to appear 18 to 21 days after inoculation and enlarged rapidly. The appearances of the inoculated plants were typical of the disease as observed under natural conditions. Lesions on Shinano walnut appeared 21～25 days after inoculation.

Conidiophores and conidia typical of the *Cercosporella* were abundantly formed on the lesions resulting from the inoculation, whereas all the check plants remained healthy. Results of the experiment examined on July 21 are summarized in table 17.

It is evident from the data mentioned already and in table 17 that pathogenicity of

* The origin is obscure, but it may be a strain of Franquett.

Table 17. Results of the inoculation experiment with the *Cercosporella* isolate to three kinds of walnut trees (June 18～July 21, 1951)

Seedling No.	Tree species	Treatment	Number of leaves inoculated	Number of leaves infected	Incubation period (day)
1	*Juglans Sieboldiana* (Oni-gurumi)	Inoculated	22	22	21
2			24	24	
3			24	24	
4			20	20	
5			25	25	
6		Check	27	0	
7			23	0	
8	*J. Sieboldiana* var. *cordiformis* (Hime-gurumi)	Inoculated	16	16	18～21
9			29	29	
10			24	22	
11			24	24	
12			34	34	
13		Check	18	0	
14			29	0	
15	Shinano walnut tree (Shinano-gurumi)	Inoculated	18	18	21～25
16			34	34	
17			18	6	
18			18	5	
19			29	25	
20		Check	24	0	
21			15	0	

the fungus to all of the walnut trees tested was determined by inoculation experiment, though there were some differences in the incubation period among the kinds of walnut trees.

Experiment—2. On September 15, another inoculation experiment was performed by the same method as in the previous experiment to three kinds of walnut trees. As inocula, two cultures were used, namely: Isolate from single conidium of the *Cercosporella* stage and that from single ascospore of the *Sphaerulina* stage.

Symptoms and signs induced by this experiment were quite similar to those obtained in the previous test. Typical conidiophores and conidia were produced not only on the leaves inoculated with the isolate from the *Cercosporella*, but also on those inoculated with the isolate from the *Sphaerulina*. The length of the incubation period in Shinano walnut tree was, also in this case, somewhat longer than that in the other two. All check plants remained free from infection. Results of the experiment obtained on October 23 are briefly presented in table 18.

Mature perithecia of *Sphaerulina* usually have been found in the spring on artificially inoculated leaves that had been left out of doors over the winter.

Table 18. Results of the inoculation experiment with the *Cercosporella* and the *Sphaerulina* isolates to three kinds of walnut trees (Sept. 15～Oct. 23, 1951).

Seedling No.	Tree species	Treatment	Number of leaves inoculated	Number of leaves infected	Incubation period (day)
31	*Juglans Sieboldiana* (Oni-gurumi)	Inoculated (*Cercosporella*)	71	71	14～17
32			64	64	
33			—	—	
34		Inoculated (*Sphaerulina*)	77	77	
35			88	88	
36			57	57	
37		Check	41	0	
38			30	0	
39	*Juglans Sieboldiana* var. *cordiformis* (Hime-gurumi)	Inoculated (*Cercosporella*)	46	46	14～17
40			42	42	
41			38	38	
42		Inoculated (*Sphaerulina*)	40	40	
43			40	40	
44			78	78	
45		Check	35	0	
46			—	—	
47	Shinano walnut tree (Shinano-gurumi)	Inoculated (*Cercosporella*)	28	1	17～27
48			24	18	
49			56	23	
50		Inoculated (*Sphaerulina*)	72	12	
51			42	9	
52			34	7	
53		Check	37	0	
54			23	0	

2. Inoculation experiment to various plants

Pathogenicity of the fungus to various plant species was tested by spraying seedlings and stocks with sterile water suspensions of conidia from culture in the same manner as in the previous experiments. In this study, plants of 28 species, representing 19 genera in 15 families, were inoculated with the fungus. Results of the two experiments made in 1952 and 1953 are briefly given in tables 19 and 20, respectively.

As has clearly been shown in tables 19—20, no evidence has yet been obtained that all of the plant species except the genus *Juglans* can be infected by the fungus. It therefore seems clear that the fungus is selectively pathogenic toward walnut trees (Plate 2, C, D: Plate 3, A, B, C, D).

Table 19. Results of the inoculation experiments with *S. juglandis* to various kinds of trees (May 15～June 7, 1952).

