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A COMPARATIVE STUDY OF CERTAIN PYCNIDIAL FUNGI FROM VITIS

BY

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THESIS

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I. INTRODUCTION

It is the purpose of this investigation, to compare the morphological characters of a number of fungi, ascribed to the form genera Phoma, Phyllosticta, and Macrophoma, which have been described from Vitis, and also to compare them with certain Phoma and Phyllosticta individuals which were isolated from Vitis and grown in pure culture under different environmental conditions.

II. "SPECIES" OF PHOMA, PHYLLOSTICTA, AND MACROPHOMA DESCRIBED FROM VITIS.

A search through the literature reveals that there have been at least forty-two so called "form species" of fungi described from Vitis which have been assigned to one or the other of the three "form genera" treated here, viz Phoma, Phyllosticta and Macrophoma. Since cross-inoculations have established that Guignardia bidwellii (Ellis) Viala et Ravaz, is capable of infecting both, individuals of Vitis and Parthenocissus (26), three additional fungi described from the latter host have been included in this study for comparative purposes. Of these forty-five fungi, there are only three or four which cause diseases of economic importance and which have, because of this fact, been given considerable attention by various investigators. Most of the others have not been studied critically except by the investigators who originally described them.

If we disregard the, admittedly, entirely artificial characters which are used to separate the three "form genera" of fungi mentioned above, and if we treat all of these so-called species as so many groups of more or less similar individuals, for the purpose of comparison, it becomes evident, after a study of the available descriptions, that many of these "species" are indistinguishable from each other or that the differences between them are very minute.

For the purpose of convenience, individuals are grouped into composite units which, in the Biological Sciences, are designated as species. How great the differences between two

individuals should be before they can be regarded as belonging to different species, is, and probably always will be, a matter of dispute among systematists, under our present system of classification. The taxonomist who regards a species as a distinct natural group of organisms is apt to have a much different conception of a "specific character" than Hochreutiner, for instance, who states (29) that the only tangible reality in the organic kingdom is the totality of living organisms which inhabit our planet.

Before deciding, therefore, whether these forty-five "species" of fungi with which this study will deal in part, need re-classification, it is necessary to find out what the differences are between them which seemed great enough to the men who originally described them to warrant noting in a formal specific description.

For the purpose of comparison, the descriptions of the forty-two "species" of Phoma, Phyllosticta, and Macrophoma which have been described from Vitis, and the three which have been described from Parthenocissus (Ampelopsis) quinquefolia and P. hederacea, are alphabetically listed below as found in the original publications, or in cases where these were not available, as found in Saccardo's "Sylloge Fungorum". The source of each description is designated by the name of the author of the original description, or "Saccardo", in parenthesis, at the end of each description.

In the few cases where an original description was later amended, both descriptions are given.

Macrophoma acinorum Pass.

Nuovo Gior. Bot. It. 1890, p. 48

Sacc., Syll. Fung. 10; 198.

"Maculis subdiscoides, fuscis; peritheciis sparsis vel subgregariis, punctiformibus, erumpentibus, fuscidulis, nitidis, acutis, contextu submembrenaceo, minuto cellulose; sporidis elongato-fusiformibus, integris, nubilosis, hyalinis plurriguttatis, 20-28 X 6-7.5; basidiis filiformibus, sporulis subaequantibus,

"Hab. in baccis maturis Vitis viniferae. Viglieffio pr. Parmam Ital. bor." (Saccardo).

Phoma ampelinum B. et C.

North Amer. F. n. 380, Grevillea, 2:81, 1873

Thümen, von, Felix, Pilze die Weinstockes, p. 31, 1878

Massalongo, C., Malpighia 8:197, 1894

Sacc., Syll. Fung., 3:79

"Subcuticulare, hysteriforme, sporis fusiformibus. On shoots of vine. Pennsylvania, Michigan. No. 4094.

"Growing beneath the cuticle which swells into little hysteriiform pustules; spores oblong, subfusiform, .0005 long." (B. et C.).

"peritheciis 170:190μ; sporulis subfusoideis 6:9 X 2.5:3.5μ, basidiis 12:26 X 1.5:28.-

"In sarmentis siccis Vitis viniferae "Tregnago"; Febr. 1889". (Massalongo).

Phoma ampelocarpa Pass.

Nuovo Gior. Bot. It. 1890 n.1

Revue Mycologique 1890, p. 86

Sacc., Syll. Fung., 10:152

"Peritheciis in centro maculae discoidae brunneae gregariis, erumpentibus, cuticula vix fissa cinerescente tectis; sporulis oblongo-ellipsoides, enucleatis, hyalynis, 7.5 X 2.5

"Hab. in baccis maturis Vitis Moscatello di Spagna dictae in Ital. bor." (Saccardo).

Phoma ampelogena Sacc.

Sacc., Syll. Fung., 3:110

Coniothyrium ampelinum Cooke

Grevillea 6:134, 1878

"Sparsa; peritheciis minutis, inconspicuis, brunneis, membranaceis, hemisphaericis; sporulis ovatis, hyalinis, 4 X 3

"Hab. in foliis Vitis aestivalis, socia Peronospora viticola, Aiken in Carolina." (Saccardo).

Macrophoma ampelopsidis (C. et E.) Berl. et Vogl.

Atti Soc. Veneto- Trentina, 1886, p. 174

Sacc., Syll. Fung., 10:189

Sphaeropsis ampelopsidis C. et E.

Grevillea 1876, Tab. 99, f. 8.

Phoma ampelopsidis (C. et E.) Sacc.

Sacc., Syll. Fung., 3:79

"Gregaria, tecta; peritheciis subglobosis, atris, cortice elevatis, papillatis; sporulis lanceolatis vel subellipticis, utrinque rotundatis, hyalinis, 30-35 X 12

"Hab. in ramis Ampelopsidis quinquefoliae, New Jersey, in Amer. bor." (Saccardo).

Phoma baccae Catt.

Pir. F. Vitigni p. 56., 1877

Sacc., Syll. Fung., 3:149

"Peritheciis globosis, atris, maculis brunneis circumdatis, ostiolo minuto pertusis, subcutaneo-erumpentibus nucleo-albido; sporulis ovoideis, utrinque rotundatis, 1-3 nucleatis, hyalinis, 12μ longis; basidiis clavatis, radiantibus, apice 2-3, sterigmata sporophora gerentibus.

"Hab. in baccis submaturis in Etruria (Targioni-Tozzeti). 'An dendrophoma?'" (Saccardo).

Phyllosticta badhami Cooke

Jr. Roy. Hort. Soc., 5:93, 1878; 28:314, 1903.

"...all that we discovered was a great profusion of very minute, almost linear, spores, exactly like those of the specimens in 'Fungi Britannici' no. 226, not more than .006 mm. long ..." (Cooke).

"...the conidia or sporules were minute (6-7 X 2 μ)..."(Cooke). On Vitis vinifera.

Phyllosticta bizzozeriana C. Mass.

Contr. Mic. Ver. p. 79, t.I, f. II., 1889.

Sacc., Syll. Fung., 10:107

"Peritheciis punctiformibus, nigris, hypophyllis vel sub-amphigenis, globosos-conicis, prominulis, poro pertusis, erumpentibus. 50-80μ in maculis parvis, irregularibus, exaridis dense aggregatis; sporulis perexiguis, hyalinis, breviter bacillaribus, continuis, utrinque obtusis, 2.5-3.5 X 1.5.

"Hab. in foliis Vitis viniferae, Calavena prope Tregnago agri Veronensis. A Ph. viticola et Ph. Vitis satis diversa". (Saccardo).

Phoma cicinnoides Fautr.

Rev. Mycol. 1893, p. 69.

Sacc., Syll. Fung. 11: 483

"Périthèces très petits, 100, 120μ diamètre, concolores a l'écorce, enfoncés, peu proéminents, aplatis, bien ouverts, contexture du Ciccinobolus. Spores ovées ou ovales, 5.6 X 2½-3.

"Sur vrilles de vigne attaquee par l'Oidium Tuckeri; à Frécy (Cote-d'Or), décembre 1892." (Fautr.)

Phoma confluens B. et C.

N. Amer. F. n. 383, Grevillea, 2:82, 1873.

Sacc., Syll. Fung., 3:80.

"Maculis elevatis marginatis, peritheciis confluentibus; sporis brevibus. On branches of vine No. 5616.

"Forming little raised hysteriiform spots with a distinct margin. Perithecia black, confluent; spores shortly oblong, .0002 long." (B. et C.)

"Maculis elevatis marginatis; peritheciis nigris confluentibus; sporulis brevibus, oblongis, 5μ longis.

