樹木炭疽病の研究—V

ニセアカシアの炭疽病菌

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昭和25年（1950年）7月，秋田市，県森連金照寺苗畑で採集された罹病ニセアカシア苗が秋田支場佐藤邦彦技官から著者らに届けられ，その病名鑑定を求められた。鏡検および分離実験の結果，これは炭疽病菌 *Colletotrichum* によるものであることがわかつた。その後，東京都目黒区下目黒林業試験場構内苗畑および世田ヶ谷区成城町東京営林局砧苗畑においても同一の病徴を呈する多数の被害苗ならびに幼・壮令木を見い出し，病状もはげしく，特に苗木に対しては軽視しえないほどの被害を与えていることがわかつた。

ニセアカシア類の炭疽病について述べられた文献はわが国では皆無であり，また海外においてもほとんど見られないので，ひととおり研究しておく必要があるものと考え，ただちに着手した。

ニセアカシアに炭疽病をおこすものとしておのおの異なる3種の *Colletotrichum* 菌と，1種の *Guignardia* 菌がえられた。そして比較検討の結果，これらの *Colletotrichum* 菌はそれぞれ，*Gloeosporium* (*Colletotrichum*) *revolutum* Ell. et Ev., *Colletotrichum destructivum* O'Gara および *Colletotrichum glycines* Hori と同定され，なお *Guignardia* 菌は *Gloeosporium* (*Colletotrichum*) *revolutum* の子嚢時代であることが立証されたのであるが，本菌にがい当する菌を文献上に見い出すことができなかつたので，新たに *Guignardia robiniae* K.Ito et T. Kobayashi, sp. nov. と命名することにした。

本報文はこれら3種の炭疽病菌の形態，生理および病原性に重点をおいて行つた実験結果の概要をとりまとめたものである。

この研究を実施するにあたり，御指導をいただいた保護部長今関六也氏，ダイズ炭疽病菌およびインゲン炭疽病菌をこころよく分譲され，ニセアカシアの菌との比較検討に多大の便宜をたまわつた国立衛生試験所技官倉田　浩氏（当時農業技術研究所），自ら検定された炭疽病に罹病性のダイズおよびインゲンの種子を恵与された横浜植物検疫所技官沢田啓司氏（当時北海道大学農学部），ニセアカシアの炭疽病を最初に発見してその被害苗を著者らに示し，本病研究のきつかけをつくつてくださつた秋田支場佐藤邦彦技官ならびに原図作成を援助された当場中川道夫氏の方々に心からお礼を申しあげる。

***Guignardia robiniae* sp. nov.**

(*Gloeosporium revolutum* Ell. et Ev.)

病　徴　はじめ小葉上に灰色～淡褐色で，周縁部に淡緑色の暈帯を伴う多数の微細な斑点が形成される。病斑はしだいに大きさをまし，円形～不定形，中心部は淡灰色，周縁部は褐色を呈する。はげしく侵

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された小葉は巻き込み，やがて乾燥して落下する。小葉柄および葉柄もまた侵され，病斑がこれらを一周すると急激に枝梢はしおれ，やがて落葉して枯死を招く。湿潤な場合には病斑上に淡桃色の本菌の分生胞子塊が生成される。

まき付床では発芽してまもなく子葉が暗色を呈し，やがて幼茎も侵されて腐敗枯死することがある。

本病は苗のみならず成木にも発生し，はげしい落葉をもたらすので，人目をひき，またはなはだしい落葉によつてその成長は阻害される（Plate 1. A, B)。

形　態　(1) *Colletotrichum* 世代　夏～秋に患部に認められる。

分生子堆は散生あるいは群生，赭褐色～淡桃色，直径 48～99 μ。分生子梗は無色，円筒形～紡錘形，11～22×1～4 μ。剛毛は分生子梗の間に形成され，単～2細胞，黄褐色，直あるいはすこしく彎曲，31～42×2～4 μ。分生胞子は無色，直，両端円く単細胞，10～15×2～5 μ（Plate 2. A; Text-fig. 1. A, B)。

(2) *Guignardia* 世代　9月中旬ごろ，罹病した小葉柄および葉柄上に *Colletotrichum* の分生子堆に近接して *Guignardia* 菌の子嚢殻が形成される。

子嚢殻は孤生あるいは群生，球形，すこしく突出してわずかに乳頭状を呈し，大きさ 76～133×78～168 μ。子嚢は棍棒状あるいは長楕円形，側糸を欠き8胞子を含み，36～56×7～11 μ。子嚢胞子は無色，広楕円形～楕円形，2列に並び単細胞，大きさ 10～17×4～6 μ（Plate 2. B, C; Text-fig. 1. D, E)。

***Colletotrichum* と *Guignardia* の同根関係**　*Colletotrichum* と *Guignardia* の間に同根関係のあることが予想されたので，これを立証するために次の諸実験を行つた。

培養基上の特徴　*Colletotrichum* の分生胞子と *Guignardia* の子嚢胞子からそれぞれ単個培養を行い，馬鈴薯寒天，2% 蔗糖寒天，斎藤氏醤油寒天，Waksman 氏寒天，Czapek 氏寒天，ニセアカシア葉の煎汁寒天，ダイズ煎汁寒天およびインゲン煎汁寒天に培養して両者の比較を行つた。

その結果，各種培養基上における両者の菌叢の特徴にはいちじるしい差は認められず，また培養基上に形成される分生胞子の形状，大きさもよく一致した。ただし，*Guignardia* の子嚢胞子から分離された菌株は培養基上に子嚢殻を形成したが *Colletotrichum* の分生胞子からの菌にはこれが認められなかつた。

なお，本菌の培養基上の特徴はインゲン炭疽病菌 *Colletotrichum lindemuthianum* (Sacc. et Magn.) Bri. et Car. とはいちじるしい差がみとめられた。

生　理　(1) 胞子の発芽　*Colletotrichum* の新鮮な分生胞子は数時間内に発芽し，24時間後には 90% の発芽率を示した。発芽にさきだち胞子は隔膜によつて2細胞になり，やがて各細胞から発芽管が伸長する。付着器の形成がしばしばみとめられた（Text-fig. 1. C; Text-fig. 4. A)。

Guignardia の子嚢胞子もまたよく発芽し両端から発芽管を出すのが普通である（Text-fig. 1. F)。

(2) 温度と菌糸の発育　*Colletotrichum* 菌および *Guignardia* 菌の両者はともに 13～30° C でよく発育し，25～28° C を適温とし，その傾向には差がみとめられないが，しかし菌糸の成長は *Colletotrichum* 菌が *Guignardia* 菌よりもややすみやかであつた。

なお，インゲン炭疽病菌 *C. lindemuthianum* は最低，最高ともにかなり低く，ニセアカシアの菌とはその傾向にややいちじるしい差が認められた。

(3) pH と菌糸の発育　pH の菌糸の発育におよぼす影響は極端（pH 3）な場合を除き，そう大きなものではなく，また *Colletotrichum* と *Guignardia* 両菌の間に差がみとめられなかつた。

病原性 *Colletotrichum*, *Guignardia* 両菌の病原性を比較するために数回にわたつて接種試験を行つた。

接種試験—1 ニセアカシア当年生苗に対して実施した結果は4日後に初期病徴がみとめられ，さらに2日後には両菌の場合とも分生子堆が形成され，病徴，菌の形態はいずれも野外でみとめられるものと同一であつた（Plate 1. D)。なお，*Guignardia* 菌を接種した場合には後に子嚢殻が形成された。

接種試験—2 両菌をニセアカシア，ヤマハギ（シロハギ)，イタチハギ（クロバナエンジユ)，インゲン（テナシナガウズラ）およびダイズ（ツルノコ）のおのおのに接種した。その結果は *Colletotrichum* 菌はダイズを除くすべてに病原性を示し，また *Guignardia* 菌はダイズとインゲンに病斑を形成しなかつた。

接種試験—3 ニセアカシア，ヤマハギ，イタチハギ，ダイズに対して *Colletotrichum* 菌，*Guignardia* 菌およびインゲン炭疽病菌 *C. lindemuthianum* を接種した。その結果は，*Colletotrichum*, *Guignardia* 両菌はダイズを除くすべての樹種に病原性を示したが，インゲン炭疽病菌はダイズだけを侵した。

接種試験—4 *Colletotrichum* 菌，*Guignardia* 菌およびインゲン炭疽病菌をダイズとインゲンに接種した。その結果，*Colletotrichum* 菌はインゲンに対しては微弱な病原性を現わしたが，ダイズではまつたくこれがみとめられず，また *Guignardia* 菌はインゲン，ダイズとも陰性の結果を示した。インゲン炭疽病菌はインゲンに対しては強い病原性を現わしたが，ダイズにはきわめて微弱であつた。

以上の形態，生理および病原性をしらべた結果から，*Colletotrichum* 菌と *Guignardia* 菌の間には明らかに同根関係が存在する。すなわち，*Guignardia* 菌は *Colletotrichum* 菌の子嚢時代（完全時代）ということができる。

分 類 1. 分生胞子世代 分生胞子の大きさ，病原性および菌糸発育の適温からみて，本菌とインゲン炭疽病菌 *C. lindemuthianum* との間にはいちじるしい差がみとめられる。

フサアカシア炭疽病菌 *Physalospora acaciae* K. Itô et Shibukawa (Itô & Shibukawa 1956)の *Colletotrichum* 世代は本菌と類似しているが，完全時代と病原性において両者は明らかにおのおの別種である。

文献によるとニセアカシア上に記載された炭疽病菌あるいはその近縁の菌として *Gloeosporium revolutum* Ell. et Ev. (Saccardo 1892), *Myxosporium russelii* (B. et Br.) Sacc. (Saccardo 1884) および *M. robiniae* Karst et Har. (Saccardo 1892) の3種がある。著者らの菌はこれらのうち，*Gloeosporium revolutum* とその分生胞子の大きさがよく一致する。病徴においていささか相違する点があるので確かなことはいえないが，著者らの菌を *G. revolutum* と一応同定しておくことにする。

2. 子嚢世代 *Glomerella cingulata* (Stonem.) Spauld. et v. Schrenk がルーピン（Weimer 1943), クズ（Tiffany & Gilman 1954), ハギ（Weimer 1943）などマメ科植物をおかすことが報告されている。最近 von Arx (1957) は *Glomerella cingulata* の分生胞子世代 *Colletotrichum gloeosporioides* Penz の Synonym としてひじように多くの *Colletotrichum* および *Gloeosporium* をかかげ，*C. acaciae* de Urries, *G. acaciae* Mc Alp., *C. camelliae* Massee および *G. revolutum* Ell. et Ev. なども，これに包括させている。彼のモノグラフからは，はたしておのおのについてその子嚢時代まで調べてこのような取りあつかいをしたかどうか知ることができない。

著者らの菌はその形態的特徴からして *Glomerella* 属ではなく，*Guignardia* 属とすべきものと考えら

れる。従来おおやけにされたものと比較したが本菌に一致するものがないので，これを未記載の菌と考え，新たに次のとおり命名することにした。

***Guignardia robiniae* K. ITO et T. KOBAYASHI, sp. nov.**

Syn. *Gloeosporium revolutum* ELL. et Ev. (1889)

Colletotrichum revolutum (ELL. et Ev.) comb. nov.