Tree species		Pathogenicity
Scientific name	Japanese name	
Ginkgoaceae		
Ginkgo biloba	Ichô	−
Salicaceae		
Populus monilifera	Monirihera-yamanarashi	−
P. Simonii	Shimoni-doro	−
Juglandaceae		
Juglans Sieboldiana	Oni-gurumi	+
J. Sieboldiana v. *cordiformis*	Hime-gurumi	+
Carya sp.	Pekan	−
Betulaceae		
Alnus firma v. *Sieboldiana*	Yashabushi	−
A. japonica	Han-noki	−
Fagaceae		
Lithocarpus edulis	Mateba-shii	−
Quercus acuta	Aka-gashi	−
Q. acutissima	Kunugi	−
Q. dentata	Kashiwa	−
Q. myrsinaefolia	Shira-kashi	−
Q. serrata	Konara	−
Q. variabilis	Abemaki	−
Ulmaceae		
Zelkowa serrata	Keyaki	−
Moraceae		
Broussonetia kazinoki	Kôzo	−
Magnoliaceae		
Liriodendron tulipifera	Hantenboku	−
Magnolia praecocissima	Kobushi	−
Platanaceae		
Platanus acerifolia	Momijiba-suzukake	−
Leguminosae		
Lespedeza bicolor v. *japonica*	Shiro-hagi	−
Robinia pseudoacacia	Nise-akashia	−
Anacardiaceae		
Rhus verniciflua	Urushi	−
Celastraceae		
Euonymus japonica	Masaki	−
Theaceae		
Camellia japonica	Tsubaki	−
Cornaceae		
Aucuba japonica	Aoki	−
Oleaceae		
Fraxinus japonica	Toneriko	−

Table 20. Results of the experiment with *S. juglandis* to several kinds of trees (Aug. 8～Sept. 4, 1953).

Tree species		Pathogenicity
Scientifec name	Japanese name	
Jugulans Sieboldiana	Oni-gurumi	+
Juglans ("Shinano"—walnut)	Shinano-gurumi	+
Carya sp.	Pekan	−
Robinia pseudoacacia	Nise-akashia	−
Euonymus japonica	Masaki	−
Aucuba japonica	Aoki	−
Fraxinus japonica	Toneriko	−

Morphology and taxonomy of the fungus

From the foregoing presenting complete agreement in physiological and parasitological characters of cultures isolated from conidia with those from ascospores, there can be no doubt as to the genetic connection between these two stages. The conclusion to be drawn is, therefore, that the *Sphaerulina* found on the overwintered leaves is the perfect stage of the *Cercosporella* as exists on the living leaves.

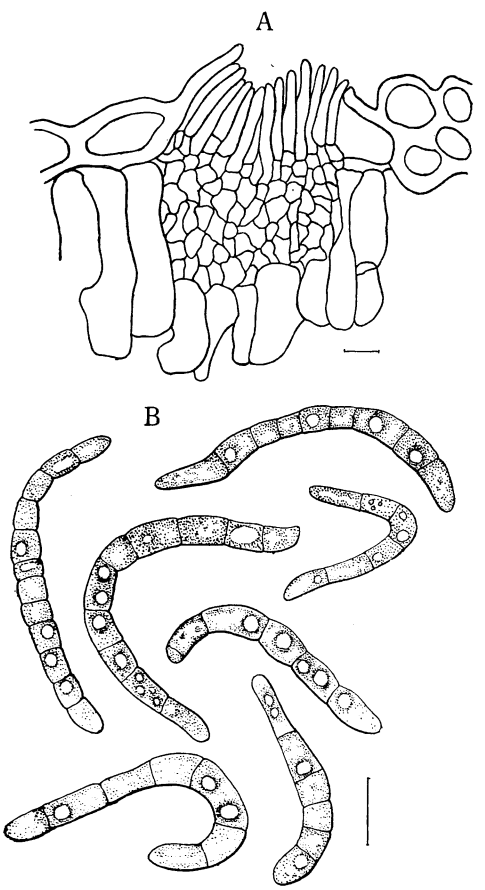
1. Conidial stage (*Cercosporella*)

Maculae epiphyllous, brown, circular or irregular, with watersoaked borders, in powdery tuft.

Stromatic mycelia hyaline aggregated in the stomatal cavities, 55～71 μ in diameter. Conidiophores differentiated from the fruiting stroma, short, erect or slightly curved, simple, hyaline, 32～68 × 3～5 μ. Conidia, usually curved or flexuous, sometimes straight, obclavate, hyaline, 1～10 septate, distal end round, contents homogeneous with small globules in the larger cells, 32～68 ×3～5 μ (Text-fig. 7).

Results of the measurement for the fruit bodies on several kinds of walnut trees are given in table 21.

As fungi causing foliage diseases of *Juglans* many species belonging to Fungi Imperfecti have been known as follows: *Phyllosticta juglandina* SACC., *P. jug-*



Text-fig. 7 Conidiophores and conidia of *Sphaerulina juglandis* sp. nov. (—=10μ). A, stroma and conidiophores; B, conidia.

Table 21. Dimension of *S. juglandis* in the *Cercosporella* stage.