"Hab. in sarmentis Vitis, in America boreali." (Saccardo).

Phoma cookei Pir.

Pir., F. Vit., p. 55, tab. XII, f. 21., 1877

Sacc., Syll. Fung., 3:80

"Peritheciis minutis, sparsis, punctiformibus, nigris, globosis subglobosisve, primum epidermide tectis, dein erumpentibus, stipatis, flexuosis, hyalinis; sporulis baculiformibus, curvatis, apicibus obtusissimis, simplicibus, hyalinis 13 X 4.5μ.

"Hab. ad ramulos Vitis viniferae prope nodos, in Britannia." (Saccardo).

Phoma cordifolia Brun.

Act. Soc. Linn. Bordeaux, XXXIII 1878.

Sacc., Syll. Fung. 10; p. 152.

"Peritheciis remotis, secus ligni fibras seriatim dispositis, siccis depressis, nigris, per epidermidem nigrificatam saepe irregulariter laceratam erumpentibus; sporulis oblongo-elongatis, rectis 7-9 X 2.5-3, continuis, 2-guttulatis.

"Hab. in sarmentis Vitis cordifoliae, Rockefort Galliae." (Saccardo).

Phoma descissens Oud.

Hedwigia 37: 314, 1898.

Sacc., Syll. Fung., 16, p. 852.

"Auf bleistiftdicken Aesten von Vitis vinifera; Juni 1898; Beins.

"Peritheciis semper peridermate tectis, solitaris, gregariis vel confluentibus, et tunc quasi binis, ternis, quaternis vel pluribus in stromata communi, nigro, nunc lineari vel lanceolato et axi longitudinali ramorum parallelo, nunc vero magis orbiculari, infossis, valde prominentibus, centro pertusis. Sporulis breviter lanceolatis, utrinque rotundatis, biocellatis 9-10 X 3, basidiis filiformibus, 12-23u longis, suffultis." (Oud.)

Phoma farlowiana Viala et Sauvr.

Jr. Bot., 1891 p. 363, fig. 10, Pl. V.

Sacc., Syll. Fung., 10:153

"Les seuls organes de reproduction que nous ayons observés pour cette espèce sont des pycnides.

Le mycélium, lorsqu'il est en filaments isolés, surtout dans le tissu spongieux de la feuille, est moins cloisonné, moins toruleux, et à diamètre un peu plus grand que celui de l'espèce precédente. Le violet de gentiane le colore d'une facon remarquable.

"Les pycnides sont immergées presque entièrement dans les tissus, et leur ostiole affleure au dehors à travers l'épaisse cuticule déchirée de la face supérieure. Elles sont de forme nettement ovoïde, avec le grand axe parallèle au plan de la feuille; elles mesurent 132μ sur 110μ. Leur enveloppe épaisse est d'un noir très foncé. L'ostiole est largement creusée dans un renflement sessile de la paroi, qui est seulement plus épaisse dans cette région. Les basides ne présentent pas de caractère bien particulier, elles sont très courtes et filiformes.

"Les stylospores (fig 10), sont incolores, simples, allongées subovoides, arrondies et fortement atténuées aux deux bouts; le pourtour de la membrane est régulièrement curviligne. La membrane est mince, hyaline. Le contenu des spores, au moment où elles se détachent de la baside, est très homogène, hyalin ou faiblement granuleux. Quand elles sortent mûres du conceptacle, on distingue des vacuoles rondes ou ovoides, peu réfringentes et situées irrégulièrement dans le protoplasm qui

est devenu plus grumeux. Elles mesurent 21μ de long sur 5μ .4 de diamètre. Quelques spores sont faiblement aplaties à une de leurs extrémites, et dans cette region la membrane est un peu plus épaisse." (Viala et Sauvr.)

Macrophoma flaccida (Viala et Ravaz) Cav.

Cavara, Atti Ist. Bot. Pavia, ser. 2, 1:317, 1888

Sacc., Syll. Fung., 10:198.

Phoma flaccida Viala et Ravaz

Viala, P. et Ravaz, L., Le Black Rot, p. 55., 1886.

"Le pustules sont constituées par deux sortes de conceptacles: des pycnides et des spermogonies. Les premiers, formés d'unes enveloppe de couleur brune foncées contiennent des stylospores en forme de fuseau raccourci, à protoplasma homogène, et mesurant de 16μ à 19μ de longueur sur 6μ de diamètre. Elles naissent au sommet de fine basides qui lapissent toute la paroi interne de la pycnide.

"L'enveloppe des spermogonies presente la même structure que celle des pycnides. Les basides portent des spermaties tres petites, en forme de courts bâtonnets obtus, et dont les dimensions ne dépassent pas 1μ de longueur sur 1μ 7 de largeur.

"Certains conceptacles renferment à la fois des stylospores et des spermaties". (Viala et Rav.)

"Hab. in baccis siccis Vitis viniferae in Pyrenais et Stradella et Voghera Italiae.- Phomae rimisedae affinis, sed ostiolo epapillato, sporulis et basidiis minoribus diversa." (Saccardo).

Phyllosticta frankiana Sacc. et Syd.

Sacc., Syll. Fung., 16:826

Frankiella viticola

N. Speschnew, Fungi paras. transcavc. 1900, p. 7.

"Maculis epiphyllis exaridis, rufo-marginatis, raris, rotundatis, 5-8 mm. diam., pallescentibus; peritheciis epiphyllis, punctiformibus, nigrescentibus, 100-120 μ diam. pertusis; basidiis valde obsoletis, latis, apiculatis, 16 X 2; sporulis oblongis, continuis, utrinque rotundatis, chlorinis, inflexis, 12-17 X 3.5-4, episporio .5 μ cr.

"Hab. in foliis vivis Vitis viniferae, Kahetia Transcaucasi. Plures Phyllostictae species gaudent basidii manifestis sporulisque chlorinis; hinc genus Frankiellae pro tempore non accipiendum". (Saccardo)

Phoma globigera Cke. et Mass.

Grevillea, 17:75, 1889

Sacc., Syll. Fung., 10:152

"Peritheciis gregariis, numerosis, punctiformibus, atris, sursum nudis, convexis; sporulis globosis, continuis, hyalinis, 5-6 μ diam.

"On twigs of Vitis vinifera (Mende) in America?" (Cke. et Mass.)

Macrophoma hederacea P. Brun.

Bull. Soc. Bot. Fr. 40:223, 1893.

Sacc., Syll. Fung., 11:496.

"Périthèces réunis ou rapprochés, bruns ou brun noir, subglobuleux, érumpents à la fin. Sporules oblongues-allongées, continues, hyalines, long. 15, larg. 3-3½, à deux grosses gouttelettes.-" Sur les branches mortes de l'Ampelopsis hederacea". (Brun.).

"Saintes, Galliae". (Saccardo).

Guignardia bidwellii (Ellis) Viala et Ravaz

Bull. Soc. Myc. France, 8:63, 1892

Phyllosticta labruscae Thüm. (See also Phoma uvicola B. et C.)

Thümen, von, Felix, Pilze die Weistockes, p. 189, 1878.

Sacc., Syll. Fung., 3:20

"Ph. peritheciis mediis, dense gregariis, numerosis, sine ordine dispositis, epiphyllis, hemisphericis, prominentibus, atris in macula suborbiculari, distincte limitata, lineam brunneam cincta, exarida, rufo-fusca, epiphylla, in pagina inferiore maculam pallidam fuscescentem, indistinctam formans; sporis numerosis, oblongis, regularibus, utrinque rotundatis, diaphanis, episporio tenui, achrois, intus grumulosis, 9-11mm. long., 6-7mm. crass.

"In foliis vivis Vitis Labruscae Lin. prope Newsfield, New Jersey, America septentrionalis. Aestate 1877. (J. B. Ellis)". (Thumen).

Phoma lenticularis Cavara

Cavara, F., Int. al Dissecc. dei Grap. della vite, Atti del Ist. Bot. Pav., Ser. 2, 1: , 1888, Tav. 5, fig. 4.

Sacc., Syll. Fung., 10:152

"Periteciis gregariis, interdum confluentibus, lenticularibus, epidermide tectis, diametro 180-220μ poro minuto pertusis; sporulis ellypticis ver cylindraceis, utrinque rotundatis, tipice biguttulatis, hyalinis, 7.5-8.5 X 3-3.5μ basidiis filiformibus 20-22μ longis.

"Hab. in baccis Vitis viniferae. In collibus transpadanis (Stradella, Codevilla). (Cavara).

Phyllosticta leveillei Cooke

Jr. Roy. Hort. Soc., 5:92, 1878

Septoria vitis Lev.