Colletotrichum destructivum O'GARA

まれではあるが，真直の分生胞子を有し，*Colletotrichum revolutum* (=*Guignardia robiniae* sp. nov.) とは異なるいま1種の *Colletotrichum* 菌を見い出した。この菌はしばしば *C. revolutum* と混在することもあるが，形態的特徴に大きな差がある。

形　態　分生子堆は小さく，直径 25～59 μ，散生あるいは群生。分生子梗は無色，円筒形～紡錘形，11～16×3 μ。剛毛は少数で分生胞子の間にあり，褐色，真直～わずかに彎曲し，3～4個の隔膜を有し，53～90×3～5 μ。分生胞子は無色，真直で両端円く，単細胞，14～19×3～4 μ (Text-fig. 2)。

生　理　1．分離および培養　分生胞子の単個培養をえて種々の培養基上の特徴をみると，本菌は *C. revolutum* およびインゲン炭疽病菌と全く異なる。

2．温度と菌糸の発育　本菌は 6～35° C で発育し最適温度は 25° C で，*C. revolutum* およびインゲン炭疽病菌よりも発育の温度範囲が広い。

3．pH と菌糸の発育　極端な場合 (pH 3) を除き菌糸の発育におよぼす pH の影響は顕著でない。

病原性　本菌の病原性をたしかめ，なお *C. revolutum* およびインゲン炭疽病菌と比較するために，ニセアカシア，ヤマハギ，イタチハギ，インゲンおよびダイズに対して接種試験を行つた。その結果，本菌は供試植物のすべてに対して病原性を示し，特にニセアカシアに対しては *C. revolutum* よりも強烈であつた。しかし，インゲンとダイズに対してその病原性は微弱であつた (Plate 3. A, B; Plate 5; Plate 6)。

分　類　本菌の形態的特徴は，最初クローバー上に記載された *Colletotrichum destructivum* O'GARA (O'GARA 1915) によく一致する。TIFFANY & GILMAN (1954) によれば，これは多犯性でハギ，ダイズを含む多数のマメ科植物に炭疽病をおこすという。

著者らの菌はハギに対しては明らかな病原性を示すが，ダイズに対してはきわめて微弱である。それでこれを *C. destructivum* の一系統としておくことにする。

Colletotrichum glycines HORI

昭和 25 年の夏，林業試験場構内苗畑においてニセアカシア苗にはげしい病害が発生し，その 80 % は罹病，多数の苗が枯死した。

著者らは病斑上にダイズ炭疽病菌 *Colletotrichum glycines* HORI ときわめてよくにた，彎曲した分生胞子を有する菌を見いだしたのであるが，翌 26 年9月には世田ヶ谷区成城町砧苗畑において同一菌による病害を認めた。

病　徴　初期病徴は6月上旬に認められる。そして夏期，特に6月下旬～8月中旬にははなはだ激しい病状になる。

最初，小葉，小葉柄および葉柄に微細な紫褐色斑点が多数形成され，一葉に 50 個以上をかぞえることもある。斑点ははじめ円形で黄色の暈帯でとりまかれ直径 0.3 *mm*，のち急速に拡大して 1～2 *mm* に達する。葉柄，茎では病斑は円形ないし不定形で多数近接して線状に配列することがある。湿潤な環境下では灰色の分生胞子塊が病斑上に多数形成される（Plate 3. C; Plate 4. A, B）。

病斑が葉柄を一周すると枝梢は急速にしおれ，やがてはげしい落葉がおこる。

生　理　本菌の分生胞子は培養基上で数時間内に発芽する（Text-fig. 3. C; 4. B）。分生胞子から単個培養をえて種々の培養基上の特徴をしらべた結果，これはダイズ炭疽病との区別がほとんどつかなかつた。

1．温度と菌糸の発育　ダイズ炭疽病菌と比較しながら，本菌の発育におよぼす温度の影響をしらべた。その結果，本菌は 8～35°C で発育し，適温は 25～28°C，ダイズ炭疽病菌のそれとよく一致する。

2．pH と菌糸の発育　本菌菌糸の発育におよぼす pH の影響はあまり顕著でなく，なおこれはダイズ炭疽病菌においても同様であつた。

病原性　本菌の病原性をたしかめ，なおダイズ炭疽病菌との異同を知るために次の接種試験を行つた。

接種試験—1　ニセアカシアとイタチハギに対する試験結果は，接種4日後にははやくもニセアカシアの小葉に病斑が形成され，病斑は急速に拡大してゆき，その病徴は野外でみられるものと同様であつた。しかし，イタチハギでは病徴の発現がややおくれ，10 日後に形成された。やがて罹病して落下した葉柄上に多数の分生子堆が確認された。

接種試験—2　ニセアカシア，ヤマハギ，イタチハギ，インゲンに対して接種を行つた。その結果本菌はインゲンを除くすべての植物に病原性を現わし，特にニセアカシアに激しい病状をもたらした（Plate 3; Plate 4. C, D; Plate 5; Plate 6）。

接種試験—3　本菌とダイズ炭疽病菌をニセアカシア，ヤマハギおよびイタチハギに接種した。試験結果は両菌とも全供試植物に病原性を現わした。

接種試験—4　本菌とダイズ炭疽病菌をダイズとインゲンに接種した。その結果，本菌はダイズに対しては病原性を示したが，インゲンには病原性なく，またダイズ炭疽病菌は両者に病原性をしめした。ただし，ダイズ炭疽病菌でもインゲンに対する病原性は微弱であつた。

上の接種試験結果から，おのおの分離源寄主に対してより強い病原性を示すことは明らかであるが，本菌とダイズ炭疽病菌の病原性にはいちじるしい差がないということができる。

形態と分類　1．形態　分生子堆は線状あるいは広楕円形，群生，直径 54～111 μ。剛毛は多数形成され剛棘状，尖端鋭，2～3個の隔膜を有し，褐色，84～225×5～7 μ。分生子梗は叢生，円筒形～紡錘形，無色，11～20×3～4 μ。分生胞子は彎曲して尖端鋭，1～4個の空胞を含み，無色，単胞，22～32×2～4 μ（Text-fig. 3. A, B; Plate 2. D）。

2．分類　本菌の形態的特徴はダイズ炭疽病菌 *Colletotrichum glycines* Hori とよく一致する（Hemmi 1920）。また生理的性質，病原性もきわめて近似するので本菌を *C. glycines* Hori と同定する。

Lehman & Wolf（1926）は *C. glycines* の子嚢時代を発見し，これを *Glomerella glycines*（Hori）n.n. と命名した。しかし Tiffany & Gilman（1954）によれば *Glomerella glycines* は *C. destructivum* の子嚢時代であつて *C. glycines* とは無関係だということである。

TIFFANY & GILMAN (l. c.) は *C. glycines* HORI を *C. truncatum* (SCHW.) ANDRUS et MOORE の Synonym として取りあつかい，また VON ARX (1957) は *C. truncatum* を *C. dematium* の品種 (forma) としているが，著者らはわが国で広く使用されている学名 *C. glycines* を採用しておく。

ニセアカシアを侵す炭疽病菌として著者らは3種の *Colletotrichum* と1種の *Guignardia* を得た。これら *Colletotrichum* のうち2種は真直の分生胞子を持ち，1種は彎曲した鎌状の分生胞子を有しており，おのおの *Gloeosporium revolutum* ELL. et EV., *C. destructivum* O'GARA および *C. glycines* HORI と同定した。なお *Guignardia* 菌は *Gloeosporium* (*Colletotrichum*) *revolutum* の子嚢時代であることが明らかになり，これに対して新たに *Guignardia robiniae* K.ITÔ et T.KOBAYASHI, sp. nov. という名を与えた。

G. robiniae sp. nov. はもつとも多くみとめられ，ついで *C. glycines* が，そして *C. destructivum* はまれにしか見い出されなかつた。

これら3種の菌およびダイズからの *C. glycines* およびインゲン炭疽病菌 *C. lindemuthianum* の，数種のマメ科植物に対する病原性を概括すると次表のとおりである。

菌名＼植物名	ニセアカシア	ヤマハギ	イタチハギ	ダイズ	インゲン
G. robiniae sp. nov. (*Colletotrichum* 世代)	++	+	+	−	+
G. robiniae sp. nov. (*Guignardia* 世代)	++	+	+	−	−
C. destructivum	+++	+	+	±	±
C. lindemuthianum (インゲン炭疽病菌)	−	−	−	+	+++
C. glycines (ニセアカシア)	+++	++	++	++	−
C. glycines (ダイズ)	+++	++	++	+++	+

図　版　説　明

Plate 1.

A．*Guignardia robiniae* sp. nov. に侵されたニセアカシア
B．*G. robiniae* sp. nov.（*Colletotrichum* 世代から分離した菌）に侵されたニセアカシアの葉（接種試験による）
C．*G. robiniae* sp. nov. の子嚢殻を形成しているニセアカシアの葉柄
D．*G. robiniae* sp. nov.（*Guignardia* 世代から分離した菌）のニセアカシアに対する接種試験結果

Plate 2.

A．*G. robiniae* sp. nov. の分生子堆（ニセアカシア）×310
B．*G. robiniae* sp. nov. の子嚢殻（ニセアカシア）　×310
C．接種試験によつてニセアカシアの葉柄に形成された *G. robiniae* sp. nov. の子嚢殻　×150
D．*Colletotrichum glycines* の分生子堆（ニセアカシア）　×150
E．2% ブドウ糖寒天上に形成された *C. glycines* の分生子堆　×2

Plate 3.

A．*Colletotrichum destructivum* に侵されたニセアカシアの葉（接種試験による）
B．*C. destructivum* に侵されたイタチハギの葉（接種試験による）
C．*Colletotrichum glycines* に侵されたニセアカシアの葉
D．同上（接種試験による）

Plate 4.

A．*C. glycines* に侵されたニセアカシアの枝梢
B．*C. glycines* に侵されたニセアカシアの茎　×1.4
C．*C. glycines* に侵されたヤマハギの葉（接種試験による）
D．*C. glycines* に侵されたイタチハギの葉（接種試験による）

Plate 5.

A．数種の炭疽病菌のニセアカシアに対する接種試験結果
　a．*Guignardia robiniae* sp. nov.（*Colletotrichum* 世代から分離した菌）
　b．*G. robiniae* sp. nov.（*Guignardia* 世代から分離した菌）
　c．*Colletotrichum destructivum*
　d．インゲン炭疽病菌 *C. lindemuthianum*
　e．*C. glycines*（ニセアカシアから分離した菌）
　f．ダイズ炭疽病菌 *C. glycines*
B．数種の炭疽病菌のイタチハギに対する接種試験結果
　a．*G. robiniae* sp. nov.（*Colletotrichum* 世代から分離した菌）
　b．*G. robiniae* sp. nov.（*Guignardia* 世代から分離した菌）
　c．*C. destructivum*
　d．インゲン炭疽病菌 *C. lindemuthianum*
　e．*C. glycines*（ニセアカシアから分離した菌）
　f．ダイズ炭疽病菌 *C. glycines*

Plate 6.

A．数種の炭疽病菌のダイズに対する接種試験結果
- a．*G. robiniae* sp. nov.（*Colletotrichum* 世代から分離した菌）
- b．*G. robiniae* sp. nov.（*Guignardia* 世代から分離した菌）
- c．*C. destructivum*
- d．インゲン炭疽病菌 *C. lindemuthianum*
- e．*C. glycines*（ニセアカシアから分離した菌）
- f．ダイズ炭疽病菌 *C. glycines*
- g．対照

B．数種の炭疽病菌のインゲンに対する接種試験結果
- a．*G. robiniae* sp. nov.（*Colletotrichum* 世代から分離した菌）
- b．*G. robiniae* sp. nov.（*Guignardia* 世代から分離した菌）
- c．*C. destructivum*
- d．インゲン炭疽病菌 *C. lindemuthianum*
- e．*C. glycines*（ニセアカシアから分離した菌）
- f．ダイズ炭疽病菌 *C. glycines*
- g．対照

Studies on Some Anthracnoses of Woody Plants—V*
Anthracnose fungi of black locust

Kazuo Itô and Takao Kobayashi

Introduction

In July, 1950, specimens of black locust (*Robinia pseudoacacia*) seedlings which had been collected in Akita City, bearing what appeared on causal examination to be a species of *Colletotrichum*, were received from Mr. Kunihiko Satô. Since this material was accompanied by a request for identification, the authors made some researches on the fungus.

In August of the same year, the authors observed a serious seedling disease of black locust in a nursery at the Government Forest Experiment Station, at Meguro, Tokyo, almost all of the seedlings being severely defoliated. Since then the disease has appeared at the station in succeeding years, and has been found in other localities in metropolitan Tokyo, which has afforded opportunity for a more intensive study of it.