Host		Diam. of stroma (μ)	Conidiophore		Conidium		
			Length (μ)	Width (μ)	Length (μ)	Width (μ)	Number of septum
J. Sieboldiana (in nature)	Range	55～71	21～32	2～3.2	32～55	2.5～4.5	3～10
	Average	62	25	2.5	41	3.5	
Ditto. (by artificial inoculation)	Range	43～71	11～16	2.5～3	40～68	2.5～3	3～8
	Average	57	14	2.7	53	2.7	
J. Sieboldiana v. *cordiformis* (*Ditto.*)	Range	50～62	11～16	2.5～3	43～68	2.5～3	3～9
	Average	56	13	2.7	53	2.6	
"Shinano" walnut (*Ditto.*)	Range	56～78	11～17	2.5～3	25～47	2.5～3	2～6
	Average	61	14	2.6	33	3.1	

landis (DC) SACC., *Ascochyta juglandis* BOLTSH., *Marssonia* (*Marssonina*) *juglandis* (LIB.) P. MAGN.=*Gnomonia leptostyla* (FR.) CES. et DE NOT., *Cylindrosporium juglandis* WOLF, *Phleospora cultimaculans* HEALD et WOLF, *Septoria juglandis* B. et C.=*Rhabdospora juglandis* (SCHW.) SACC., and *Septogloeum juglandis* HARA, etc.

Cercospora Juglandis KELL. et SAW., which was described on leaves of *Juglans nigra* in America, is quite different from the fungus under consideration in many respects (SACCARDO 1892[14]). Numerous Cercosporellae have been described on various woody plants as follows: *C. Evonymis* ERIKSS. on *Euonymus europae* (SACCARDO 1892[14]), *C. pirina* ELL. et EV. on *Pirus coronariae* (SACCARDO 1892[14]). *C. prolificans* (ELL. et EV.) SACC. on *Sambucus glauca* (SACCARDO 1895[15], 1900[16]), *C. ulmicola* v. HÖHNEL on *Ulmus* (SACCARDO 1906[18]), *C. Aceris* DEARN. et BARTH. on *Acer macrophyllum* (DEARNESS 1917[1]), *C. Alni* DEARN. et BARTH. on *Alnus rubra* (DEARNESS 1917[1]), *C. Mori* PECK on *Morus* (WOLF 1936[21]), *C. maculans* (BERENG) WOLF=*Mycosphaerella Mori* (FUCKEL) WOLF on *Morus* (WOLF 1936[21]), *C. arachnoidea* WOLF=*Mycosphaerella arachnoidea* WOLF on *Morus rubra* (WOLF 1936[21]), *C. rubi* (WINTER) PLAKIDAS on *Rubus* (PLAKIDAS 1937[10]), *C. persica* SACC. =*Mycosphaerella persica* HIGGINS et WOLF on *Prunus* (SACCARDO 1886[12], TSUJI 1919[20], HIGGINS & WOLF 1937[5]), *C. caryigena* (ELL. et EV.) HÖHN.=*Mycosphaerella caryigena* DEM. et COLE on *Carya* (DEMAREE & COLE 1932[2]) and *C. theae* PETCH=*Calonectria theae* LOOS on *Thea sinensis* (PETCH 1923[9], LOOS 1950[8]). None of these fungi is accordant with the authors' fungus in morphology and parasitism.

2. Spermogonial stage

An examination of the under surface of fallen leaves with a hand lens reveals numerous slightly raised black spots, the spermogonia. They measure 65～113 μ in diameter, and numerous spermatia, 2.8～3.8×0.4～0.7 μ, ooze out. Attempts to induce germination of spermatia met with consistent failure. Results of

Table 22. Dimension of spermogonium and spermatium of *S. juglandis* on the host plant (μ).

	Spermogonium		Spermatium	
	Height	Diameter	Length	Width
Range	84～102	65～113	2.8～3.8	0.4～0.7
Average	89	80	3.3	0.6

the measurement for spermogonia and spermatia are given in table 22 (Plate 4, A, B).

3. Perithecial stage (*Sphaerulina*)

The perithecia are present chiefly on the under surface of overwintered fallen leaves. They are first noted among the spermogonia, and no means has been determined of knowing which of these are destined to become spermogonia or perithecia when they are young.

Perithecia scattered or in groups, globose, membranaceus, papillate, 99～118×71～99 μ; asci cylindrical clavate, rounded at the end, without paraphyses, 8-spored, 43～56×8～9 μ; spores arranged in 2- or 3-rows, cylindric-fusiform, slightly curved, 3～7 septate, not constricted at septum, hyaline, 24～35×3～4 μ (Plate 4, C, D, E, F, G; Text-fig. 8).

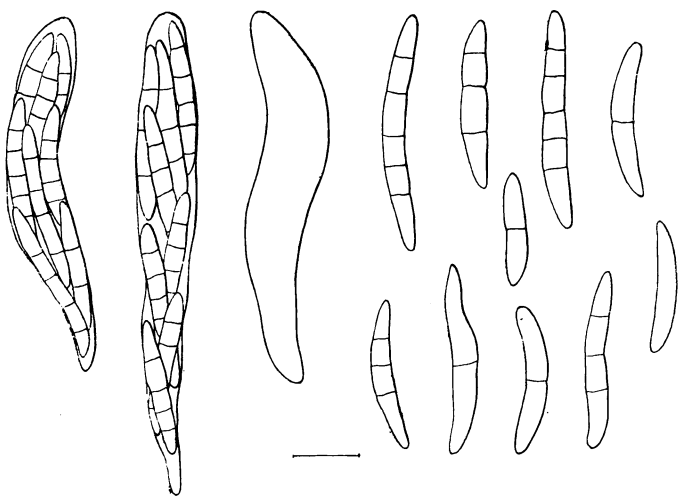
Results of the measurement for the fungus in the ascigerous stage are given in table 23.