Ann. Sc. Nat., 1846, P. 279

Sacc., Syll. Fung., 11:540

"Hab. in foliis adhuc vivis Vitis viniferae in Gallia et Belgio.-Maculae amphigenae, nigricantis; perithecis hypophylla, minutissima, gregaria; sporulae fusoideae, curvulae, continuae, hyalinae." (Saccardo).

"The spores are narrowly oblong, and less than .01mm. in length." (Cooke).

Macrophoma longispora (Thüm.) Berl. et Vogl.

Atti Veneto-Trentina 1886 p. 188

Sacc., Syll. Fung. 10:201

Leptothyrium longisporum Thüm et Pass.

Thümen, von, Felix, Pilze die Weinstockes, p. 153, 1878

Phoma longispora (Thum. et Pass.) Cooke

Grevillea

Sacc., Syll. Fung., 3:79

"L. peritheciis parvulis, dense gregariis, in epidermidis partibus expallescentibus, imposito-prominulis subcompresso-globosis, atris, sporis sterigmatibus fasciculatis, plus minus longis, rectis, tenuibus, hyalinis fultis, cylindraceis, subrectis vel minime curvatis, simplicibus, utrinque obtuso-rotundatis, achois, 20mm. long. 4-4.5 mm. crass.

"In Vitis viniferae Lin. sarmentis aridis, Italia: Parma, Vere 1877 (Passerini)." (Thumen).

Phyllosticta microspila Passer.

Nuovo Gior. Bot. Ital. 1875

Diagn. F. V. 4:8

Sacc., Syll. Fung., 10:107

"Maculis irregularibus, parvulis, fuscis, brunneo-limitatis; peritheciis epiphyllis, atris, aparsis; sporulis oblongis, ad apices attenuatis, hyalinis, 10X3.

"Hab. in foliis Vitis viniferae silvestris in silvis Collecchio pr. Parma Ital. bor. Phoma viticola, quamvis sporularum magnitudine proxima, differt maculis amplis interdum confluentibus, peritheciis amphigenis vel hypophyllis et sporulis at apices non attenuatis." (Saccardo)

Phoma negrianum Thüm.

Thümen, von, Felix, Pilze die Weinstockes, p. 185, 1878

Sacc., Syll. Fung., 3:110

"Ph. peritheciis minutis, epiphyllis, dense gregariis, hemisphaerico-subprominulis, glaucis, nigris in foliorum partibus maximis, irregularibus, exaridis, griseo-albicantibus, subctus fuscis, sine ordine dispositis; sporis cylindro-ellipticis, untrinque subrotundatis, plerumque anucleatis, raro cum nucleo in utroque polo, simplicibus, diaphanis, 5-7 mm. long, 3-3.5 mm. crass.

"Ad folia viva, morbo "Gialluma" laborantis Vitis viniferae Lin. prope Casale Monferrato in Pedemontio, ubi ligit Advocat F. Negri, Octobri 1876, misit clar. G. Passerini." (Thümen).

Phyllosticta neurospilea Sacc. et Berl.

Misc. Myc. II n. 37, 1885.

Sacc., Syll. Fung., 10:107

"Maculis epiphyllis, nervis exacte limitatis, hinc angulosis, rufoochraceis; peritheciis globoso-depressis, arcissimis, innatoprominulis, punctiformibus; sporulis ovato-oblongis, 8-10 X 5-6, intus granulosis, hyalinis; basidiis brevibus e basi stromatica, densiuscula oriundis.

"Hab. in foliis Vitis antarcticae, Logan Australiae" (Saccardo).

Phoma nidulans Greg.

Diaporthe

Sphaeria

Michelia 2:341, 1880

Sacc., Syll. Fung. 3:80

"In ramis corticatis Ampelopsidis hederaceae, Pau. R. Perithecia hinc approximata, oblonga, erumpentia, intus grisca; spermatia fusoidea 8-10 X 2½, biguttata hyalina, basidiis filiformibus 25-30 X 1½ fasciculatis e basi proligera lutescenti oriundis suffulta." (In Michelia).

Phoma pallens B. et C.

N. Amer. F. n. 381, Grevillea, 2:81, 1873.

Sacc., Syll. Fung., 3:80

"Subcuticulare hysteriiforme; sporophoris sporis utrinque apiculatis sub-aequalibus. On shoots of vine which it renders pallid. Car. Inf. No 5015. Also on Celastrum scandens, Penn. Michener. No 4127.

"Growing beneath the cuticle, sporophores equal in length to the spores which are oblong-fusiform pointed at either end, .0002-.0003 long." (B. et C.).

"Subcuticularis, hysteriiformis, sporulis oblongo-fuscoideis, utrinque apiculatis sub-equalibus, 5-7.5μ long.

"Hab. in ramis tenellis dealbatis Vitis, Ampelopsidis, nec non Celastri scandentis, in Pennsylvania, regione Amer. bor." (Saccardo).

Phoma parvula P. Brun.

Act. Soc. Linn. Bordeaux, 1898, p. 11, extr. Sacc., Syll. Fung., 14:871

"Peritheciis numerosis, dense gregariis, globoso-conicis, nigris, perforatis, erumpentibus subsuperficialibusque; sporulis ovoideis hyalinis, minutis, 3-4 X 3.

"Hab. in fructibus maturis Vitis viniferae pluribus locis in Gallia." (Saccardo).

Macrophoma peckiana (Thüm.) Berl. et Vogl.

Atti Soc. Veneto-trentina 1886, p. 174.

Sacc., Syll. Fung., 10:189

Sphaeropsis peckiana Thüm.

Thumen, von, Felix, Pilze die Weinstockes, 149, 1878.

Phoma peckiana (Thüm.) Sacc.

Sacc., Syll. Fung., 3:80

"Sph. peritheciis gregariis, primo epidermide tectis, denum erumpentibus, liberis, conico-globosis, papillatis, mediis, nigris; sporis non pedicellatis, longe ellipsoideis vel oblongo-ovatis, utrinque obtuso-rotundatis, medio non vel minime inflexis, simplicibus, uni-vel trinucleatis, nucleis magnis, 30 mm long., 10-12 mm. crass, hyalinis.

"In sarmentis emortuis Vitis labruscae Lin. Albany, N. Y., America septentr. Vere 1877 (Peck.)" (Thumen).

Phyllosticta pilispora N. Spesch.

N. Speschnew, Fungi transcaspici et Turkestanici, 1901, p. 17, tab II, fig. 12-15.

Sacc., Syll. Fung. 18:226

"Maculis irregularibus, 2-8 mm. diam., fuscis, quandoque subzonatis; pycnidiis immersis, dilute coloratis; ostiolo plus minus amplo; sporulis initio hyalinis, dein dilutissime flavescentibus 4-5.5 X .5-1.5.

"Hab. in pag. sup. foliorum Vitis viniferae in regione transcaspica. A Phyllosticta osteospora Sacc. sporulis minoribus contextu pycnidii etc. differt." (Saccardo)

Macrophoma reniformis (Viala et Ravaz) Cav.

Atti Ist. Bot. Pavia, Ser. 2. 1:315, 1888

Phoma reniformis Viala et Ravaz

Viala, P. et Ravaz, L., Le Black Rot, p. 57, 1886.

Sacc., Syll. Fung., 10:204.

"Les pycnides emergent à peins de la surface de la baie, elles sont allongées, surbaissées, un peu déprimées vers la partie où est creussée l' ostiole, et mesurent 363μ de longueur, sur 253μ de hauter; leur membrane est d'un roux clair. Les spores apparaissent au sommet de basides droits et tres nombreux. Elles sont allongées, à contour un peu ondulé, obtuses à chaque extremité et renflées au centre. Elles mesurent 22μ de longuer sur 6μ de largeur.

"Le mycélium est très ramifié, flexueux, mais non variquex, cloisonné de loin en loin, blanchâtre et de dimensions variable. (Dim 1μ.5 à 4μ.5)." (Viala et Rav.).

"Peritheciis sparsis, epidermide tectis, globosis, atro-fuscis, poro albido pertusis; sporulis cylindraceis, rectis v. curvulis, utrinque obtusis, hyalinis, granuloso-farctis, 22-28 X 6-8; basidiis

brevibus.

"Hab. in fructibus siccis Vitis viniferae, Herault Galliae et Stradella, Casteggio et Codevilla Italiae. Habitus Macrophomae rimisedae et M. longisporae." (Saccardo).

Macrophoma rimiseda (Sacc.) Berl. et Vogl.

Atti Soc. Veneto-Trentina, 1886, p. 1888, t.III f. XXI

Sacc., Syll. Fung. 10:200

Phoma rimiseda Sacc.