The microscopic examination and the isolation test showed that the disease was caused by a *Colletotrichum* which was very similar to Akita's fungus. The fungus has been found identical with one described by Ellis and Everhart in 1889 as *Gloeosporium revolutum* on leaves of *Robinia pseudoacacia* in Newfield, America (Saccardo 1892).

An ascomycete belonging to the genus *Guignardia* occurred often in the anthracnose lesions. The genetic relationship between the *Colletotrichum* and the *Guignardia* was verified by the authors. The name *Guignardia robiniae* sp. nov. is proposed for the ascigerous stage of the fungus.

Another straight-spored *Colletotrichum* has been collected by the authors, though rarely, on the same plant species at Seijô, Tokyo. This fungus resembles closely *C. destructivum* O'Gara.

In the course of studying the anthracnose disease of black locust, the authors have found a *Colletotrichum* bearing curved-spores, which was identified as *Colletotrichum glycines* Hori causing a soy bean anthracnose.

A search through the relevant literature failed to show that anthracnose fungi of black locust had previously been described or reported from Japan. In this paper the authors deal with the anthracnose fungi of black locust with special emphasis on their morphology, physiology and pathogenicity. A portion of this paper has already been presented at the Annual Meeting of the Phytopathological Society of Japan, Tokyo, April, 1955 (Itô & Kobayashi 1956).

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Guignardia robiniae sp. nov., the ascigerous stage of *Gloeosporium revolutum* ELLIS et EV.

Since the first collection in Akita City, in 1950, this fungus has been frequently obtained by the authors in Tokyo. It no doubt occurs in other districts where black locusts are grown, but the definite distribution of the fungus still remains obscure. This fungus has been found more commonly than the others, *Colletotrichum destructivum* and *C. glycines*, which will be described in a later part of this paper.

During the early autumn of 1950 and 1951 while studying the anthracnose disease, an ascomycetous fungus was observed by the authors. The fungus, although not of general occurrence, was found only in the anthracnose lesions, and its perithecia were usually accompanied by acervuli in which were produced typical *Colletotrichum* conidia. This led to the belief that there might be a possible connection between the fungous forms, and some experiments were conducted in order to verify this assumption.

Symptoms

Though seedlings are apparently the most seriously affected, nursery stocks, saplings and adult trees are also subject to the disease.

The first evidence of the disease is numerous spots on the leaflets. They are at first minute, gray or light brown, and surrounded by a faint yellow halo. As the lesions enlarge, they develop in a roughly circular or irregularly shape, and have light gray centers with brown margins. Severely diseased leaflets are curled, dried, and later they fall. The petiolules and petioles are also affected by the disease. The lesions very frequently girdle entire petiolules and petioles, causing a rapid wilting, early defoliation and subsequent death of the shoot. Under moist conditions, pinkish conidial masses are abundantly produced on the lesions.

In black locust nurseries, it has been observed frequently that seedlings sometimes are killed by the anthracnose fungus soon after emergence. Infection first appears on the cotyledon as darkened lesions and gradually extends downward to the hypocotyl. The young stem rots and after a short time collapses.

Many of the lower branches on a large number of trees, even on adults, are completely defoliated. Defoliation brings about a decrease in growth increment, and may result in the death of the seedlings. The symptoms and effects of the disease are so striking that the severely diseased trees attract attention from a distance (Plate 1. A, B).

Morphology

(1) *Colletotrichum*　The *Colletotrichum* may be found at any time throughout the entire summer and the early part of autumn, when new lesions appear.

Acervuli erumpent, scattered or gregarious, larger and irregular when confluent, ochraceous buff to light pink, 48～99 μ in diameter; conidiophores hyaline, cylindrical or fusoid, 11～22×1～4 μ; setae among the conidiophores, 1- or 2-celled, cinnamon-brown,

straight or slightly curved, 31～42×2～4 μ; conidia hyaline, straight with rounded ends, 1-celled, 10～15×2～5 μ (Plate 2. A; Text-fig. 1. A, B).

Results of the measurement for the dimension of the *Colletotrichum* are shown in table 1.

Table 1. Dimension of *Colletotrichum* stage of *Guignardia robiniae* sp. nov. on black locust in nature (μ)

Locality	Width of acervulus	Size of seta	Size of conidiophore	Size of conidium
Akita City Jnly, 1950	48～90	31～38×2～3	15.5×2*1 (11～22×1～3)*2	11.5×3.5 (11～13×2～4)
Meguro Tokyo Aug. 28, 1950	61～88	27～38×3～4	12×2.5 (11～13×2～3)	12.5×4 (10～14×3～5)
Meguro Tokyo Sept. 21, 1950	53～99	32～42×3～4	11.5×2.5 (10～14×2～4)	12.5×4 (11～15×4～5)

Notes: *1....Averaged, *2....range.

(2) *Guignardia* By mid-September matured perithecia of an ascomycete are found near acervuli of the *Colletotrichum* on the diseased petiolules and petioles (Plate 1. C). The morphological features of the fungus are clearly the same as those that characterize the genus *Guignardia*.

Mature perithecia single or in groups, partially erumpent, globose, slightly papillate, 76～133×78～168 μ; asci clavate or ovato-oblong, aparaphysate, 8-spored, 36～56×7～11 μ; ascospores hyaline, ovate or elliptical, arranged biseriately, 1-celled, 10～17×4～6 μ (Plate 2. B, C; Text-fig. 1. D, E).

Dimensions of the *Guignardia* are presented in table 2.

Table 2. Dimension of *Guignardia robiniae* sp. nov. collected on black locust in nature (μ)

Locality	Host plant	Size of perithecium	Width of perithecium wall	Size of ascus	Size of ascospore
Meguro, Tokyo Sept. 14, 1950	Adult tree	84.2×93.2 (80～90×78～111)	7.9 (6～11)	40.4×8.8 (36～44×7～11)	13.8×5.4 (12～17×4～6)
Meguro, Tokyo Sept. 21, 1950	Seedling	95.3×130 (76～105×113～168)	12.0 (8～15)	40.5×9 (36～50×8～11)	12×4.6 (10～15×4～5)
Seijô, Tokyo Sept. 11, 1951	Sapling	106.8×112.4 (86～133×89～130)	13.2 (9～19)	49.3×10 (43～56×9～11)	12.8×5.2 (11～16×4～6)

Genetic relation between the *Colletotrichum* and the *Guignardia*

The possibility of a genetic connection between the *Colletotrichum* and the *Guignardia* was presumed by the authors, and some experimental work was undertaken.

Isolation By a modification of Yoshii's (1933) method using 2 per cent aqueous solution of copper sulphate to avoid bacterial contamination, single-spore isolates were obtained from the conidium of the *Colletotrichum* and ascospore of the *Guignardia*, respectively.

Characteristics on agar media The isolate from the *Colletotrichum* and that from the *Guignardia* were cultured on the following agar-media, respectively: Potato sucrose agar, 2 per cent dextrose agar, Saito's soy agar, Waksman's solution agar, Czapek's

solution agar, black locust leaves decoction agar, soy bean decoction agar and common bean decoction agar.

As regards the macroscopic appearances of the colonies on various agar media, there were no remarkable differences between the isolate from the conidium and that from the ascospore, though there might be a slight difference in the color of the colony. In shape and size, conidia of the ascosporous isolate produced on agar media were particularly accordant with those of the conidial isolate.

It is noteworthy that the ascosporous isolate produced abundant mature perithecia on agar media, while the conidial isolate did not produce any at all. The dimensions of the *Guignardia* produced on agar media are shown in table 3.

Table 3. Dimension of *Guignardia robiniae* sp. nov. produced on agar medium

Size of perithecium	Width of perithecium wall	Size of ascus	Size of ascospore
150×141 (122～215×116～185)	——	44.1×8.2 (51～76×7～8)	16.5×4.5 (13～21×4～5)

Characteristics of the mycelial colony of the fungus on agar media were quite different from those of *Colletotrichum lindemuthianum* (Sacc. et Magn.) Bri. et Car.*, a straight-spored fungus causing an anthracnose disease of common beans.

Physiology

1. Germination of spores　Fresh conidia of the *Colletotrichum* began to germinate in a few hours with over 90 per cent germination in 24 hours. As an initial step in germination a median septum was generally formed. This was followed by the formation of germ tubes. Appressoria, dark colored chlamydospore-like bodies, were very frequently formed on germ tubes (Text-fig. 1. C; Text-fig. 4. A).

Ascospores of the *Guignardia* germinated well, and sent out usually 2 germ tubes from each end of the spore. The germination of both types of spores was markedly similar (Text-fig. 1. F).

2. Effect of temperatures on the mycelial growth　The relation of temperature to growth of the mycelium was tested by the Petri dish method using potato sucrose agar. For inocula bits of mycelial colonies originated from each of the conidium and ascospore were cut and transplanted to the center of each plate, and then plates were placed in incubators regulated at desired temperatures. Diameters of the mycelial colonies at each temperature measured and averaged after 6 days are presented in tables 4～6.

Table 4. Relation between temperature and the mycelial growth of *Guignardia robiniae* sp. nov. (1)

Origin of isolate	Experiment No.	Diameter of colony (*mm*)				
		0～1°C	6～7°C	13～14°C	18°C	20°C
Colletotrichum	I	0	0	11	27	37
Guignardia		0	0	18	36	53
Colletotrichum	II	0	0	13	35	43
Guignardia		0	0	14	39	53

* The culture was kindly supplied by Mr. Hiroshi Kurata, of the National Hygienic Laboratory.

Table 5. Relation between temperature and the mycelial growth of *Guignardia robiniae* sp. nov. (2)

Origin of isolate	Experiment No.	Diameter of colony (*mm*)					
		20° C	25° C	28° C	30° C	35° C	40° C
Colletotrichum Guignardia	I	41 59	51 75	51 81	14 33	0 ±	0 0
Colletotrichum Guignardia	II	44 53	51 67	47 79	16 40	0 ±	0 0

Table 6. Relation between temperature and the mycelial growth of *Guignardia robiniae* sp. nov. (3)

Origin of isolate	Diameter of colony (*mm*)									
	Temperature (°C)									
	0	6～8	13	18	20	25	28	30	35	40
Colletotrichum	0	±	13	37	45	53		21	0	0
Guignardia	0	±	11	40	52	68	76	48	0	0

It is clear from tables 4～6 that both of the isolates grow favorably at the temperatures ranging from 13 to 30°C, with an optimum 25～28°C, and that the cardinal temperatures for the mycelial growth of the *Colletotrichum* are about the same as those of the *Guignardia*, though the growth of the former is smaller than that of the latter.

Effect of the temperature upon the mycelial growth of *C. lindemuthianum* and the *Colletotrichum* from black locust was tested by the same method as that above, and results obtained are shown in tables 7～9.

Table 7. Relation between temperature and the mycelial growth of *Guignardia robiniae* sp. nov. and *Colletotrichum lindemuthianum* (1)

Fungus	Experiment No.	Diameter of colony (*mm*)				
		0～1° C	6～7° C	13～14° C	18° C	20° C
G. robiniae *C. lindemuthianum*	I	0 0	0 0	11 14	27 23	37 28
G. robiniae *C. lindemuthianum*	II	0 0	0 0	13 11	35 28	43 34

Table 8. Relation between temperature and the mycelial growth of *Guignardia robiniae* sp. nov. and *Colletotrichum lindemuthianum* (2)

Fungus	Experiment No.	Diameter of colony (*mm*)					
		20° C	25° C	28° C	30° C	35° C	40° C
G. robiniae *C. lindemuthianum*	I	41 30	51 30	51 11	14 0	0 0	0 0
G. robiniae *C. lindemuthianum*	II	44 30	51 30	47 15	16 0	0 0	0 0

Table 9. Relation between temperature and the mycelial growth of *Guignardia robiniae* sp. nov. and *Colletotrichum lindemuthianum* (3)

Fungus	Diameter of colony (*mm*) Temperature (°C)									
	0	6～8	13	18	20	25	28	30	35	40
G. robiniae	0	±	13	37	45	53		21	0	0
C. lindemuthianum	0	7	15	26	27	31	11	±	0	0

From tables 7～9, it is indicated that the minimum and maximum for *C. lindemuthianum* are lower than those of the *Colletotrichum*.