Table 23. Dimension of *S. juglandis* in the ascigerous stage.

Host		Perithecium			Ascus		Ascospore		
		Height (μ)	Diameter (μ)	Width of wall (μ)	Length (μ)	Width (μ)	Length (μ)	Width (μ)	Number of septum
J. Sieboldiana (June 15'51)	Range	99～118	71～99	9～12	43～56	8～9	24～35	3～4	3～7
	Average	107	83	11	52	8.8	27	3.8	
Ditto. (June 23, 53)	Range	96～124	78～99	8～12	43～56	9～12	24～39	3～4	2～6
	Average	101	91	10	48	9.8	30	3.2	

So far as is known by the authors, there has been no account concerning the occurrence of any species of *Sphaerulina* on members of the genus *Juglans*. Sphaerulinae inhabiting woody plants which have been described by earlier investigators and their morphological characteristics are summarized in table 24.

Among the fungi shown in table 24, *S. fraxinea* SACC. et SPEG. described on wilting leaves of *Fraxinus ornus* closely resembles the fungus under consideration. However,



Text-fig. 8 Asci and ascospores of *Sphaerulina juglandis* sp. nov. (—=10 μ).

Table 24. Sphaerulinae inhabiting woody plants described hitherto by ealier workers.

Fungus and host	Perithecium	Ascus	Ascospore	Author
S. myriadea (DC.) SACC. *Quercus* et *Fagus* —fallen leaf—	aggregatis epiphyllis, innato-prominulis 90～100μ in diam.	subfusoides, 60×6μ	distichis elongatis 3-septatis, utrinque acuti-usculis, 30～35×2～3μ	SACCARDO (1883)[11]
S. serograpta (DURR. et MONT) SACC. *Quercus coccifera*e —dry leaf—	hypophyllis, ovatis globosis, 200～220μ latis et altis	creberrimis, cylindricis, breviter, stipitatis	3～4-serialiter stipatis, fusiformi-cylindricis, leviter flexuosis, 3-septatis vix constrictis, 34～36×3μ	SACCARDO (1883)[11]
S. fraxinea SACC. et SPEG. *Fraxinus ornus* —wilting leaf—	hypophyllis, sparsis, lenticulari-globulosis, 1/8*mm*. diam.	fasciculatis cylindraceo-clavulatis, 60×9～10μ	2～3-stichis, cylindraceo-fusoideis, leniter curvulis, typice 3-septatis, quandoque 4～6-reptatis, 28～30×4μ	SACCRDO (1883)[11]
S. intermixta (B. et BR.) SACC. *Rubus fruticosa* —bark—	gregariis, leniti-cularibus ambitu circularibus v. ovatis, 80～100μ diam.	oblongo-clavatis 45～55 ×12～14μ. brevissime stipitatis apice tunica	distichis, clavato-obpyriformibus, 16～18×6～8μ, rectis v, curvulis, 3～4-septatis, ad septa leniter constrictis	SACCARDO (1883)[11]
S. camelliae PASS. *Camellia japonica* —dry twig—	sparsis v. laxe gregariis, globosis, tectis, atris, ostiolo minuto vix emerso	cylindraceis, subclavatis, subssessilibus,	distichis, subfusoideis, 3-septatis, medio leniter constrictis, septo medio magnis distincto, guttulatis, 17.5×5μ	SACCARDO (1891)[13]
S. phellogena D. SACC. *Acer campestris* —bark of twig—	laxe gregariis, innato-erumpentibus, papillulatis, globulosis, 100～200μ diam.	rosulatis, clavatis, subsessilibus sinuatis, apice rotundatis, 70～75 ×15～18μ,	inaequaliter distichis, oblongo-clavulatis, 3-septatis, raro 4, ad septum medium constrictis, apice rotundatis, 22～24×8μ	SACCARDO (1900)[16]
S. tilliaris FAUTR. et LAMB. *Tilia* sp. —twig—	epidermide velatis, applanatis, irregularibus, minutis	piriformibus, 20～30× 20～22μ	cumulatis, conico-truncatis, 4 septatis, septis constrictis, 18～20×5～7μ	SACCARDO (1900)[16]
S. Pruni MCALP. *Amygdalis communis* —dead twig—	sparsis v. subcongestis, erumpentibus, globosis, ostiolo papillatis, 120μ diam.	breve stipitatis, oblongis, 45～50×10μ	distichis, oblongo-ovatis, rectis, 3-septatis ad septa non constrictis, 15～16×4.5～5μ	SACCARDO (1905)[17]
S. Aucubae SHIRAI et HARA *Aucuba japonica* —leaf—	scattered, immersed, globose or hemisphaerical, papillate, 120～150 ×120～160μ.	clavate, cylindrical or fusiform, rounded at end, 40～80×10～13μ.	arranged in 2-rows or obliquely 1-seriate, fusiform, ellipticall or oblong, 2～3 septate, not constricted, 20～27×6～8μ	SHIRAI and HARA (1911)[19]