Phoma rimicola Sacc.

Michelia 2:380, 1880.

Sacc., Syll. Fung., 3:78

"Peritheciis longitudinaliter dense aggregatis, e corticis rimis erumpentibus, globosis, papillatis, ½ mill. d., nigris intus pallidis; contextu parenchymatico solido, fuligineo; spermatiis oblongo-fusoideis, 18-21 X 4½-6½, utrinque acutiusculis, varie guttulatis nubilorisque, hyalinis, basidiis bacillaribus dense fasciculatis 20 X 2 suffultis. In rimis ramorum corticatorum Vitis viniferae, Padova, Febr. 1881 (Bizzozero). A ceteris Phomae et Sphaeropsidis speciebus in Thuem. "Pilz. d. Weinst." descriptis spermatiis multo majoribus dignoscenta." (Sacc. in Michelia).

Macrophoma sicula Scalia

Pr. Contr. alla Conosc. della Fl. Micol. di Catania, 1899, p. 18.

Sacc., Syll. Fung., 16:880

"Peritheciis sparsis, primo epidermide tectis, dein eam perforantibus, globosis v. subglobosis, 200-230 μ diam., atris osteolatis, sporulis ellipsoideis v. oblongo-ellipsoideis, continuis, 13-20 X 5-8, eguttulatis, initio hyalinis, maturitate pallide olivaceis.

"Hab. in ramis Vitis viniferae in Sicilia". (Saccardo).

Phyllosticta spermoides Peck.

Kept. St. Mus. New York, 40:48

Sacc., Syll. Fung., 10:107

"Maculis subcorticularibus, brunneis v. cinereis, margine brunneo donatis v. subinde confluentibus; peritheciis minutis, 35-62μ diam., numerosis, hypophyllis, nigriscentibus; sporulis minutis, cylindraceis, 5μ long.

"Hab. in foliis vivis Vitis ripariae, Gansevoort Amer. bor." (Saccardo).

Phoma succedanea Passer.

Erb. critt. Ital. II n 1378

Sacc., Syll. Fung., 3:110

"Peritheciis epiphyllis, superficialibus, in maculis exaridis, fuscis, irregularibus, gregariis vel crebre sparsis, globulosis, atris; sporulis ellipticis, nucleolatis, hyalinis vel, acervatim visis, fumidulis, 5 X 2.5.

"Hab. in foliis languentibus Vitis viniferae, in maculis Peronosporae, Vigheffio prope Parma (Passerini)". (Saccardo).

Phyllosticta turmalis E et Ev.

Erythrea, 2; New West.-Amer. Fungi 23, 1894

Sacc., Syll. Fung., 11:473

"Hab. in foliis Vitis ripariae, Kansas Amer. bor.-Maculae sparsae, brunneolae, atro-marginatae; perithecia 60-75μ diam. nigra; sporulae teriti-oblongae, utrinque paullo crassiores, 3.5-5 X 1, hyalinae." (Saccardo).

Phoma ustulatum B. et C.

N. Amer. F. n. 384, Grevillea, 2:82, 1873.

Sacc., Syll. Fung., 3:110.

"Maculis helvolis, peritheciis punctiformibus; sporis utrinque attenuatis. On leaves of Vitis aestivalis. No. 4842.

"Spots broad, red brown; perithecia minute punctiform; spores attenuated at either end, shortly fusiform, .0003 long." (B. et C.).

"Maculis helvolis seu rufo-brunneis; peritheciis minutis, punctiformibus; sporulis utrinque attenuatis, breve fusoides, 7-8μ longis.

"Hab. in foliis Vitis aestivalis, in America boreali." (Saccardo).

Phoma uvarum (B. et C.) Sacc.

Sacc., Syll. Fung., 3:149

Sphaeropsis uvarum B. et C.

N. Am. F. n 417, Grevillea, 3:1

"Peritheciis innumeris, collabascentibus, cuticula membranacea tectis, hyalinis, centro nigris; sporulis breviter cymbaeformibus leniterve curvis, 7-10 X 5.

"Hab. in baccis Vitis (Scuppernong), in Carolina infer." (Saccardo).

Guignardia bidwellii (Ellis) Viala et Ravaz

Bul. Soc. Myc. France, 8:63, 1892

Phoma uvicola B. et C. (See also Phyllosticta labruscae Thüm.)

N. Amer. F. n. 382, Grevillea, 2:82, 1873

Sacc., Syll. Fung., 3:149

"Irregulare cuticula anguste cinctum, sporis oblongis. On berries of Vitis. Car. Inf. No. 3286.

"Produced beneath the cuticle, through which it bursts, and by which it is surrounded; spores oblong, .0003 long." (B. et C.).

"Irregularis subcutaneo-erumpens et tunc cuticula anguste cincta; sporulis oblongis, 7-8μ longis.

"Hab. in baccis Vitis, in Carolina infer. Amer. bor." (Saccardo).

Phoma uvicola Berk. et Curt. var. labruscae Thüm.

Thümen, von, Felix, Pilze die Weinstockes, p. 16, 1878.

Sacc., Syll. Fung.,

"A forma typica differt sporis regularibus, semper subglobosis, majoribus, 7-8 mm. diam.

"Ad Vitis labruscae Lin. acinos maturos, adhuc pendulos sed corrugatos.- America septentrionalis, New Jersey (J. B. Ellis)". (Thumen).

Phyllosticta vitea Sacc.

Sacc., Syll. Fung., 3:20

Septoria viticola Berk et Curtis sec. Ravenel

Phyllosticta viticola Thüm.

Thümen von, Felix, Die Pilze die Weinstockes, p. 188, 1878

"Ph. peritheciis amphigenis, sed praecipque hypophyllis, in pagina superiore prominulis, in macula magna, exarida, plus minus orbiculata, interdum confluentia, subter fusca, superne fusco-purpurea, exacte limitata, densissisme gregariis, minutis, globosis, epidermide tectis, postremo

aliquando liberis, numerosissimis; sporis minutis, paucis, brevi-ellipsoideis, utrinque rotundatis, simplicibus nucleatis, nucleo uno maximo in medio parte, 8-9mm long., 4 mm crass., achrois.

"Ad Vitis vulpinae Lin. folia viva. America septentr.: Aiken, Carolinae australis. (H. W. Havenel). (Thümen).

Macrophoma viticola (Cooke) Berl. et Vogl.

Atti Soc. Veneto-Trentina, 1886, p. 192

Sacc., Syll. Fung. 10:203

Sphaeropsis viticola Cooke

Grevillea, 12:22, 1883

Phoma viticola (Cooke) Sacc.

Phoma vitea Sacc.

Sacc., Syll. Fung. 3:110

"Foliicola gregaria; peritheciis subglobosis, demum centro depressis, fuscis; sporulis sublanceolatis, rectis, curvulisve, hyalinis, 22-24 X 5-6.5.

"Hab. in foliis Vitis, in Carolina austr. (Ravenel)". (Saccardo).

Phoma viticola Sacc.

Michelia, 2:92, 1880.

Sacc., Syll. Fung., 3:79

Phoma viniferae Cooke

Grevillea 13:92, 1885

Sacc., Syll. Fung., 10:152

"Peritheciis sparsis, epidermide velatis, globosis, papillatis, vix 1 6 mill. d.; contextu laxe parenchymatico fuligineo; spermatiis ellipsoideis, subinaequilateralibus, 7 X 4, eguttulatis hyalinis, basidiis filiformibus subaequantibus suffultis.--In sarmentis Vitis viniferae. Mb.- Ab affinis Ph. Vitis Bon. differt spermatiis duplo majoribus. (Sacc. in Michelia).

Phyllosticta viticola Sacc. et Sp.

Michelia 1:155, 1878

Sacc., Syll. Fung., 3:19

"Maculis vagis sinuosis, arescendo candidis, anguste fuscosinctis, epiphyllis; peritheciis punctiformibus, lenticularibus, pertusis; spermatiis ellipsoideis, 5 X 2½, eguttulatis, e hyalino olivaceis.

"Hab. in foliis Vitis viniferae in agro Tarvisino Aut. 1876." (Sacc. et Sp.).

Phoma vitis Bon.

Abhandl. Myc. p. 14, 1870

Pirotta F. Vit. p. 54, 1877.

Sacc., Syll. Fung., 3:79

"Peritheciis minutis, punctiformibus, sparsis, depresso-globulosis nigris; ostiolo minuto, primo conico, epidermidem perforante; sporulis ovato-ellipticis, 3-3.5 X 1-2, hyalinis; basidiis

simplicibus, subventricosis.