3. Effect of H-ion concentrations on the mycelial growth　The relation of H-ion concentration to the mycelial growth was studied with potato sucrose agar in Petri dishes. By addition of certain amounts of normal NaOH or HCl solutions, the H-ion concentration of the medium after sterilization was varied as follows: 3, 3.6, 4.6, 5.4, 6.2, 6.6, 7.0, 7.2 and 7.4. Effects of pH value on the mycelial growth were determined by taking the averaged diameter of the colonies at the end of 6 days at 25°C. Results of the experiment are presented in table 10.

Table 10. Relation between H-ion concentration and the mycelial growth of *Guignardia robiniae* sp. nov.

Origin of isolate	Diameter of colony (*mm*) pH								
	3	3.6	4.6	5.4	6.2	6.6	7.0	7.2	7.4
Colletotrichum	20	46	56	57	52	55	56	53	51
Guignardia	17	55	70	75	77	78	79	79	79

From table 10, it is known that the influence of H-ion concentration on the mycelial growth is not considerable, so far as a test by such an experimental method shows.

Pathogenicity　In order to ascertain the pathogenicity of both of the *Colletotrichum* and the *Guignardia*, artificial inoculations to some leguminous plants were conducted in 1951～1953.

Inoculation experiment—1　On July 26, 1951, healthy potted 1-year-old seedlings of black locust were inoculated by atomizing with the conidial suspension under greenhouse conditions. As inocula the monosporous isolate from the *Colletotrichum* and that of the *Guignardia* were used. After inoculation, the seedlings were covered with bell-jars and kept in a moist condition for about 48 hours. The check plants were sprayed with sterile water instead of the fungous suspension.

On the inoculated seedlings, the first symptoms of the disease appeared 4 days after inoculation, and numerous acervuli were formed after a further 2 days. On the seedlings inoculated with the *Guignardia* isolate, perithecia of the fungus were produced within 3 weeks after inoculation. The appearances of the diseased seedlings were characteristic of the disease as observed under natural conditions (Plate 1. D). In check plants no sign of the disease showed on any of the tested plants even after 3 weeks.

There were no differences in pathogenicity between the *Colletotrichum* isolate and the *Guignardia* isolate. Results obtained of the experiment are briefly summarized in table 11.

Table 11. Inoculation experiment with *Guignardia robiniae* sp. nov. to black locust (July 26, 1951)

Date of observation	Origin of isolate					Check	
	Colletotrichum		*Guignardia*				
	Lesion	Acervulus	Lesion	Acervulus	Perithecium	Lesion	Acervulus or perithecium
July 30	+	−	+	−	−	−	−
Aug. 1	+	+	+	+	−	−	−
Aug. 16			+	+	+	−	−

Re-isolation cultures were made from conidia of the artificially inoculated seedlings and the original fungus recovered.

Inoculation experiment—2 On June 14, 1952, the inoculation test was made on each of the following leguminous plants utilizing the same method as in the previous experiment: Black locust, *Lespedeza bicolor* var. *japonica*, *Amorpha fruticosa*, common beans (f. "tenashi-naga-uzura")*1 (*Phaseolus vulgaris*) and soybeans (f. "tsurunoko")*2 (*Glycine max*). Results of the experiment are briefly presented in table 12 (Plate 5 , 6).

Table 12. Inoculation experiment with *Guignardia robiniae* sp. nov. to leguminous plants (June 14, 1952)

Plant species	Origin of isolate		Check
	Colletotrichum	*Guignardia*	
Robinia pseudoacacia	++	++	−
Lespedeza bicolor v. *japonica*	+	+	−
Amorpha fruticosa	+	+	−
Glycine max	−	−	−
Phaseolus vulgaris	+	−	−

As shown in table 12, the *Colletotrichum* isolate is pathogenic to all of the leguminous plants tested except soy bean, whereas the *Guignardia* isolate seemed unable to attack both common bean and soy bean.

Inoculation experiment—3 On July 24, 1952, by the same method as in the previous one, the inoculation test with the *Colletotrichum*, the *Guignardia* and *C. lindemuthianum* was conducted on the following plants: Black locust, *Lespedeza bicolor* var. *japonica*, *Amorpha fruticosa* and soy bean. Results of the experiment are summarized in table 13.

Table 13. Inoculation experiment with *Guignardia robiniae* sp. nov. and *Colletotrichum lindemuthianum* to leguminous plants (July 24, 1952)

Plant species	*Guignardia robiniae*		*Colletotrichum lindemuthianum*	Check
	Colletotrichum-isolate	*Guignardia*-isolate		
Robinia pseudoacacia	++	++	−	−
Lespedeza bicolor v. *japonica*	+	+	−	−
Amorpha fruticosa	+	+	−	−
Glycine max	−	−	+	−

*1, *2 Seeds of these plants were kindly supplied by Mr. Keiji SAWADA, of Yokohama Plant Quarantine Office.

From table 13, it is known that both of the *Colletotrichum* and the *Guignardia* attack all of the plants tested except soy bean, while *C. lindemuthianum* is pathogenic to soy bean only.

Inoculation experiment—4　During the summer and the autumn of 1953, a series of inoculation experiments with the *Colletotrichum*, the *Guignardia* and *C. lindemuthianum* to soy bean and common bean were undertaken.

The *Colletotrichum* isolate was slightly pathogenic to common bean, but not to soy bean. The pathogenicity of the *Guignardia* to these plants was negative. *C. lindemuthianum* attacked the common bean severely, but only slightly the soy bean. Results of the experiments are briefly given in table 14.

Table 14. Inoculation experiment with *Guignardia robiniae* sp. nov. and *Colletotrichum lindemuthianum* to soy bean and common bean

Plant species	*Guignardia robiniae*		*Colletotrichum lindemuthianum*	Check
	Colletotrichum-isolate	*Guignardia*-isolate		
Glycine max	−	−	+	−
Phaseolus vulgaris	+	−	##	−

From the results of the inoculation experiments 1～4, it is clear that there are no remarkable differences in pathogenicity between the *Colletotrichum* isolate and the *Guignardia* isolate, and that these isolates are quite different from *C. lindemuthianum* in pathogenicity.

Morphology of the *Guignardia* in the conidial stage　In order to compare the *Colletotrichum* with the conidial stage of the *Guignardia*, each of the two isolates was artificially inoculated to several leguminous trees. Morphological characters of the conidial stage produced on the diseased plants inoculated with the *Guignardia* isolate are quite similar to those inoculated with the *Colletotrichum* isolate, and, as can be seen in tables 15 and 16, respectively, there are no differences in dimension between these two.

Table 15. Dimension of *Colletotrichum* stage of *Guignardia robiniae* sp. nov. on the plants inoculated artificially (μ) (1)

Plant species	Width of acervulus	Size of seta	Size of conidiophore	Size of conidium
Robinia pseudoacacia	56～78	28～43×4～5	8.1×2.4 (8～9×2～3)	12.9×3.7 (9～19×3～6)
Lespedeza bicolor v. *japonica*	28～68	47～84×3～4	9.5×2.9 (8～11×3)	13.1×4.3 (11～15×4～6)

Table 16. Dimension of *Colletotrichum* stage of *Guignardia robiniae* sp. nov. on the plants inoculated artificially (μ) (2)

Plant species	Width of acervulus	Size of seta	Size of conidiophore	Size of conidium
Robinia pseudoacacia	28～84	34～60×3	11.2×2.9 (9～14×3)	13×4.2 (11～15×4)
Amorpha fruticosa	28～50	43～109×3～4	10.7×3.2 (8～12×3～4)	12.2×4.1 (11～14×3～4)
Lespedeza bicolor v. *japonica*	22～47	34～77×3	10×3 (8～12×3)	13×4.1 (11～17×3～4)

Since the foregoing presents complete agreement in morphological, physiological and parasitological characters of cultures isolated from the conidium of the *Colletotrichum* with those from the ascospore of the *Guignardia*, there can be no doubt as to the genetic connection between the two fungous forms. The conclusion to be drawn is, therefore, that the *Guignardia* is the ascigerous stage of the *Colletotrichum*.

Taxonomy

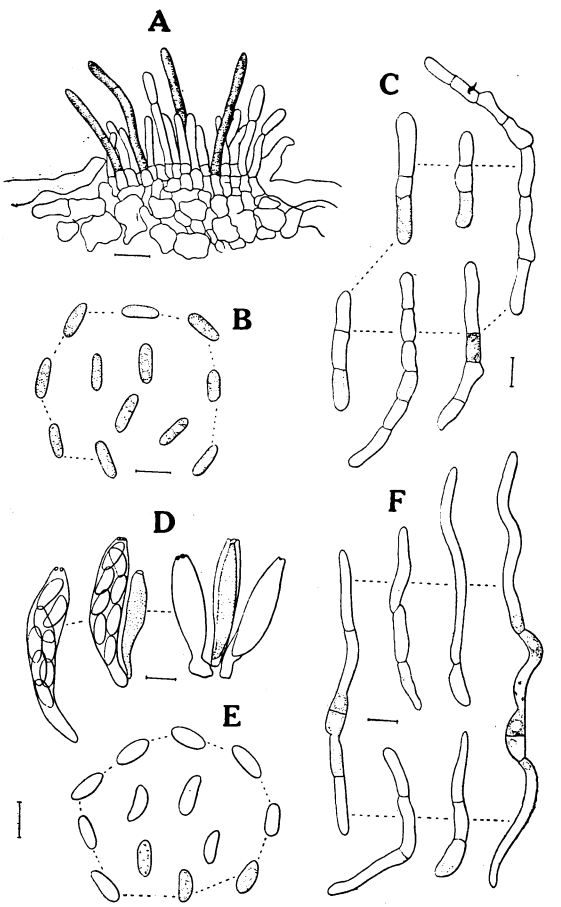
1. The conidial stage In size of conidium, pathogenicity, and the cardinals for mycelial growth, there are remarkable differences between the *Colletotrichum* and *C. lindemuthianum*, the common bean anthracnose fungus.

The *Colletotrichum* stage of *Physalospora acaciae* K. Itô et Shibukawa is very similar to the fungus under consideration in morphological characteristics, but the former's perfect stage and pathogenicity are quite different from those of the latter (Itô & Shibukawa 1956).

There have been described 3 anthracnose or allied fungi on black locust as follows : *Gloeosporium revolutum* Ell. et Ev. (Saccardo 1892), *Myxosporium russelii* (B. et Br.) Sacc. (Saccardo 1884), and *M. robiniae* Karst et Har. (Saccardo 1892). Among them, *Gloeosporium revolutum* is most similar to the authors' fungus, and, according to the description by Saccardo (1892), its characteristics are as follows: Acervulis marginem folii dein flaventem et revolutum efficientibus; conidiis oblongis, 12～15×3～4.5.....In statu juniore habitus Taphrinae.

Size of conidium of *Gloeosporium revolutum* is quite accordant with that of the *Colletotrichum* in question, though there are seen some differences in symptoms and signs between these two fungi. The fungus is here treated by the authors as *G. revolutum* Ell. et Ev., but it has not been identified with certainty.

2. The ascigerous stage It has been reported that *Glomerella cingulata* (Stonem.) Spauld. et v. Shrenk attacks some leguminous



Text-fig. 1 *Guignardia robiniae* sp. nov. on black locust (—=10 μ)
A～C : Conidial stage (*Colletotrichum*)
D～F : Ascigerous stage (*Guignardia*)
A, Acervulus; B, Conidia; C, Germinating conidia; D, Asci; E, Ascospores; F, Germinating ascospores.

plants as follows: Lupines (WEIMER 1943), "Kudzu" (*Pueraria thunbergiana*) (TIFFANY & GILMAN 1954) and lespedeza (WEIMER 1946), etc.