S. Rubi-moriforiae HARA *Rubus Wrightii* —fallen leaf—	scattered on both leaf surfaces, globose or subglobose, papillate	cylindrical or clavate, small stalked, rounded at end, 40～50×15～18μ	fusiform or elliptical, acute in both direction, 3-septate, often constricted at septum, 15～18×5～6μ	HARA (1918)[4]
S. Fuji HARA *Kraunhia floribunda* var. *typica* —fallen leaf—	scattered or in group on the under surface of leaf, globose or subglobose, papillate, 50～70μ diam.	oblong-clavate or elliptical, rounded at end, small stalked, 28～30×13～15μ	arranged in 2-rows, elliptical or fusiform, straight or curved, 3-septate, containing oil drops, 12～15×2～3μ	HARA (1918)[4]
S. Euptelaceae HARA *Euptella polyandra* —fallen leaf—	scattered on the under surface of leaf, globose, or subglobose, somewhat erumpent, 60～100μ diam.	cylindrical or clavate, small stalk, 40～50×10～13μ	arranged in 2-rows, fusiform or oblong, 3-septate, straight or slightly curved, usually not constricted at septum, rarely constricted, hyaline or light yellow, 13～15×3～3.5μ	HARA (1918)[4]
S. Magnoliae-Kobusii HARA *Magnolia praecocissima* —fallen leaf—	scattered or in group on the under surface of leaf, globose or subglobose, papillate, 50～80μ diam.	clavate or cylindrical, rounded at end, small stalked, 35～45×7～9μ	arranged in 2-rows, oblong or fusiform, rounded at both ends, 3-septate, not constricted at septum, 24～2(?)0×4～5μ	HARA (1918)[4]
S. rubi DEMAREE et WILCOX *Rubus strigosi* —fallen leaf—	scattered or in groups, mostly hypophyllous, black, conical, ostiolate-papillate, 88～140×86～120μ	fasciculate, sessile, clavate-cylindrical, curved or straight, 49～70×10～15μ	cylindrical, usually curved, pointed at both ends, slightly more so at apex 6～8 celled, normally 4, hyaline, 32～78×3.5～5.8μ	DEMAREE & WILCOX (1943)[3]
Sphaerulina of the authors *Juglans Sieboldiana* —fallen leaf—	scattered or in clusters, globose, papillate, 99～118×71～99μ	cylindrical clavate, round at end, 43～56×8～9μ	arranged in 2- or 3- rows, cylindric-fusiform, slightly curved, not constricted at septum, 3～7 septate, 24～35×3～4μ	

judging from the fact that the authors' fungus did not infect *Fraxinus*, this is to be treated as a different species from *S. Fraxinea.*

In view of the morphological features and the parasitism of the fungus, the authors consider it a new species and propose the following name;

Sphaerulina juglandis K. ITÔ et T. KOBAYASHI, sp. nov.

Syn. *Cercosporella juglandis* K. ITÔ et T. KOBAYASHI, nom. nov.

Peritheciis sparsis vel gregariis, innato-erumpentibus, globosis, membranaceis, ostiolo minuto papillutatis, 99～118×71～99 μ; ascis cylindraceo-clavulatis, apice rotundatis, aparaphysatis, octosporis, 43～56×8～9 μ, sporidiis 2～3-stichis, cylindraceo-fusoideis, leniter curvulis, ad septa non constrictis, 3～7-septatis, hyalinis, 24～35×3～4μ.

Hab. in overwintered fallen leaf of *Juglans Sieboldiana* MAXIM. (Oni-gurumi) (June 15, 1951, Meguro, Tokyo, on the material collected at Kamabuchi, Yamagata Pref., on Oct. 15, 1950, by K. ITÔ & T. KOBAYASHI*1; June 23, 1953, Meguro, Tokyo, on the material collected at Kamabuchi, Yamagata Pref., on June 23, 1952, by K. ITÔ; Oct. 1, 1950, Nakano, Yamanashi Pref., by T. KOBAYASHI*2; Sept, 1952, Meguro, by T. KOBAYASHI*2; Sept. 21, 1953, Miyamura, Miyagi Pref., by K. ITÔ*2), and *J. Sieboldiana* MAXIM. var. *cordiformis* MAKINO (Hime-gurumi) (Oct. 25, 1950, Imajuku, Saitama Pref., by T. KOBAYASHI*2).

Status conidicus: Statum conidicum *Cercosporella juglandis* sistit. Maculis epiphyllis, circularibus vel angulosis, brunneis, distincte marginatis, caespitulis, pulvereis albis; conidiophoris brevis, simplicibus, hyalinis, 32～68×3～5μ; myceliis stromaticis hyalinis; conidiis curvatis vel flexuosis, raro rectis, obclavatis, hyalinis, 1～10 septatis, 32～68×3～5 μ.

Hab. in living leaf of *Juglans Sieboldiana* MAXM., *J. Sieboldiana* MAXM. var. *cordiformis* MAKINO and *Juglans* sp.*3 (Shinano walnut).