"Hab. in sarmentis Vitis viniferae, in Italia, Germania, Austria et Britannia." (Saccardo).

Phyllosticta vitis (Fckl.?) Sacc.

Michelia 1:135, 1878

Sacc., Syll. Fung., 3:20

"Maculis vagis, sinuosis arescendo dealbatis, fusco-marginatis; peritheciis remotis, punctiformibus, lenticularibus, 70 diam., pertusis, contextu laxiuscule parenchymatico fuligineo; spermatiis oblongo-ovoideis, 6 X 3, 2-guttulatis hyalinis.

"Hab. in pag. sup. foliorum Vitis viniferae a Casale Monferrato, Aut. 1876. (F. Negri); a Selva, Aut. 1874-76.

"Ob. Phyll. Vitis Fckl. et Phyll. Vitum Dmz, in Hoffm. Ind. mycol. p. 96 citatae, mihi ignotae sunt." (Sacc. in Michelia)

A study of the above descriptions shows that the fungous characters which are used in differentiating between organisms which fall into the three genera treated here, are: Shape of pycnidium, size of pycnidium, type of ostiole, length of spores, width of spores, shape of spores, and presence or absence of guttulae in the spore. That the size and shape of

spores are considered as perhaps the most reliable criteria, is shown by certain statements included in some of the descriptions, which point out what the investigator evidently regards as the main differences between the organism at hand and certain other forms which closely resemble it.

Thus, we find Saccardo pointing out for Macrophoma flaccida Viala et Ravaz (Syll. 10:198): "Phomae rimisidae affinis, sed ostiolo epapillato, sporulis et basidiis minoribus diversa"; for Macrophoma rimiseda Sacc. (Mich., 2:380): "A ceteris Phomae et Sphaeropsidis speciebus in Thüemen, 'Pilz. d. Weinst.' descriptis spermatiis multo majoribus dignoscenta"; for Phoma viticola Sacc. (Mich., 2:292): "Ab affinis Ph. Vitis Bon. differt spermatiis duplo majoribus"; for Phyllosticta microspila Pass. (Syll., 10:107): "Phoma viticola, quamvis sporularum magnitudine proxima, differt maculis amplis interdum confluentibus, peritheciis amphigenis vel hypophyllis et sporulis at apices non attenuatis". Speschnew (Syll., 18:226) indicates that Phyllosticta pilispora Spesch. differs from Phyllosticta osteospora Sacc. as follows: "A Phyllosticta osteospora Sacc. sporulis minoribus contextu pycnidii etc. differt".

Pycnidial shapes in these fungi may be grouped under three main divisions: (a) Globose - including all those described as globosis, globoso-depressis, subglobosis, conico-globosis, ovoideis- (b) Hemisphaerical, and (c) Lenticular - to include convex. Shapes of spores in these forms, have been described as bacillary, cylindric, elliptic, elliptic-cylindroid, oblong-ellipsoid, ovate-elliptic, lanceolate-

subelliptic, lanceolate, sublanceolate, oblong, oblong-elongate, oblong-fuseoid, fuseoid, oblong-ovoid, oblong-ovate, elongate-subovoid, ovate, ovoid, subglobose, globose, etc.

It is now necessary, before discussing the classification of such a group of organisms, to evaluate these characters which have been considered important by taxonomists, and discover how stable they remain under different environmental conditions.

III. EXPERIMENTAL METHODS AND RESULTS.

A. Definition of terms; purpose.- It is well known that fungi show considerable differences in their physiological constitution as evidenced by their response to different environmental conditions, by the degree of parasitism which they exhibit on the host, etc.

Several names have been applied to these "physiological units" by different investigators. Stevens (58) gives a list of terms which have been employed, as follows: physiological species, species sorores, biologische Spezies, biologiske Arten, schwester Arten, biologische Rassen, specialisirte Formen, formae speciales, Gewohnheitsrassen, races specialiees, microspecies, biotypes, elementary species, pure lines, biological forms, biological races. To these may be added the terms: strains, physiological strains, and biological strains.

Because many of these names have been used by different investigators to mean sometime one thing and sometime another, the term "clone" will be used throughout this paper to designate an individual which was originally isolated as a single pycnidiospore and then entirely perpetuated by asexual means.

In certain fungi, definite individuals or groups of individuals are recognized, which differ from each other, at least in so far as is known, only in their physiological behavior with reference to the hosts which they are capable of parasitizing. In many other fungi, these units, in addition to the differences in physiologic behavior, also exhibit

certain distinct morphological differences. These differences are supposed to be of so small a degree so as to make it inadvisable to asign them specific importance. On the other hand, when taxonomic literature is critically studied, the fact that new species of fungi have been created on as small a difference in character as that found between two individuals which are asigned to one and the same species, is inescapable.

The purpose of the experimental work undertaken in connection with this investigation is to make a careful study of the effect of environmental conditions on the morphological and physiological characters of different individuals which the taxonomist would presumably include in one species, and compare any morphological differences which these individuals or the clones resulting from them exhibit, with those existing between the ~~fifty~~ forty-five so-called species of Phoma, Phyllosticta, and Macrophoma which have been described by various investigators from Vitis, and Parthenocissus.

It was at first proposed to isolate and study as many individuals as possible of Guignardia bidwellii Viala and Ravaz, the organism which causes the black rot disease of the grape. It was found, however, that this organism did not readily produce spores on artificial media and since spore characters are considered to be the most important ones, in delimiting species of fungi belonging to the genera which are treated in this paper, it became necessary, for the purpose of this investigation, to work with an organism which readily

sporulates on a variety of media in a comparatively short period of time.

During the course of isolation work, several individuals which closely resembled each other were isolated from grape material received from different parts of the country. It was found that these could readily be grown on almost any kind of a standard medium and that they sporulated freely. The general characters which they exhibited placed them in the genera Phoma and Phyllosticta of the Deuteromycetes and it was decided to use these as a basis for the experimental work which was to be carried on. All of these individuals were started from single spores and were asexually propagated during the course of the investigation over a period of three years by either a transfer of spores produced abundantly in pycnidia in culture, or by mycelial transfers.

The results herein given, therefore, are those of the behavior of these different clones under the different environmental conditions to which they were subjected in the laboratory.

These results include observations made on a clone of Macrophoma flaccida (designated as PH. F.)*, isolated from grape bark which was kindly supplied by Prof. L. Ravaz, Director of Viticulture at the Ecole Nationale d'Agriculture de Montpellier, France. Unfortunately, this organism did not freely sporulate in culture and the observations relating to it

* If we accept the present distinction made between the genera Phoma and Macrophoma, the name for this organism would be Macrophoma flaccida (Viala et Ravaz) Cavara. Prof. Ravaz, however, uses the name Phoma flaccida and for this reason the clone is here designated as "PH. F."

are confined to macroscopic characters such as rate of growth, zonation, pigment, etc.

B. Material and Methods.- The origin of the different clones which will be treated in this paper, is indicated in Table 1.

Table 1. Origin of Clones Used.

Clone	Locality	Material from which isolation was made
G6	Mississippi	Fruits of Vitis sp.
G6M	Mutant	Sector from G6 in culture
G20	Washington, D. C.	Fruits of Vitis
G72	Connecticut	Fruits of Vitis
G72M	Mutant	Sector from G72 in culture
G81	Illinois	Leaf of *Vitis labrusca*
G81M	Mutant	Sector from G81 in culture
PH. F.	Montpellier, France	Bark of *Vitis vinifera* (?)

Isolations were made by transfering some of the spores which readily exuded from a pycnidium when this was placed in a drop of sterile water, to corn meal agar. When the colonies resulting from these spores sporulated, a single pycnidiospore was isolated and transfered to a sterile corn-meal agar plate. From the colony thus formed, a transfer was made to a stock bottle of the same medium which was kept in the laboratory at room temperature.

All measurements of colonies were made with a millimeter ruler and the longest diameter of the colony was taken as the standard. Pycnidial measurements were taken under the low power objective; all spore measurements were made

under the oil immersion lens with a 1.8 mm. objective.

For agar cultures, 20 cc. of melted Difco agar were always used to the petri dish. For liquid media cultures, 25 cc. of solution were used to each 150 cc. Erlenmeyer flask. In the experiments with ultra-violet irradiation, a Hanovia mercury vapor lamp was utilized.

C. Experimental Results.- In order to determine whether any appreciable differences exist between the different clones, they were all grown under as nearly identical conditions as possible under a variety of environmental conditions. It was found that they could be differentiated by the following macroscopic criteria: Rate of growth, pycnidial formation, color of colony, zonation, amount of aerial mycelium, thermal relations, photic relations, and response to ultra-violet irradiation. The following microscopic criteria were also found to be useful in differentiating these clones: Mean size of spores, color of mycelium, shape of mycelial cells, and vacuolation of mycelium.