As synonyms of *Colletotrichum gloeosporioides* PENZ, the conidial stage of *Glomerella cingulata*, VON ARX (1957) has recently described a large number of *Colletotrichum* and *Gloeosporium* containing *C. acaciae* DE URRIES, *G. acaciae* McALP., *C. camelliae* MASSEE and *G. revolutum* ELLIS et Ev. From his monographic paper, the present authors were unable to ascertain whether he studied the ascigerous stage of each of these fungi or not.

Considered from the fundamental generic interpretation of *Glomerella* which are usually based on the presence of beaks with tuft of coarse brown hairs, the morphological features in the perithecial stage of the authors' fungus are doubtlessly accordant with those of the genus *Guignardia*.

Since a search of literature has failed to disclose any species inhabiting leguminous plants identical with the fungus, the authors consider it a new species and propose the following name:

Guignardia robiniae K. ITO et T. KOBAYASHI, sp. nov.

Syn. *Gloeosporium revolutum* ELL. et Ev. (1889)

Colletotrichum revolutum (ELL. et Ev.) comb. nov.

Peritheciis sparsis vel aggregatis, erumpentibus, globosis, papillula minima prominente, 76～105×113～168 μ; asci clavatis vel ovato-oblongis, breve stipatis, 36～50×8～11 μ, aparaphysatis, octosporis; sporidiis biseriatis, 1-cellularibus, ovatis vel ellipticis, hyalinis, 10～15×4～5 μ.

Hab. on leaflets and petioles of *Robinia pseudoacacia*[*1] (Sept. 14, 1950, Meguro, Tokyo, by T. KOBAYASHI; Sept. 21, 1950, Meguro, Tokyo, by T. KOBAYASHI; Sept. 11, 1951, Seijô, Tokyo, by T. KOBAYASHI), *Amorpha fruticosa*[*2] and *Lespedeza bicolor* var. *japonica*[*3].

***Colletotrichum destructivum* O'GARA**

Another species of *Colletotrichum* bearing straight conidia has on rare occasions been collected by the authors on the anthracnose lesions of black locust at Seijô, Tokyo. This fungus existed often together with *C. revolutum* on the diseased plants, but its morphological characteristics were so different from those of *C. revolutum* that some studies were undertaken by the authors.

Morphology

Acervuli small, 25～59 μ in diameter, scattered or gregarious; conidiophores hyaline, cylindrical or fusoid, 11～16×3 μ; setae between conidia, few, brown, straight or slightly curved, 3- or 4-septate, 53～90×3～5 μ; conidia hyaline, straight with rounded ends, 1-celled, 14～19×3～4 μ (Text-fig. 2).

Morphological features of the fungus are very similar to those of *C. destructivum* O'GARA which was originally described on *Trifolium pratense* in America (O'GARA 1915).

[*1] The type specimen has been deposited in the Herbarium of the Gov. For. Exp. Sta., Meguro, Tokyo, Japan.

[*2], [*3] By artificial inoculation.

Physiology

1. Isolation and culture Single-spore isolations of conidia were obtained in the manner described already.

Characteristics in the mycelial colony of the fungus on various agar media were quite different from those of *C. revolutum* and *C. lindemuthianum*.

2. Effect of temperatures on the mycelial growth The relation of temperatures to growth of the mycelium was tested by the Petri dish method, and results obtained are presented in tables 17～19.

Table 17. Relation between temperature and the mycelial growth of *Colletotrichum destructivum* (1)

Experiment No.	Diameter of colony (*mm*)				
	0～1° C	6～7° C	13～14° C	18° C	20° C
I	0	±	22	36	43
II	0	9	24	46	52

Table 18. Relation between temperature and mycelial growth of *Colletotrichum destructivum* (2)

Experiment No.	Diameter of colony (*mm*)					
	20° C	25° C	28° C	30° C	35° C	40° C
I	41	45	39	33	+	0
II	44	48	43	33	+	0

Table 19. Relation between temperature and the mycelial growth of *Colletotrichum destructivum* (3)

	Temperature (°C)									
	0	6～8	13	18	20	25	28	30	35	40
Diam. of colony (*mm*)	0	12	21	41	48	56	52	49	+	0

As shown in tables 17～19, the fungus grows at the temperatures ranging from 6 to 35°C with an optimum 25°C, and the range of temperature for the growth of the fungus is wider than that of both *C. revolutum* and *C. lindemuthianum* (cf. tables 7～9).

3. Effect of H-ion concentrations on the mycelial growth The relation of H-ion concentration to the mycelial growth was studied by utilizing the same method described previously, and the results obtained are given in table 20.

Table 20. Relation between H-ion concentration and the mycelial growth of *Colletotrichum destructivum*

	pH								
	3	3.6	4.6	5.4	6.2	6.6	7.0	7.2	7.4
Diam. of colony (*mm*)	12	40	48	60	64	63	65	66	67

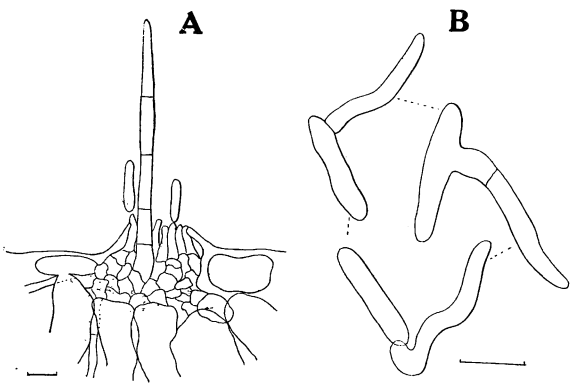
As shown in table 20, the influence of H-ion concentration on the mycelial growth is not considerable, except at a very low exponent such as pH 3, so far as a test by such an experimental method shows.

Pathogenicity

On June 14, 1952, an inoculation experiment was performed in the same manner noted previously. Plants used in this experiment were as follows: Black locust, *Lespedeza bicolor* var. *japonica*, *Amorpha fruticosa*, common bean and soy bean. Comparison with the fungus, pathogenicity of *C. revolutum* and *C. lindemuthianum* to the same plant species was simultaneously tested. Results of the experiment are summarized in table 21.

Table 21. Inoculation experiment with *Colletotrichum destructivum*, *Guignardia robiniae* sp. nov. and *C. lindemuthianum* to leguminous plants (June 14, 1952)

Plant species	*C. destructivum*	*G. robiniae*	*C. lindemuthianum*	Check
Robinia pseudoacacia	##	##	−	−
Lespedeza bicolor v. *japonica*	+	+	−	−
Amorpha fruticosa	+	+	−	−
Glycine max	±	−	+	−
Phaseolus vulgaris	±	+	##	−



Text-fig. 2 *Colletotrichum destructivum* on black locust (⊢⊣=10 μ)
A, Acervulus; B, Germinating conidia.

Table 21 shows that the fungus is capable of attacking all of the plants tested, and attacks black locust more severely than *C. revolutum*, though very slightly pathogenic to common bean and soy bean (Plate 3. A, B; Plates 5, 6).

Measurement for dimensions of the fungus formed on the plants which had been inoculated artificially was made, and the results obtained are presented in table 22.

Table 22. Dimension of *Colletotrichum destructivum* on the plants inoculated artificially (μ)

Plant species	Width of acervulus	Size of seta	Size of conidiophore	Size of conidium
Robinia pseudoacacia	37～59	40～65×3	11.2×2.8 (9～12×2.5～3)	15.4×3.2 (14～16×3～4)
Amorpha fruticosa	37～53	34～56×3	11.2×2.7 (9～12×2.5～3)	15×3.1 (14～17×3)

Taxonomy

As noted already, the fungus is very similar to *C. destructivum* in morphological features. According to TIFFANY and GILMAN (1954), *C. destructivum* is a polyxenic organism and causes an anthracnose disease of many leguminous plants containing

lespedeza and soy bean.

The fungus in question is distinctly pathogenic to lespedeza, but very weakly to soy bean. Accordingly, the authors consider it a strain of *C. destructivum* O'GARA.

Colletotrichum glycines HORI

In the summer of 1950, a severe epidemic of black locust seedlings occurred in nursery beds at Meguro, Tokyo. It was observed that as many as 80 per cent of the seedlings were infected and a great number of the seriously affected plants died.

On the lesions of the diseased seedlings the authors obtained usually a falcate-spored *Colletotrichum* which was close to *C. glycines* HORI, an anthracnose fungus of soy bean. In September of 1951, the same fungus was collected on black locust at Seijô, Tokyo.

Symptoms

The first symptoms of the disease generally appear at the early part of June in Tokyo. The disease is prevalent and causes the greatest damage during the summer season, especially around late July to mid-August.

Early symptoms appear as minute purplish brown spots on leaflets, petiolules and petioles. It is not rare that more than 50 spots a leaflet can be counted. Spots are at first circular, surrounded by a faint yellow halo, 0.3 *mm* in diameter, enlarging rapidly to 1～2 *mm* in diameter. On the petioles and stems the lesions vary from circular to linear (Plate 3. C; Plate 4. A, B). Under moist conditions grayish conidial masses of the fungus are abundantly produced on the lesions.

The most common disease symptom on young plants is the occurrence of wilted shoots which resulted when the fungus girdled the petioles, sometimes causing severe defoliation of the plants.

Physiology

Conidia of the fungus germinate in a few hours on agar media (Text-fig. 3. C; 4. B). Monosporous isolates from the conidium were easily obtained by the method noted previously.

The fungus was grown in parallel cultures with *C. glycines* isolated from soy bean*.

Characteristics in the mycelial colony of the fungus on various agar media were indistinguishable from those of *C. glycines*.

1. Effect of temperatures on the mycelial growth To compare with *C. glycines* isolated from soy bean, the relation of temperatures upon the mycelial growth of the

Table 23. Relation between temperature and the mycelial growth of *Colletotrichum glycines* (1)

Original host	Experiment No.	Diameter of colony (*mm*)				
		0° C	6～7° C	13° C	18° C	20° C
Black locust	I	0	0	9	18	28
Soy bean		0	0	11	19	28
Black locust	II	0	0	9	32	42
Soy bean		0	0	9	29	37

* This culture was kindly supplied by Mr. Hiroshi KURATA, of the National Hygienic Laboratory.

Table 24. Relation between temperature and the mycelial growth of *Colletotrichum glycines* (2)

Original host	Experiment No.	Diameter of colony (*mm*)					
		20° C	25° C	28° C	30° C	35° C	40° C
Black locust	I	25	42		48	8	−
Soy bean		25	36	45	48	12	−
Black locust	II	40	41	57	49	9	−
Soy bean		36	43	52	41	11	−

Table 25. Relation between temperature and the mycelial growth of *Colletotrichum glycines* (3)

Original host	Diameter of colony (*mm*)									
	Temperature (°C)									
	0	6～8	13	18	20	25	28	30	35	40
Black locust	0	±	9	32	39	57	57	52	±	0
Soy bean	0	±	9	29	33	54	53	50	±	0

fungus was tested by the Petri dish method. Results obtained at the end of 6 days are presented in tables 23～25.

As shown in tables 23～25, the fungus grows at the temperatures ranging from near 8°C to 35°C with an optimum 25～28°C, and the cardinals for this fungus are quite accordant with those for *C. glycines* isolated from soy bean.

2. Effect of H-ion concentrations on the mycelial growth　Results of the experiment made by the simple method noted already are presented in table 26.

Table 26. Relation between H-ion concentration and the mycelial growth of *Colletotrichum glycines*

Original host	Diameter of colony (*mm*)								
	pH								
	3	3.6	4.6	5.4	6.2	6.6	7.0	7.2	7.4
Black locust	9	23	52	63	65	66	67	68	71
Soy bean	10	25	33	41	41	39	35	35	37

From table 26 it is seen that the influence of H-ion concentration upon the mycelial growth of both fungi is not remarkable, though there are some differences in the rate of growth between the fungus and *C. glycines* isolated from soy bean.