Summary

The morphology, physiology, parasitology and cycle of development of a fungus that causes frosty mildew of walnut trees have been studied. The disease, as its name indicates, is characterized by the presence of white, powdery patches on the leaves.

The investigations have shown that, in addition to the conidial stage, *Cercosporella*, the pathogen produces spermogonia and perithecia on the fallen decaying leaves. The perithecial stage matures in spring and belongs to the genus *Sphaerulina.* Genetic relationship between *Cercosporella* and *Sphaerulina* has been verified by detailed experiments. The artificial inoculation shows that the disease is apparently limited to species and variety of the genus *Juglans.*

The fungus is considered to be a new species and it is accordingly given the name *Sphaerulina juglandis*, sp. nov.

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

*2 *Cercosporella* stage, only.

*3 By artificial inoculation.

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Explanation of plates

Plate 1

A-B. Leaves of *Juglans Sieboldiana* (Oni-gurumi) attacked by *Sphaerulina juglandis* sp. nov. collected in Yamanashi Pref. x 1.

C-D. Leaves of *J. Siedoliana* attacked by *S. juglandis* sp. nov. collected in Yamagata Pref. x 4.

Plate 2

A. Colonies of *S. juglandis* sp. nov. on potato sucrose agar. × 1. a, old culture (aerial mycelium, present). b, young culture (aerial mycelium, absent).

B. Colonies of *S. juglandis* sp. nov. on 2 per cent glucose agar (a), and potato sucrose agar (b, c). After 18 days at 20°C. × 4/5.

C. Results of the inoculation experiment with *S. juglandis* sp. nov. to *J. Sieboldiana.*

D. Results of the inoculation experiment with *S. juglandis* sp. nov. to *J. S ieboldiana* var. *cordiformis* (Hime-gurumi).

Plate 3

Results of the inoculation experiment with *S. juglandis* sp. nov. to walnut trees.

A, B, *J. Sieboldiana*; C, *J. Sieboldiana* var. *cordiformis*; D, "Shinano" walnut.

Plate 4

A. Spermogonium of *S. juglandis* sp. nov. in the fallen leaf of *J. Sieboldiana* × 310.

B. *Ditto.* × 150.

C. Perithecia of *S. juglandis* in the fallen leaf of *J. Sieboldiana* collected in Yamagata Pref. × 180.

D, E, F. *Ditto.* Stained with I-KI solution. × 310.

G. Perithecia of *S. juglandis* sp. nov. in the fallen leaf of *J. Sieboldiana* collected in Yamanashi Pref. × 310.

Plate 5

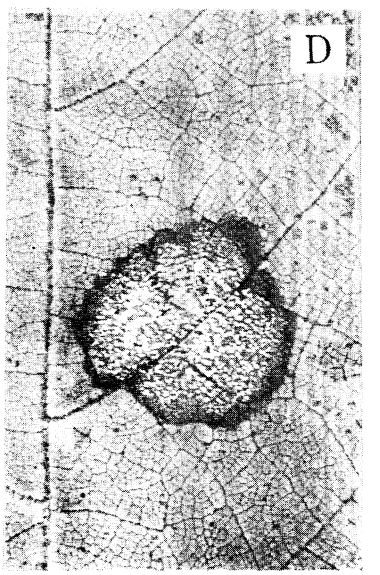
A. Relation between the growth of *S. juglandis* sp. nov. and temperatures.
C, isolate from conidium of *Cercosporella* stage; *S*, isolate from ascospore of *Sphaerulina* stage.
a, -1°C; b, 1°C; c, 6~8°C; d, 10~12°C; e, 16~18°C; f, 20°C; g, 25°C; h, 28°C; i, 30°C.

B. Effect of H-ion concentrations on the growth of *S. juglandis* sp. nov. (3 weeks after incubation, at 25°C).
C, isolate from conidium of *Cercosporella* stage; *S*, isolate from ascospore of *Sphaerulina* stage.
a, pH 3.4; b, pH 4.2; c, pH 4.6; d, pH 5.6; e, pH 6; f, pH 6.6; g, pH 7; h, pH 7.4; i, pH 7.6.


A


B


C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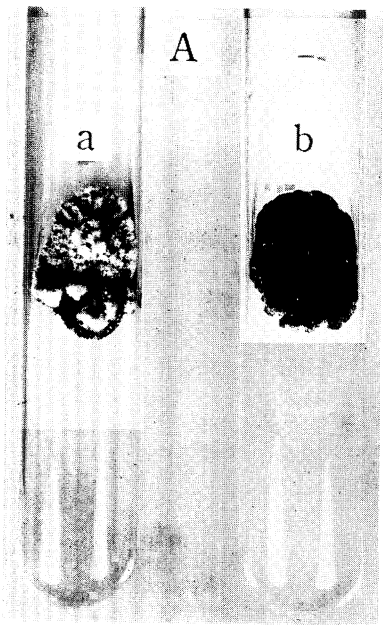
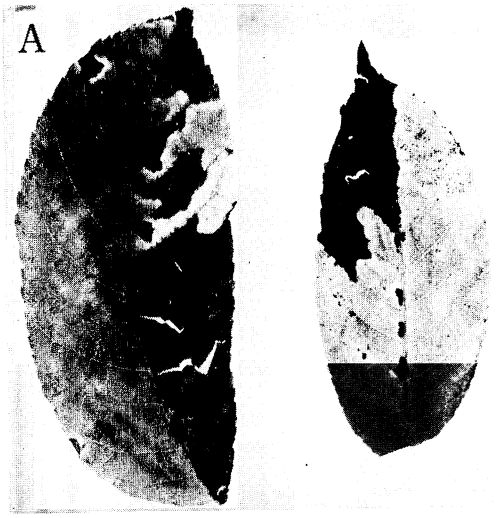
—Plate 2—