As will be pointed out, these characters are all more or less affected by changes in environment, but all except color, remain very stable as long as the clone is grown under constant conditions.

1. Rate of Growth.- In order to study the rate of growth of these clones, they were grown under as nearly identical conditions as possible at room temperature on cornmeal agar and on potato-dextrose agar. Because some of the clones originating as sectors of colonies on agar did not sporulate in culture, inoculations were made by means of mycelial transfers. All growth experiments were

carried out in duplicate. Only the averages are given in Tables 2 and 3 in which the results are summarized.

Table 2. Rate of Growth, Room temp., Corn-meal agar.

Diameter of colonies in mm.							
Clone	Age in days						Ave.
	2	3	4	9	10	11	
G6	7.0	11.5	17.5	45.0	50.0	57.0	5.1
G6M		9.2	13.5	38.0	44.0	48.0	4.3
G20		7.0	14.0	40.0	47.0	53.0	4.8
G72	7.5	11.7	17.5	47.5	52.0	59.0	5.3
G72M	9.5	14.2	20.0	51.5	61.0	64.5	5.9
G81	7.5	11.5	17.0	42.0	52.0	55.5	5.0
G81M	9.0	13.5	19.0	48.0	56.0	59.5	5.4
PH. F.	21.0	42.0	58.0				14.5

Table 3. Rate of Growth, Room temp., Potato-dextrose agar.

Diameter of colonies in mm.							
Clone	Age in days						Ave.
	2	3	4	9	10	11	
G6	6.0	9.5	13.0	31.5	37.0	40.0	3.6
G6M		9.5	15.0	42.0	49.0	53.5	4.9
G20		6.0	13.0	26.5	32.0	35.0	3.2
G72	8.0	14.5	22.0	60.0	68.0	75.0	6.8
G72M	13.5	19.5	27.5	69.5	80.0	85.0	7.7
G81	7.5	12.0	15.5	34.5	39.5	42.5	3.9
G81M	9.0	14.0	18.0	52.0	59.0	66.0	6.0
PH. F.	27.5	54.5	86.0				21.5

Plates 1, 2, 3, and 4 show the colonies at the end of their ninth day of growth. Figs. 1 and 2 show the growth curves of the different clones on corn-meal agar and potato-dextrose agar at room temperature. It is quite clear from these figures that the organism from France, PH. F. exhibits the most rapid growth, and that, among the other organisms which have much more in common in all respects, G72M grows most rapidly. If we list the clones in the order of rapidity of growth on corn-meal agar by the end of the third day, the series will be: PH. F., G72M, G81M, G6, and G81, G72, G6M, and G20. On the fourth day, G20 has overtaken G6M, but the rest grow in the same order. By the ninth day, G6 has dropped below G81 for the only change in the series. On the tenth day G72 has suddenly increased its rate of growth and by the eleventh day it has surpassed G6 and G81 coming very close to G81M, the rate of growth of which is decreasing.

On potato-dextrose agar, the differences in rate of growth are much more pronounced. PH. F. and G72M are again first and second in the series, respectively. Then come G72, G81M, G81, G6, and G20. A comparison of the growth curves on these two media, shows clearly that the rate of growth of PH. F., G72M, G81M, and G6M was accelerated by potato-dextrose agar. On the other hand, the rate of growth of G81, G6 and G20 was retarded by the richer medium.

Pycnidial production.- The clones differ markedly in their ability to produce pycnidia in culture under different conditions. The results described under this section refer to the pycnidial production of colonies grown on corn-

meal agar and potato-dextrose agar at room temperature and exposed to daylight.

Of all the clones treated here, G6N has never produced pycnidia under any circumstances. This clone originated as a sector of G6 on corn-meal agar. This sector was darker in color from the parent colony and was completely devoid of pycnidia.

G6 produces relatively few pycnidia on corn-meal agar, but a great abundance of them on potato-dextrose agar. The pycnidia on corn-meal are produced mostly in an area close to the inoculum and are generally few in number. An occasional colony, however, sporulates more freely by producing in addition, one or more zones of pycnidia on the younger mycelium. Transfers from such colonies do not remain prolific but revert to the original condition. Pycnidial production in this clone seems in some way to be associated with pigment in the mycelium. Although pycnidia may be produced on a very young colony, only three days old, which at the time shows no pigment whatever, by the time the colony fills the petri dish in which it is growing, the pycnidia will, in the majority of cases, be located on the pigmented areas only. It should be stated here that G6 produces pigment mostly in irregular areas, which radiate from the center of the colony. These areas are not definitely limited. The irregularity with which this clone produces pigment will be more fully discussed in the section on "Color of colony". The great majority of the pycnidia of G6 are produced superficially. Now and then a

few are found buried in the agar. Buried pycnidia are much more commonly found in potato-dextrose agar than in corn-meal agar.

G20 produces pycnidia abundantly on corn-meal agar and like all the other clones responds to potato-dextrose by an even more abundant fructification. On corn-meal agar there is no tendency for the pycnidia to be produced in zones. They are scattered evenly over the surface of the agar. On potato-dextrose, they are very much crowded, being formed mostly in groups of from two to five or more. This is especially true on the older part of the colony around the inoculum. On this medium there is a distinct tendency for the pycnidia to be formed in radial strands until the colony reaches a diameter of about 30 mm., after that, the majority of the pycnidia produced are formed in concentric zones.

When G72 was originally isolated, the young colonies growing on corn-meal agar developed a few pycnidia near the inoculum. As the colonies grew older, fruiting bodies were produced in concentric zones though a few could be seen in the intervening spaces. On potato-dextrose agar, the zonation of pycnidia was also noticeable. Although fruiting bodies developed in abundance, in this medium, all over the colony, their number was much greater to the unit area in these definite zones.

After the clone had been cultivated for several months on artificial media, it suddenly lost its power of pycnidial production altogether and will not fruit at present under any circumstances. It is possible that when the last

transfer was made in a stock bottle just previous to discontinuing work for the summer, a potentially sterile sector which could not be differentiated in any way from the parent colony was used in making the transfer, with the resulting loss of the original clone. It is also possible, however, that the character of the original clone may have changed after a certain period of time in culture. Such changes have been reported in the literature dealing with artificial culture of fungi by Stevens (61) for Glomerella, Burkholder (13) for Fusarium ( ), and others.

G72M which originated as a very dark brown sector of G72, produces pycnidia very abundantly on all solid media on which it has been grown. This clone is interesting in that it produces the majority of its fruiting bodies buried in the agar. A great many of the fruiting bodies of this clone contain no spores. These sterile bodies are generally much smaller than the pycnidia which contain spores but resemble the latter in all other respects.

G81 produces pycnidia very abundantly on both media under consideration. On corn meal agar, many of the pycnidia are found in a central area about two centimeters in diameter over which they are more or less evenly scattered. Most of the pycnidia on the rest of the colony are arranged in distinct zones which may be of different widths. On potato-dextrose agar, the zoning is often not very distinct because of the heavy production of pycnidia in all parts of the colony; it is unmistakably present, however. In this clone, too, the

the great majority of pycnidia are superficial.

G81M produces fewer pycnidia than its parent G81, on corn-meal agar, but considerably more on potato-dextrose agar. On the former medium the pycnidia are scattered over the colony with no definite tendency toward zonation. On the latter medium, however, there is a very definite zonation produced by a greater concentration of fruiting bodies in narrow zones which are close together.

Clone PH. F. has been seen to produce pycnidia only once. This was on a plate of corn-meal agar grown at room temperature. The few pycnidia produced formed a clump, superficially, which was estimated to contain not more than six or seven fruiting bodies. These developed and matured their spores which germinated readily.

3. Color of Colony.- An olive or yellowish-brown pigment seems to be linked with the inheritance of all the clones treated here with the exception of PH. F. Even the ones which are perfectly white on corn-meal agar when the colony is young, develop such a pigment after two or three weeks. It is very difficult to describe the color of some of these colonies accurately because it is not always exactly the same. Color seems to be influenced by the slightest change in the chemical composition of the medium and as a result, no two colonies are exactly alike.

The colors given in Table 4 are the ones in Ridgeway's Color Standards (51) which more nearly match those of the average colony for each clone at the age of twenty-

four days.