Pathogenicity

In order to make clear the pathogenicity of the fungus, some inoculations have been attempted under greenhouse conditions during the past several years.

Inoculation experiment—1　On July 26, 1951, a spore suspension from pure cultures of the fungus was atomized to the potted healthy seedlings of black locust and *Amorpha fruticosa*. The plants were placed in a moist chamber for 24 hours, after which they were removed and maintained under greenhouse conditions. Check plants were similarly treated except that they were atomized with water instead of spore suspension.

On the leaflets of black locust, spots began to appear as early as 4 days after inoculation and enlarged rapidly. The appearances of the inoculated plants were

typical of the disease as observed under natural conditions. Lesions on *Amorpha fruticosa* appeared 10 days after inoculation. On August 14, numerous acervuli of the fungus were found on petioles of the fallen leaves resulting from the inoculation, whereas all the check plants remained healthy. Results of the experiment obtained are summarized in table 27.

Table 27. Inoculation experiment with *Colletotrichum glycines* (isolated from black locust) to black locust and *Amorpha fruticosa* (July 26, 1951)

Plant species	Lesion formation	Acervulus formation
Robinia pseudoacacia	##	##
Amorpha fruticosa	##	+

Inoculation experiment—2 On June 14, 1952, another inoculation experiment with the fungus was conducted on the following leguminous plants in the same manner described previously: Black locust, *Lespedeza bicolor* var. *japonica*, *Amorpha fruticosa* and common bean. Results of the experiment are briefly presented in table 28.

Table 28. Inoculation experiment with *Colletotrichum glycines* (isolated from black locust) to leguminous plants (June 14, 1952)

Plant species	*Colletotrichum*	Check
Robinia pseudoacacia	##	−
Lespedeza bicolor v. *japonica*	##	−
Amorpha fruticosa	##	−
Phaseolus vulgaris	−	−

As shown in table 28, the fungus is pathogenic to all of the plants tested excepting common bean, and attacks most severely black locust (Plate 3 . D; Plate 4 . C, D; Plate 5 , Plate 6).

Inoculation experiment—3 In this experiment, the fungus in question and *C. glycines* isolated from soy bean were used as inocula.

On July 24, 1952, an inoculation was given to the following 3 plant species utilizing the same procedure noted already: Black locust, *Lespedeza bicolor* var. *japonica* and *Amorpha fruticosa*. Results of the experiment are given in table 29.

Table 29. Inoculation experiment with *Colletotrichum glycines* (black locust- and soy bean-isolates) to leguminous plants (July 24, 1952)

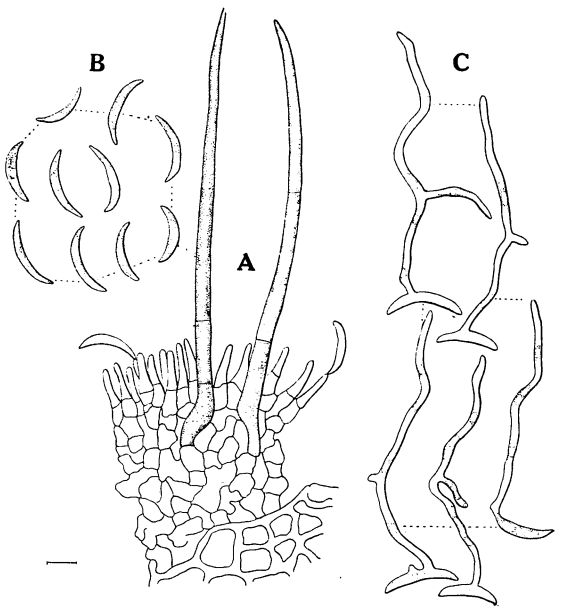
Plant species	Black locust isolate	Soy bean isolate	Check
Robinia pseudoacacia	##	##	−
Lespedeza bicolor v. *japonica*	##	##	−
Amorpha fruticosa	##	##	−

From table 29, it is clear that *C. glycines* isolated from soy bean as well as the fungus from black locust is pathogenic to all of the plants tested.

Inoculation experiment—4 Both the fungus and *C. glycines* isolated from soy bean were inoculated to soy bean and common bean. Results are presented in table 30 (Plate 6).

Table 30. Inoculation experiment with *Colletotrichum glycines* (black locust- and soy bean-isolates) to soy bean and common bean

Plant species	Black locust isolate	Soy bean isolate	Check
Glycine max	++	+++	−
Phaseolus vulgaris	−	+	−



Text-fig. 3 *Colletotrichum glycines* on black locust (⊢⊣ = 10 μ)
A, Acervulus; B, Conidia; C, Germinating conidia.

As shown in table 30, the fungus is pathogenic to soy bean, but not to common bean, while *C. glycines* isolated from soy bean is pathogenic to 2 plant species, though very weakly to common bean.

From the foregoing data of repeated inoculations, it may certainly be said that there are no remarkable differences in pathogenicity between the fungus in question and *C. glycines*, though the soy bean isolate is slightly more pathogenic on soy bean than on black locust; conversely the black locust isolate thrives parasitically more on its original host than on soy bean.

Morphology and taxonomy

Morphological description of the fungus is as follows: Acervuli linear or oval, crowded, hemispheric to truncate-conical, erumpent, 54～111 μ in diameter; setae numerous, filiform, tapering at the apex, variable, long and short intermixed, 2- or 3-septate, chestnut brown, 84～225×5～7 μ; conidiophores fasciculate, cylindrical or fusoid, hyaline, 11～20×3～4 μ; conidia falcate-lanceolate, 1～4-guttulate, 1-celled, hyaline, 22～32×2～4 μ (Text-fig. 3. A, B; Plate 2. D).

Results of the measurement for dimensions of the fungus on black locust collected in Tokyo are presented in table 31.

Table 31. Dimension of *Colletotrichum glycines* collected on black locust in nature (μ)

Locality	Width of acervulus	Size of seta	Size of conidiophore	Size of conidium
Meguro, Tokyo Aug. 11, 1950	54～111	111～225×6～7	13.7×2.6 (11～21×3～4)	26.4×3 (23～32×2～4)
Seijô, Tokyo Sept. 19, 1951	62～ 87	84～195×5～6	16.6×2.9 (14～20×3)	24.6×3 (22～31×3)

Dimensions of the fungus and *C. glycines* isolated from soy bean on the leguminous woody plants which had been artificially inoculated are presented in table 32.

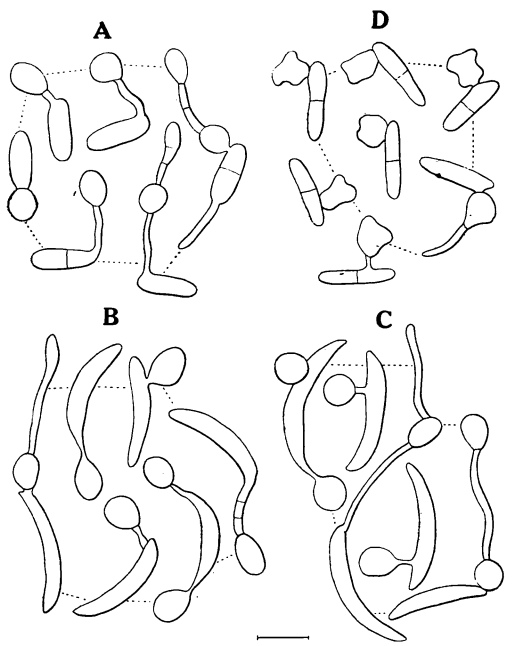
Table 32. Dimension of *Colletotrichum glycines* on the plants inoculated artificially (μ)

Plant species	Width of acervulus	Size of seta	Size of conidiophore	Size of conidium
Black locust-isolate				
Lespedeza bicolor v. *japonica*	40～65	56～124×3～6	——	23.7×3.1 (20～26×3)
Soy bean-isolate				
Robinia pseudoacacia	59～90	124～220×4～8	13.7×2.6 (11～16×2～3)	26.4×2.8 (25～28×2～3)
Lespedeza bicolor v. *japonica*	43～59	76～115×3	12.3×2.6 (9～16×2～3)	24.5×3.1 (22～26×2～3)

Morphological characteristics of the fungus in question agree well with those of *C. glycine* Hori. Hori has not published a technical description of this species, but its morphological features, accompanied by drawings, however, were given in a report by Hemmi (1920).

In physiology and pathogenicity there have been no differences between these two fungi, and the fungus is doubtlessly identical with *C. glycines*.

Lehman and Wolf (1926) found the ascigerous stage of *C. glycines* in America, and they named it as *Glomerella glycines* (Hori) n. n. Recently, Tiffany and Gilman (1954) have stated that *C. glycines* (Hori) Lehman et Wolf was not the perfect stage of *Colletotrichum glycines*, but the ascigerous stage of a straight-spored fungus, *C. destructivum*.



Text-fig. 4 Appressorium formation in conidial germination (—=10 μ)
A, *Guignardia robiniae* sp. nov.
B. *Colletotrichum glycines* from black locust
C, *Colletotrichum glycines* from soy bean
D, *Colletotrichum destructivum*

Tiffany and Gilman (l. c.) have treated *C. glycines* as a synonym of *C. truncatum* (Schw.) Andrus et Moore, and they noted that "Inoculations with the soybean isolates were successful on soy bean, alfalfa,····lespedeza vetch,····lima bean and pea".

More recently, von Arx (1957) has treated *C. truncatum* as a forma of *C. dematium*, and named it *C. dematium* (Pers. ex Fr.) Grove f. *truncata* (Schw.) v. Arx.

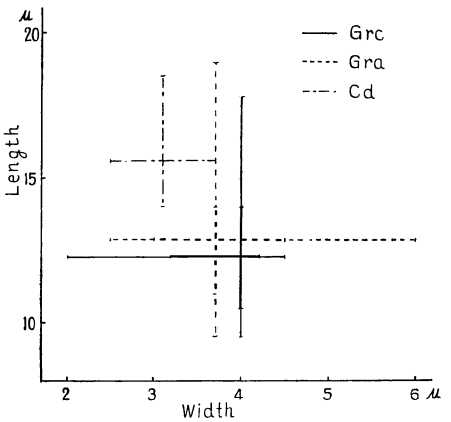
In spite of the repeated examinations, the ascigerous stage of the fungus has not been obtained by the authors on either host plants or agar media.

As stated above, several opinions on taxonomy of the fungus have been published

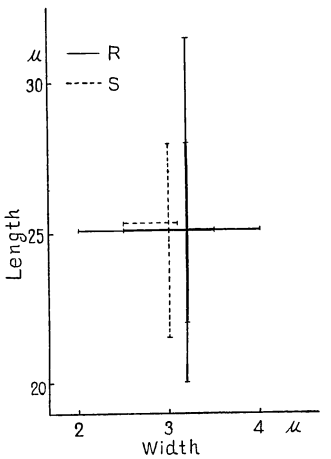
by foreign workers, but the authors adopt here the name *C. glycines* Hori, by which it has been generally known among Japanese plant pathologists.

Conclusive summary

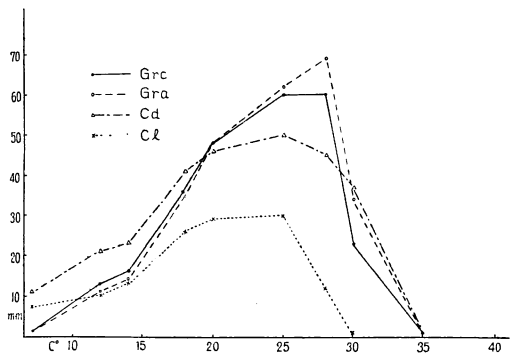
Since the first collection of black locust seedlings affected by an anthracnose in 1950, some studies on the disease have been made by the authors, with special emphasis on taxonomy and the pathogenicity of the causal organisms.