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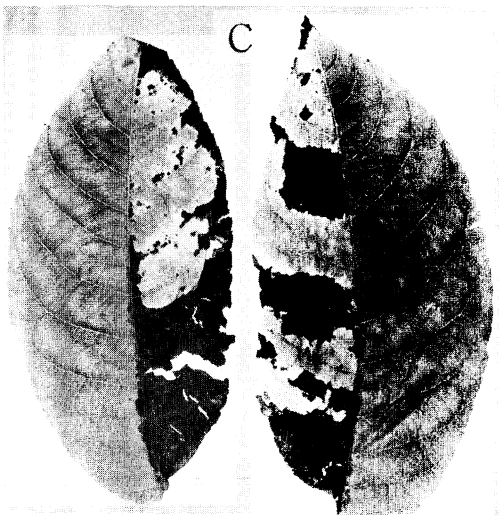
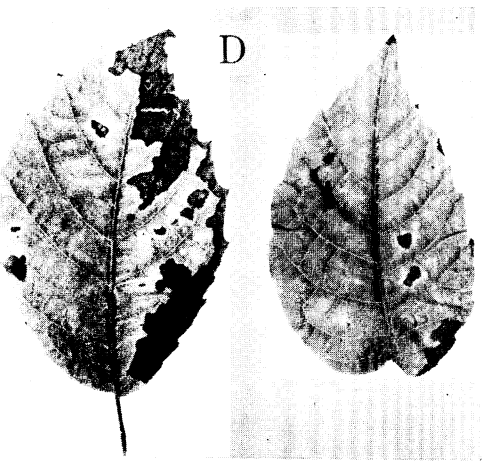