Table 4. Color of Colony

Clone	Corn-meal agar	Potato-dextrose agar
G6	Light brownish olive	Medal bronze
G6M	Buffy olive	Medal bronze
G20	Light brownish olive	Sepia to black
G72	White with Saccardo's olive center.	Cinnamon buff to pinkish buff.
G81	Saccardo's olive	Sepia to black
G81M	Light brownish olive	Buffy citrine
PH. F.	White	Black

It should be stated here that few colonies, if any, of any one clone are uniform in color throughout. The center of the colony is generally much darker than the areas nearer the edges. The very young mycelium at the edge of a colony is in every case hyaline and as a result, all the colonies which develop a pigment early, have a white border which differs in width with each clone but is fairly constant with colonies of the same clone. The greatest variation in color pattern for any one clone is found in G6. A colony from this clone on corn-meal agar is perfectly white for several days. At about the tenth day of growth a slight tinge of brownish pigment may begin to appear near the inoculum. This may spread slowly towards the edge of the colony, at first forming a pigmented central core with a wide white border till the colony fills the petri dish in which it is growing, and the pigment slowly reaches the tips of the mycelial hyphae.

On the other hand, the color may appear in irregular sectors without definite limits, which may run radially from the center of the colony to the edge, leaving perfectly white irregular spaces in between. Whether transfers are made from the white parts of the colony or from the pigmented sector, the results are the same in most cases, indicating that the majority of these sectors are probably due to micro-environmental changes. Occasionally, however, a sector may be due to a change ~~in genetic constitution~~ permanent and give rise to a new clone as was the case with G6M.

Color patterns were found to be much more constant on potato-dextrose agar than on corn-meal agar for all clones.

4. Zonation.- The character of zonation was touched when pycnidial formation was considered. It is interesting to note here that clone G72 which at the present time does not produce pycnidia, continues to present the phenomenon of zonation as a purely mycelial character as can be seen in Plate 2 in the figure at the left which is a photograph of a G72 colony on corn-meal agar. The zones here are due to a more compact mycelial growth which takes place at intervals.

The clones differ both as to the degree and the type of zonation which they present. Table 5 summarizes these characters. A small degree of zonation is represented by the figure "1" and a large degree by "2". The words "mycelial" and "pycnidial" are used to differentiate between zonation caused by a more compact growth of mycelium, and by a concentric pycnidial arrangement, respectively.

Table 5. Degree and Type of Zonation

Clone	Degree of zonation	Type of zonation
G6	1	Pycnidial when present.
G6M	2	Mycelial
G20	1	Pycnidial*
G72	2	Mycelial
G72M	2	Mycelial
G81	2	Pycnidial
G81M	2	Pycnidial
PH. F.	2	Mycelial

* Some degree of pycnidial zonation is evident on potato-dextrose agar as has been mentioned under "Pycnidial production".

5. Aerial Mycelium.- There is a tendency for aerial mycelium to be formed by most clones to some degree. Potato dextrose agar favors the formation of aerial mycelium much more than corn-meal agar. In the clones which form aerial mycelium to a slight degree only, it is invariably located in the center of the colony around the inoculum.

Two types of aerial mycelium can be recognized in these clones; these have been termed: cottony and filamentous. Table 6 summarizes these characters. The figures 0 to 5 are used to denote differences in amount of aerial mycelium from a complete absence to a very heavy growth.

Table 6. Aerial mycelium.

Clone	Amount of Aer. Mycel. Corn-meal	Amount of Aer. Mycel. Potato-dext.	Type of aer. mycel. Corn-meal	Type of aer. mycel. Potato-dext.	Color of aer. mycel. Corn-meal	Color of aer. mycel. Potato-dext.
G6	1	2	Cottony	Filamentous	White	Olive-green
G6M	2	3	" "	Cottony	Gray	Gray
G20	1	2	" "	Filamentous	Olive	Gray
G72	0	4	—	" " "	—	White
G72M	2	3	Cottony	" " "	Gray	Gray to white
G81	2	3	" "	" " "	Olive	Olive-green
G81M	1	2	" "	" " "	Olive	Gray
PH. P.	1	5	" "	Cottony	White	Gray

6. Thermal Relations.- The effect of temperature on growth was studied by inoculating the clones on corn-meal and potato-dextrose agar and incubating them in constant temperature chambers at 10°C. and 20°C. Growth took place at both temperatures, but as was expected, it was much more rapid at the higher temperature. Tables 7 and 8 show the effect of temperature upon the rate of growth.

Table 7. Effect of Temp. on Rate of Growth, Corn-meal agar

Clone	Diameter of colonies in mm. Age in days											
	3d.		4d.		6d.		7d.		9d.		Ave.	
	10°	20°	10°	20°	10°	20°	10°	20°	10°	20°	10°	20°
G6	--	13.0	Tr.	19.0	11.0	30.0	13.0	35.5	18.0	47.0	2.0	5.2
G6M	--	11.0	Tr.	17.0	8.5	26.0	11.0	31.5	15.0	42.0	1.7	4.7
G20	--	13.0	8.0	18.0	12.0	26.5	15.0	31.0	18.0	42.0	2.0	4.7
G72	--	14.0	Tr.	19.0	13.0	32.0	17.5	38.0	21.5	50.0	2.2	5.6
G72M	9.0	19.0	12.0	26.0	19.0	34.0	22.5	40.5	30.0	56.0	3.3	6.2
G81	--	17.0	10.0	19.0	12.0	29.0	16.0	33.0	18.0	45.0	2.0	5.0
G81M	--	16.0	6.0	21.0	11.0	32.5	13.0	38.0	19.0	53.0	2.1	5.9
PH. F.	13.0	45.0	19.0	62.0	32.0	--	37.0	--	51.0	--	5.7	15.6

Table 8. Effect of Temp. on Rate of Growth, Potato-dextrose agar.

Clone	Diameter of colonies in mm. Age in days										Ave.	
	3 d.		4 d.		6 d.		7 d.		9 d.			
	10°	20°	10°	20°	10°	20°	10°	20°	10°	20°	10°	20°
G6	--	15.0	10.0	17.5	13.0	23.0	15.0	27.0	17.0	34.0	1.9	3.8
G6M	--	13.0	12.0	19.5	17.0	31.0	19.5	37.0	24.0	46.0	2.7	5.1
G20	--	14.0	10.0	17.0	11.0	19.5	12.0	23.0	15.0	30.0	1.7	3.3
G72	--	19.0	14.0	27.0	21.5	42.0	26.0	50.0	38.0	63.0	4.2	7.0
G72M	9.0	29.0	20.0	39.0	30.0	55.0	36.0	62.0	47.0	77.0	5.2	8.6
G81	--	14.0	8.0	17.0	11.0	25.0	13.0	29.5	15.0	39.0	1.7	4.3
G81M	--	19.0	11.0	26.0	13.0	40.5	15.0	47.0	17.5	60.0	3.5	6.7
PH.F.	17.0	63.0	21.0	--	42.0	--	52.0	--	74.0	--	8.2	21.0

Figs. 3, 4, 5, and 6, show the growth curves of the clones on corn-meal agar and potato-dextrose agar at 10° and 20°C. Besides the differences in growth due to a difference in temperature, these graphs verify the results obtained at room temperature in regards to the effect of the medium upon the rate of growth. G6, G20, and G81, grew more rapidly on corn-meal agar, while the growth of G6M, G72, G72M, G81M, and PH. F., was accelerated by potato dextrose.

It will be noted in Fig. 6 that after the fourth day of growth, there is no change in the relative growth rate of the clones. The series on potato-dextrose agar is PH. F., G72M, G72, G81M, G6M, G81, G6, and G20, or precisely the same as it was at room temperature. Potato-dextrose agar, stimulates

the growth of certain clones to such an extent that temperature variations during the twenty-four hours of the day are not sufficient to change the relative rates of growth.

On corn-meal agar, there is a much closer relationship of rates of growth. The stimulation of the slow growers which is brought about by this medium is not sufficient to send them into the lead, nor is the retardation of the fast growers sufficient to cause them to lag behind. As a result, a much smaller range of variation is obtained, and relatively small changes in temperature may be sufficient to change the order at practically any given period within the range of the experiment.

With a constant low temperature of 10°C. the rate of growth of clones G6, G20, G81, and G81M is brought to the same level.

G72M was peculiar in that it always exhibited a distinct spiral growth when grown at constant temperature--either 10° or 20°C.--for the first five or six days, but in no case at room temperature.

Pycnidial production did not seem to be affected by the temperature factor. The clones that generally sporulate under laboratory conditions developed pycnidia normally at both 10° and 20°C. and there was no noticeable difference in the number of pycnidia developed at the two different temperatures.

The quantity or kind of aerial mycelium was not affected by the temperature factor to any extent, nor was zonation.

The effect of temperature on the microscopic characters of the clones will be treated later when these characters are discussed.