Text-fig. 5　Size range of conidia of *Guignardia robiniae* sp. nov. and *Colletotrichum destructivum*
Grc····*Colletotrichum* isolate of *G. robiniae* sp. nov.
Gra····Ascospore isolate of *G. robiniae* sp. nov.
Cd····*Colletotrichum destructivum*



Text-fig. 6　Size range of conidia of *Colletotrichum glycines*
R····Black locust isolate
S····Soy bean isolate



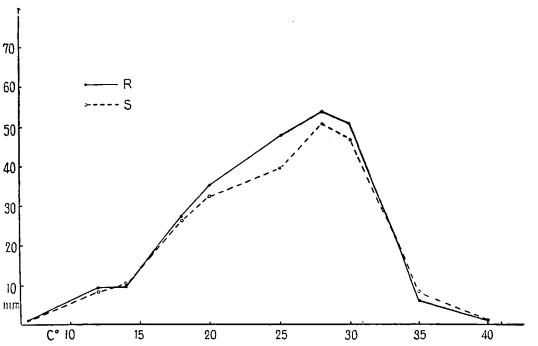
Text-fig. 7　Effect of temperature upon the mycelial growth of the fungi
Grc····*Colletotrichum* isolate of *Guignardia robiniae* sp. nov.
Gra····Ascospore isolate of *G. robiniae* sp. nov.
Cd····*Colletotrichum destructivum*
Cl····*Colletotrichum lindemuthianum*

On the diseased plants, there have been collected 3 species of *Colletotrichum* (Fungi Imperfecti) and one *Guignardia*(Ascomycete). Comparative studies of these fungi were undertaken by the authors in morphology, physiology and parasitology.

Of 3 species of *Colletotrichum*, two were straight-spored and one was curved-spored. Differences between the 2 straight-spored fungi were so remarkable that these were to be treated as species different from each other (Text-fig. 5, 7; Table 33). The small-

spored fungus was identified by the authors as *Gloeosporium* (*Colletotrichum*) *revolutum* ELL. et Ev., whereas the large-spored one was identical with *C. destructivum* O'GARA. Morphology, physiology and pathogenicity of the falcate-spored *Colletorichum* were quite agreeable with those of *C. glycines* HORI, an anthracnose fungus of soy bean.

In autumn, perithecia of an ascomycete belonging to the genus *Guignardia* were produced on the anthracnose lesions caused by *C. revolutum*. The existence of a genetic relationship between these two fungous forms was verified by the cultural and inoculation experiments. Since a search of the literature by the authors failed to disclose any species like the fungus under consideration, it was regarded as a new species, and the name, *Guignardia robiniae* sp. nov. was proposed for it.



Text-fig. 8 Effect of temperature upon the mycelial growth of *Colletotrichum glycines*
R····Black locust isolate; S····Soy bean isolate

Table 33. Summary of results of inoculation experiments with the fungi to several species of leguminous plants

Plant species / Fungus species	*Robinia pseudoacacia*	*Lespedeza bicolor* var. *japonica*	*Amorpha fruticosa*	*Glycine max*	*Phaseolus vulgaris*
C. revolutum (conidial stage of *G. robiniae*)	##	+	+	−	+
Guignardia robiniae sp. nov.	##	+	+	−	−
C. destructivum	###	+	+	±	±
C. lindemuthianum	−	−	−	+	###
C. glycines (black locust isolate)	###	##	##	##	−
C. glycines (soy bean siolate)	###	##	##	###	+

G. robiniae sp. nov. was found more commonly than *C. glycines*, and *C. destructivum* rarely occurred. The fungous isolates were apt to be more pathogenic on their original host than on the others. Artificial inoculations showed that pathogenicity of *G. robiniae* to several woody plants was slightly weaker than that of *C. destructivum* and of *C. glycines* (Table 33).

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Explanation of plates

Plate 1.

A. Defoliation of black locust caused by *Guignardia robiniae* sp. nov.
B. Leaves of black locust inoculated with *Colletotrichum* isolate of *G. robiniae* sp. nov.
C. Petioles of black locust attacked by *G. robiniae* sp. nov., producing the ascigerous stage of the fungus
D. Leaves of black locust inoculated with the ascospore isolate of *G. robiniae* sp. nov.

Plate 2.

A. Acervulus of *G. robiniae* sp. nov. on black locust ×310
B. Perithecium of *G. robiniae* sp. nov. on petiole of black locust ×310
C. Perithecia of *G. robiniae* sp. nov. on petiole of black locust produced by artificial inoculation ×150
D. Acervulus of *Colletotrichum glycines* on black locust ×150
E. Acervuli of *C. glycines* produced on 2 per cent dextrose agar ×2

Plate 3.

A. Leaves of black locust inoculated with *Colletotrichum destructivum*
B. Leaves of *Amorpha fruticosa* inoculated with *C. destructivum*

C. Leaves of black locust attacked by *C. glycines*

D. Leaves of black locust inoculated with *C. glycines*

Plate 4.

A. Shoots of black locust attacked by *C. glycines*

B. *Ditto.* ×1.4

C. Leaves of *Lespedeza bicolor* var. *japonica* inoculated with *C. glycines*

D. Leaves of *Amorpha fruticosa* inoculated with *C. glycines*

Plate 5.

A. Result of the inoculation experiment with several anthracnose fungi to black locust seedlings

a. *Colletotrichum* isolate of *G. robiniae* sp. nov.

b. Ascospore isolate of *G. robiniae* sp. nov.

c. *C. destructivum*

d. *C. lindemuthianum*

e. *C. glycines* (black locust isolate)

f. *C. glycines* (soy bean isolate)

B. Result of the inoculation experiment with several anthracnose fungi to *Amorpha fruticosa* seedlings

a. *Colletotrichum* isolate of *G. robiniae* sp. nov.

b. Ascospore isolate of *G. robiniae* sp. nov.

c. *C. destructivum*

d. *C. lindemuthianum*

e. *C. glycines* (black locust isolate)

f. *C. glycines* (soy bean isolate)

Plate 6.

A. Result of the inoculation experiment with several anthracnose fungi to soy bean

a. *Colletotrichum* isolate of *G. robiniae* sp. nov.

b. Ascospore isolate of *G. robiniae* sp. nov.

c. *C. destructivum*

d. *C. lindemuthianum*

e. *C. glycines* (black locust isolate)

f. *C. glycines* (soy bean isolate)

g. Check

B. Result of the inoculation experiment with several anthracnose fungi to common bean.

a. *Colletotrichum* isolate of *G. robiniae* sp. nov.

b. Ascospore isolate of *G. robiniae* sp. nov.

c. *C. destructivum*

d. *C. lindemuthianum*

e. *C. glycines* (black locust isolate)

f. *C. glycines* (soy bean isolate)

g. Check


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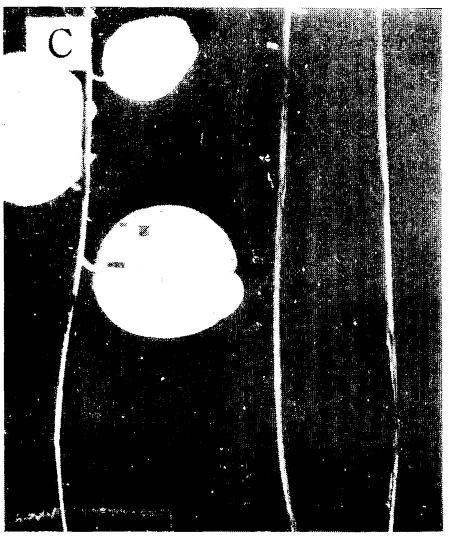
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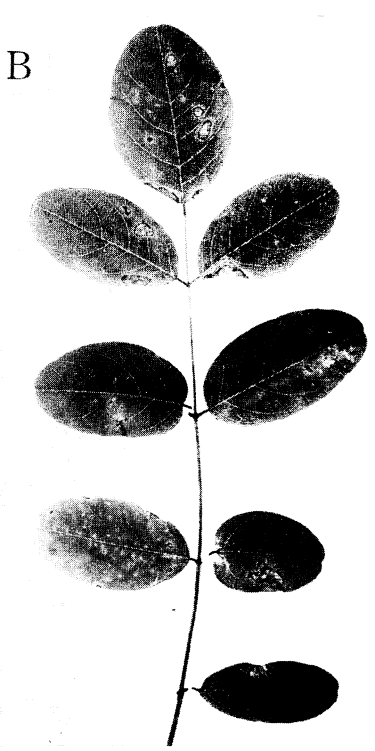
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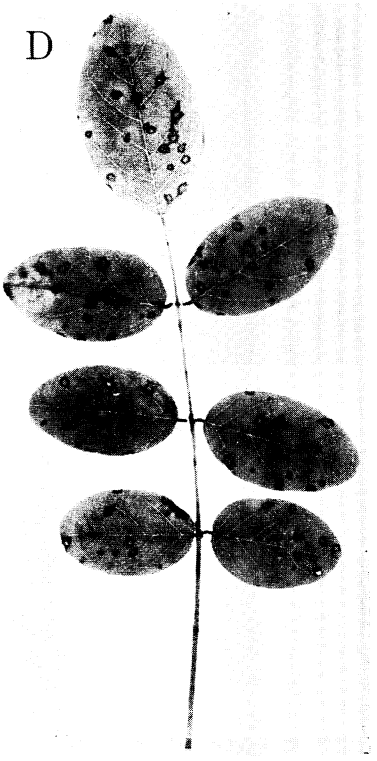
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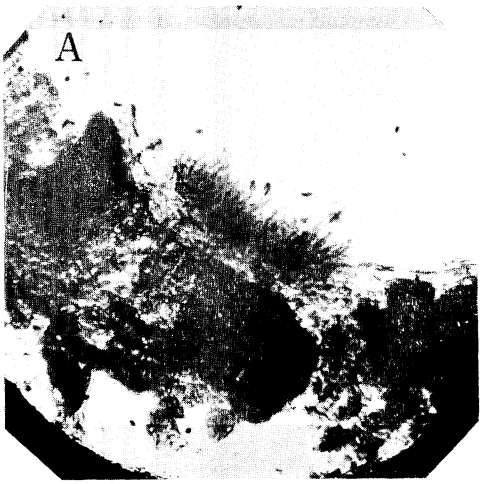