C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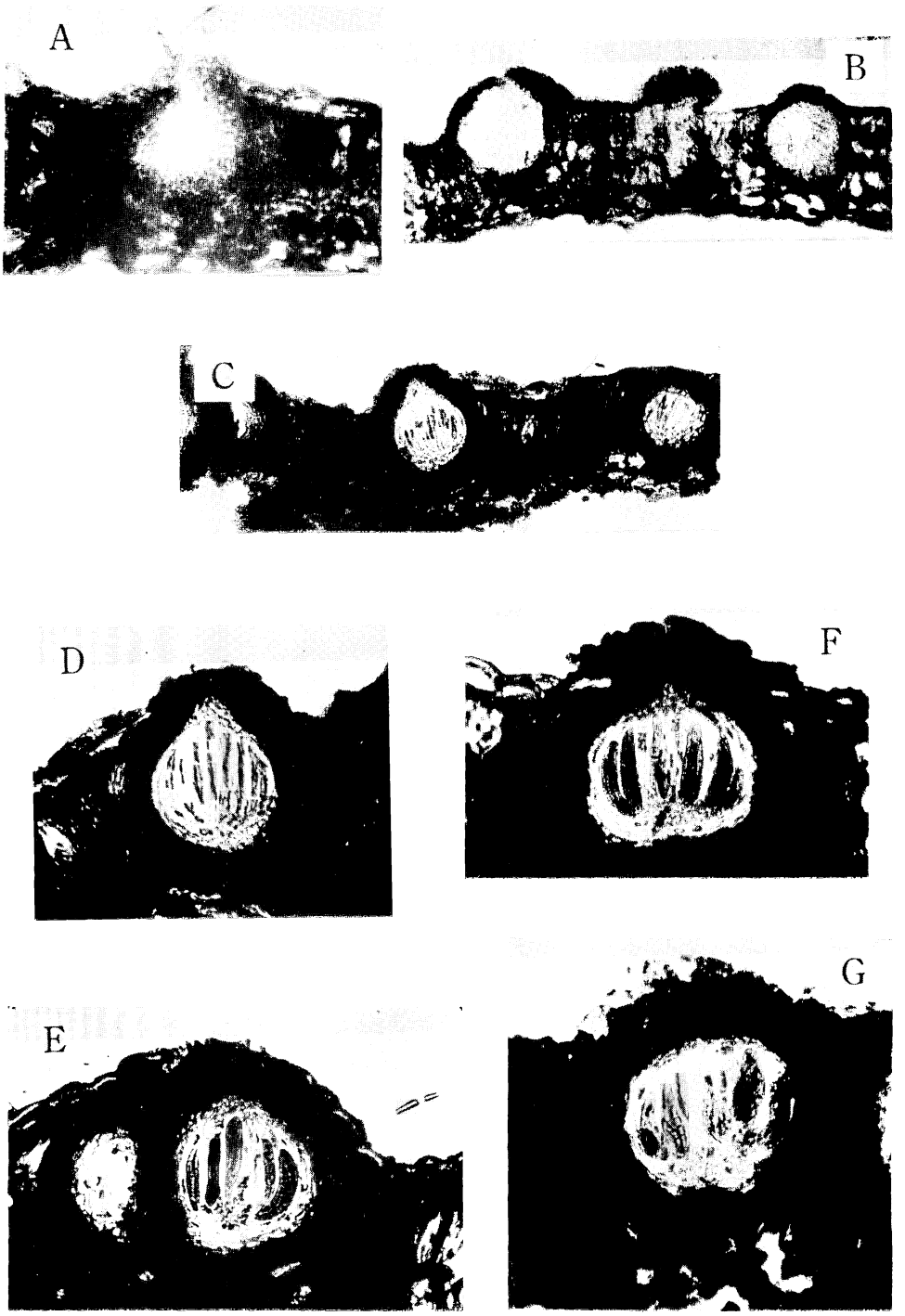
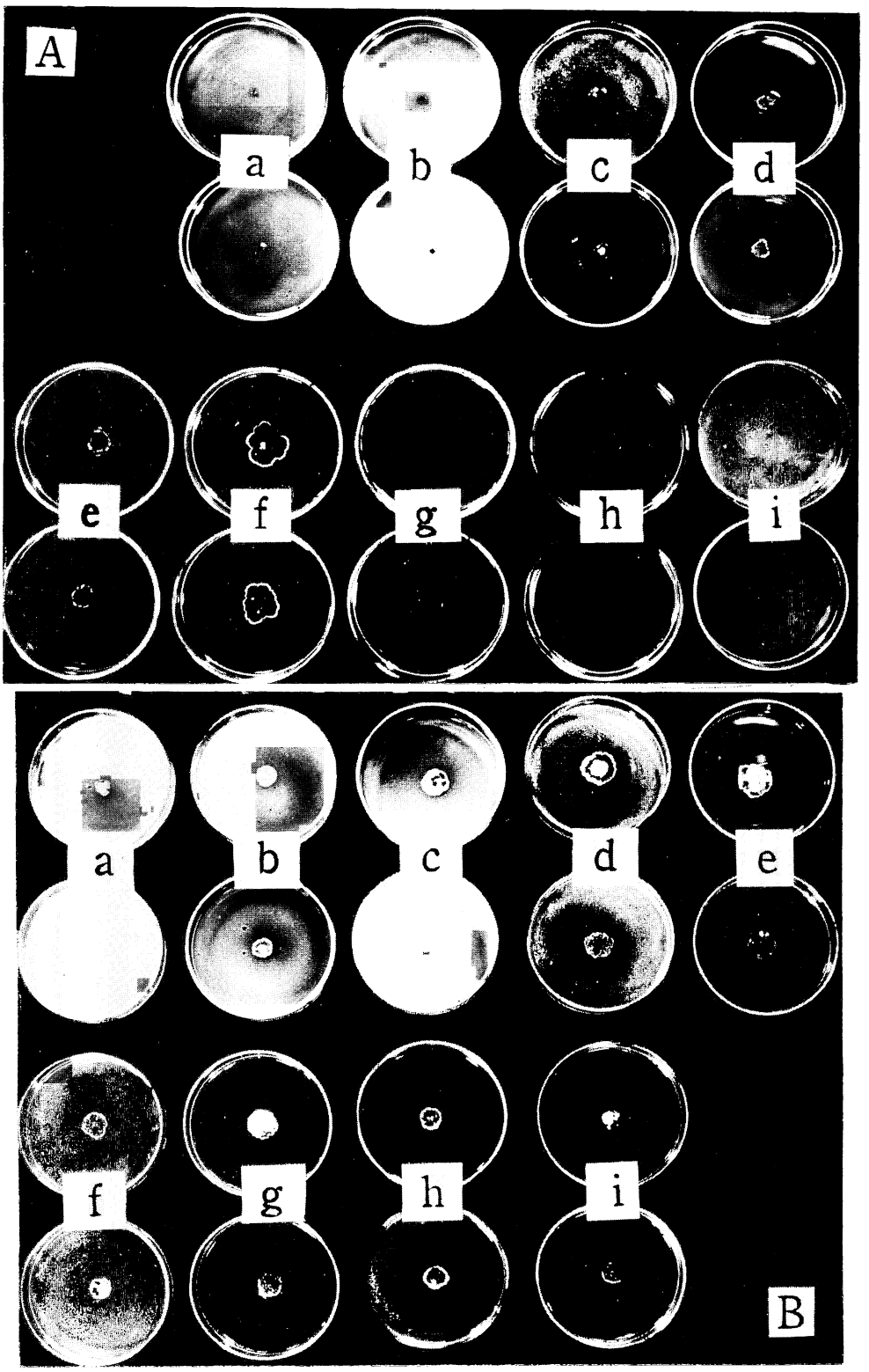
—Plate 4—

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