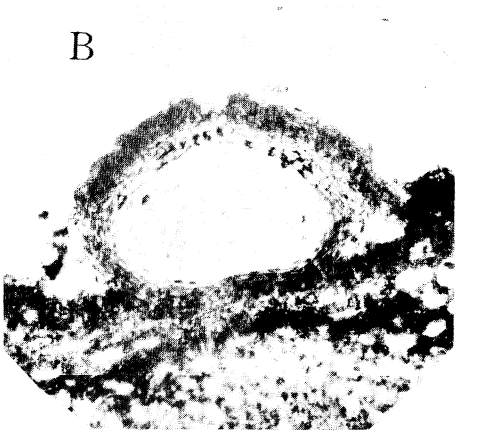

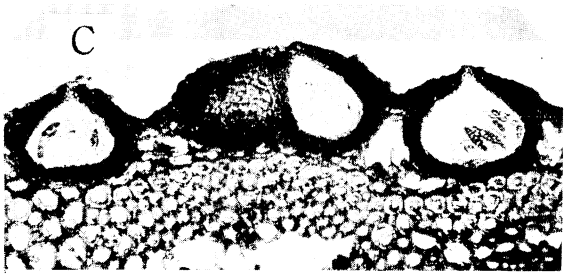

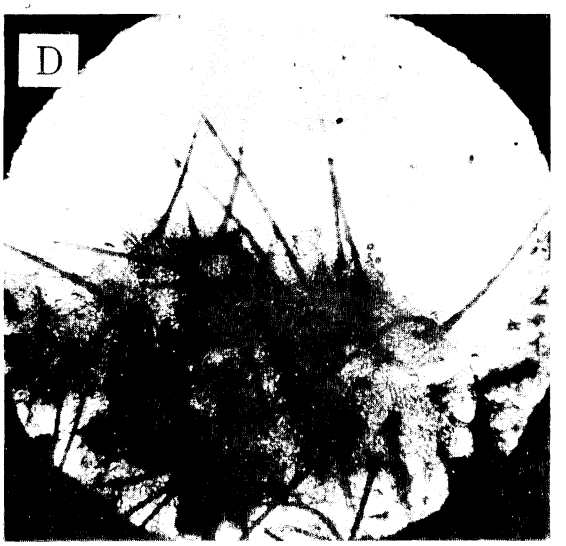

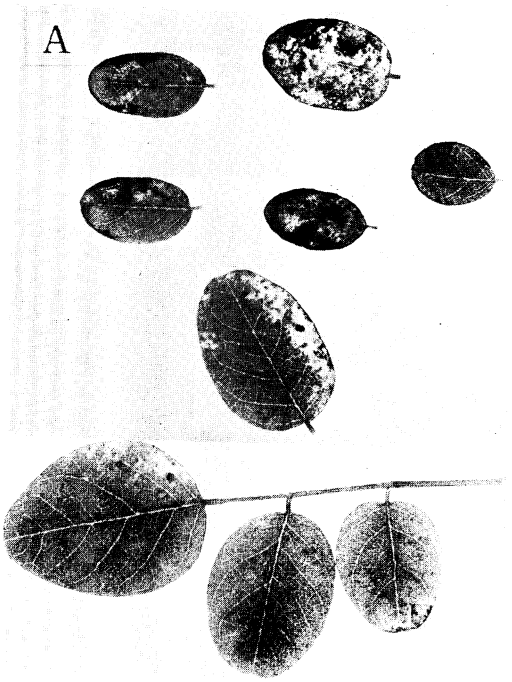
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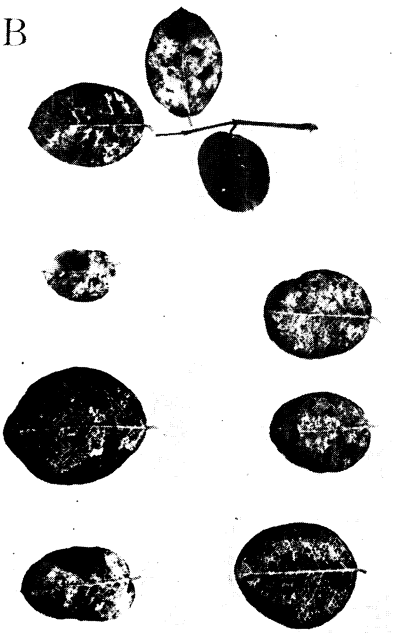
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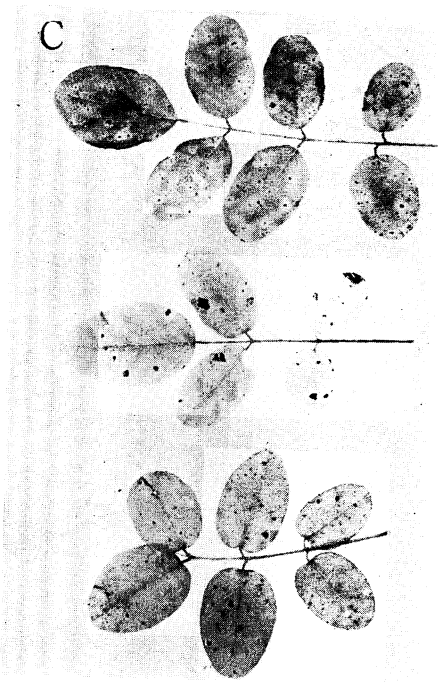
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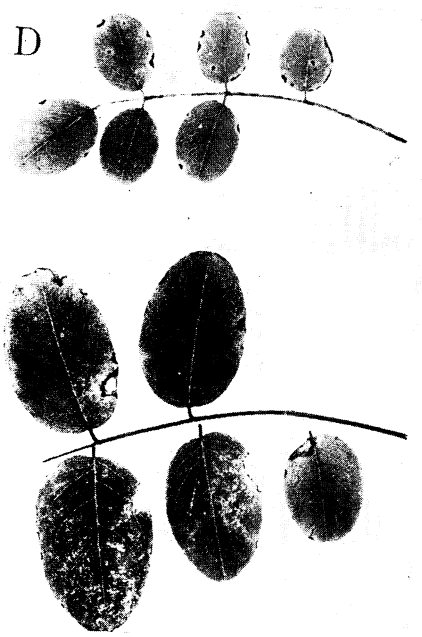
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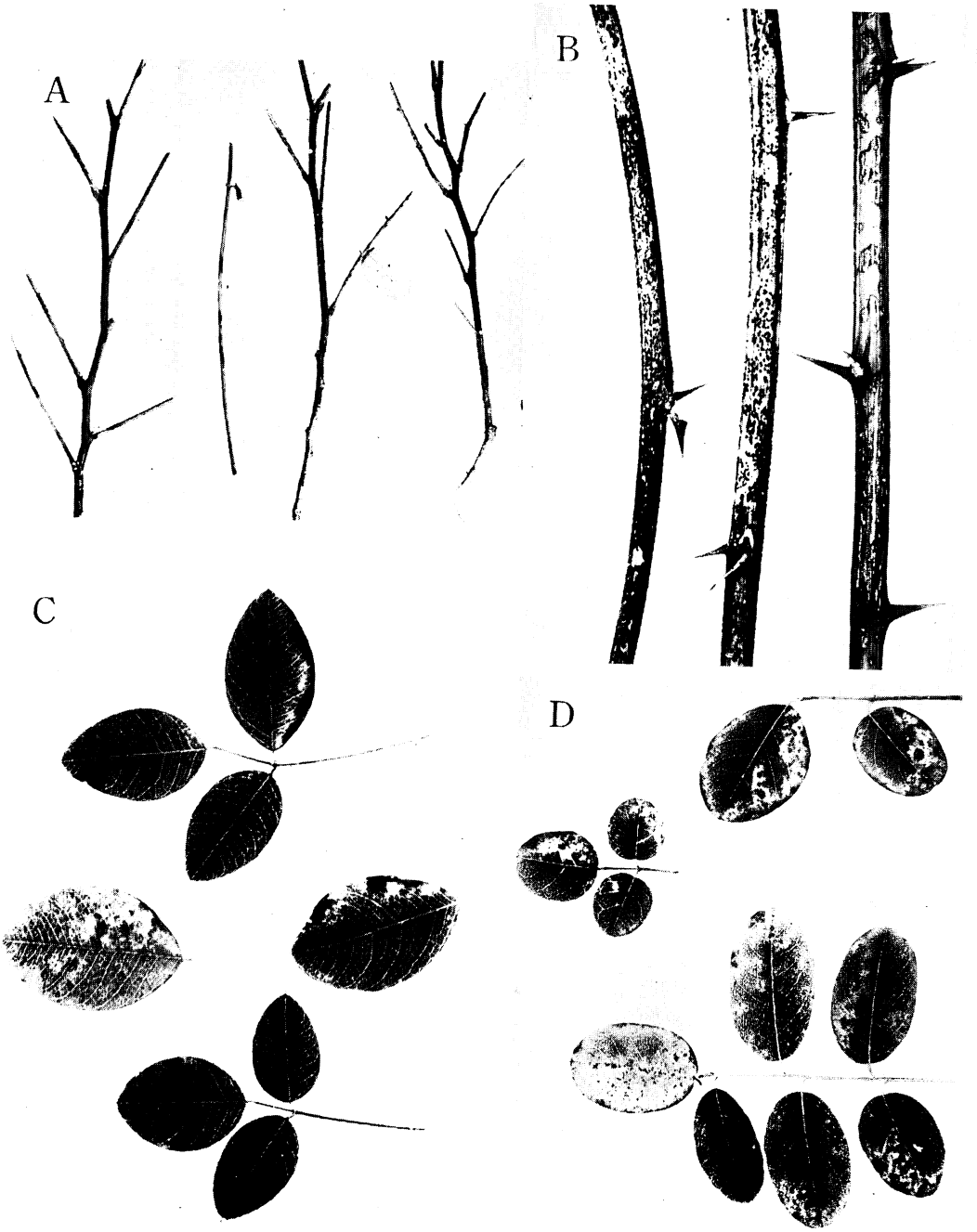

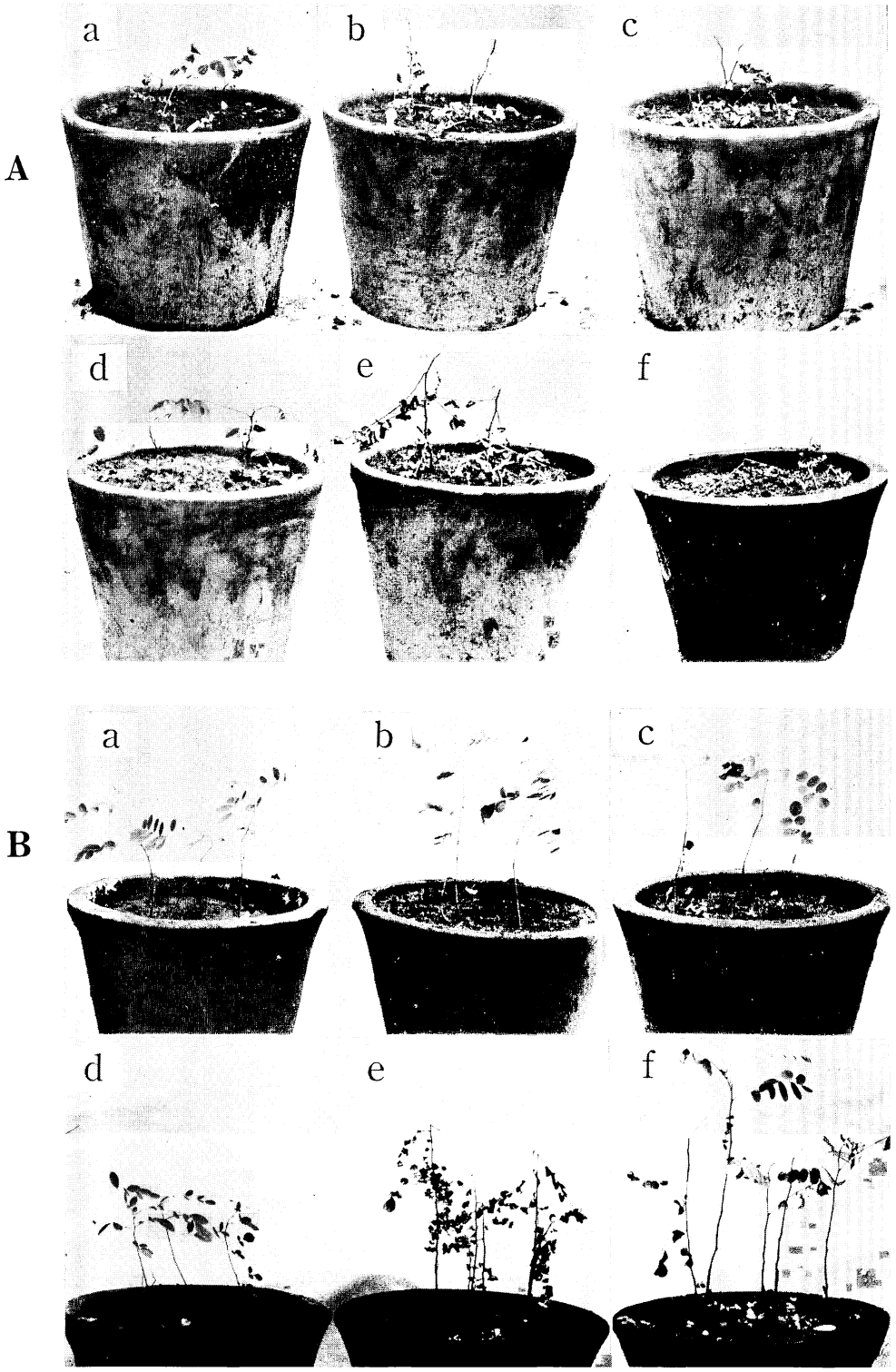
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—Plate 6—