7. Photic Relations.- In order to determine whether the clones under consideration were in any way affected by the presence or absence of light, a series of corn-meal agar plates was inoculated in quadruplicate. Two plates inoculated with each clone were kept on shelves in the laboratory and exposed to ordinary daylight while two other plates were kept in the cupboard of a desk. All openings into the cupboard were loosely plugged with cotton to exclude light, but permit circulation of air. Before the experiment was begun, a thermometer was placed in each place where the two sets of plates were to be kept during the experiment. The temperature was taken from time to time for five days and the greatest variation was found not to exceed three degrees.

The results of the experiment showed quite conclusively that pycnidial production was inhibited by the absence of light in three of the five clones which regularly sporulate on corn-meal agar. Clones G20, G81, and G81M failed to form any pycnidia in the cultures from which light was excluded, but produced fruiting bodies in the usual manner in cultures which were exposed to daylight. Plate 15 shows two cultures of G81. The upper one was kept in darkness for fourteen days. Clone G6 produced approximately twice as many pycnidia when exposed to light than it did in the absence of light. Light seemed to have no effect upon pycnidial production in the case of G72M.

Color of the colony was to some degree affected by the absence of light. The colonies of all the clones except PH. F. contained more yellow pigment when light was excluded.

Experiments with light were not tried with cultures growing on potato-dextrose agar. It is possible that the light factor simply provides a stimulus for the reproduction of these clones. In such a case, it is probable that if the clones are grown on a medium which favors a heavy production of pycnidia, light may not be necessary for reproduction.

8. Response to Ultra-violet Irradiation.- Because of the very interesting results obtained by Stevens (59, 60, 61, 62, 63, 64, 65.) with Glomerella cingulata and other fungi when he exposed them to ultra-violet irradiation, a series of experiments was begun with the purpose of determining whether the clones under consideration differ in any way in their response to irradiation.

The colonies were grown on corn-meal agar and potato-dextrose agar in the laboratory. When they were approximately 50mm. in diameter, one-half of each colony was exposed to ultra-violet irradiation the other half being shaded with a piece of composition board to serve as a check.

Stunting of growth was a universal effect on all clones treated in such a manner. It was first evident about twenty hours after irradiation and the effect lasted for three or four days after, with colonies treated for 30", the stunted mycelium usually reaching the length of the untreated mycelium before the colony filled the petri dish. With higher dosages, the stunting was more permanent the hyphal tips not resuming

their growth for several days after treatment.

In the clones which regularly produced pycnidia in culture, exposure to ultra-violet irradiation for 15" to 5' stimulated reproduction. The increase in number of pycnidia was much greater when the clones were grown on corn-meal agar than on potato-dextrose agar.

Clone 72 at first responded to ultra-violet irradiation by producing pycnidia in abundance in the spaces between the pycnidial zones as shown in Plate 6 . It will be noticed that a dark pigment in the mycelium was induced as a result of the treatment. When this clone lost its power to reproduce in culture after having been grown for a long time on corn-meal agar, ultra-violet irradiation did not cause pycnidial development.

Clone G6M which has never been seen to produce pycnidia, will not develop them with ordinary dosages (5" to 3') of ultra-violet irradiation. A colony growing on potato-dextrose which was exposed to ultra-violet light for 10', produced pycnidia-like bodies, both superficially and submerged in the agar, only on the irradiated side. These, however, did not contain spores. The experiment was not repeated and the constancy of this character is not, therefore, established. A similar colony growing on corn-meal agar did not in any way respond to a similar treatment.

Reproduction could not be induced in clone PH. F. with ultra-violet irradiation.

9. Mycelial Characters.- The mycelium was not found to be very useful in differentiating these clones with the exception of PH. F.

Plate ~~8~~ 7 shows the characters of the mycelia of the different clones. Fig. 4 represents the mycelium of G72. It can be seen that the cells are irregular in size and shape and that there is a tendency for some of them to assume a barrel shape. Fig. 5 shows the mycelium of G72M. Both types shown here are very common in all cultures from this clone. The very large oil dropplets in drawing "a" are rather characteristic for the old mycelium of this clone on all media on which it was grown. These included, corn-meal agar, potato-dextrose agar, bean-pod agar, and 3% Merck Dextrose solution. Yet, the number and size of these dropplets varies enough to make the character an unreliable one for purposes of identification. Drawing "b" Fig. 5 represents another type of mycelium found in this clone. The connection between the two types has been established. This type of mycelium has been also found in clone G81.

Color of the mycelium varies to some extent with the different clones. Generally, G72M has the darkest colored mycelium, while G72 has the lightest, the latter being completely hyaline except when very aged. G6M is always much darker in color than G6 while G81 and G81M are very similar in most respects. The mycelium of PH. F. is grayish-brown on corn-meal agar but very dark brown approaching black, on potato-dextrose agar.

10. Pycnidia.- The shape and size of the pycnidia are characters that have been used together with others in delimiting species. The extreme variation in size of pycnidia even in the same colony of a clone renders this character valueless in the identification of the clones treated in this paper. Because of the great variation, it would be necessary to measure a very large number of these structures to get at an even approximate figure denoting the mean size. Pycnidia growing side by side in a colony of G81 on corn-meal agar were found to measure 70u and 250u respectively. On potato-dextrose agar several pycnidia of the same clone were found to measure as high as 330u and as low as 58u. There seemed to be no correlation between size of pycnidia and environmental factors in any of the clones. Large and small pycnidia were scattered indiscriminately on the media on which the colonies were growing.

On potato-dextrose agar there was a distinct tendency for pycnidia to form in groups. Sometimes two to five or more pycnidia would grow together forming a large fruiting body with as many or more ostioles as there were pycnidia originally. It was very common to find a pycnidium with two or even more ostioles in all the clones the pycnidia of which were ostiolate. That this did not in many cases result from the fusion of two or more pycnidia was evident from the very regular globular shape of many of these fruiting bodies and was established by sectioning in many cases.

Measurements for individual clones are not given here because of the extreme variability in size.

The shape of individual pycnidia was found to vary considerably in the same colony from globose to acute-conic. No pycnidia were found which could be described as lenticular or hemisphaerical. Beaked pycnidia were not uncommon. The ostiole in all cases was round.

In G72M the pycnidia were in general, much smaller than in the other clones and much more uniform in size, and shape. The great majority of these are globose or nearly so. Another interesting character exhibited by the fruiting bodies of this clone is the complete absence of an ostiole. Though a large number of them were examined, no opening of any kind could be found. The mature pycnidia in all clones are very dark brown or black. The darker color is favored by potato-dextrose agar. In G6, this is especially true since the pycnidia produced on corn-meal agar are lighter in color to some degree than those produced by other clones on this medium. On potato-dextrose, however, G6 cannot be differentiated from the other clones on this character.

11. Spore Characters.- There is considerable variation in both spore size and shape in all the clones studied which sporulated in culture. The literature on fungi, as will be pointed out in the next chapter, offers much evidence that spore characters in some fungi are subject to modification by environmental conditions. The data presented below shows that the nature of the substratum on which the fungus grows, as well as the temperature at which spores are produced, are factors which influence spore size and shape. Presence or absence of guttulae is also a character which is influenced by the en-

vironment. Moreover, the variation in all these spore characters is quite large even in a single pycnidium.

The influence of nutriment and temperature on the length of spores was studied by growing the fungi on corn-meal agar and potato-dextrose agar at 20°C. and 10°C. One hundred spores were measured in each case and plotted on curves as shown in Fig. . The results of these experiments are summarized in Table

The results shown on Table 9 , indicate that both, nature of the substratum and temperature influence the mean length of spores of the four clones which were studied biometrically. Cultures on potato-dextrose agar in all cases resulted in an increase in mean length of spores from 13.0% to 22.2%. A drop in temperature of 10°C increased the mean length of spores from 6.5% to 10.5%. No general conclusions can be drawn in regards to the influence of the medium or temperature on the variability in spore length, from these figures. The coefficient of variability is about the same under all conditions, for clones G6 and G81. In clone G20 both medium and temperature seem to have induced an effect on variability while for clone G81M the nature of the medium exerted an influence but the temperature did not.

Though no statistical data were obtained for width of spores, observations revealed that, as a rule, the spores were of a considerably larger diameter on potato-dextrose agar than on corn-meal agar and at 10°C than at 20°C on potato-dextrose.

Shape of spores was very variable even within the same pycnidium. The majority of the spores in all clones except

Table 9a. Effect of Medium and Temperature on Mean Length of Spores in .

Clone	20°C			10°C	
	Corn-meal	Potato-dextrose	% increase due to medium	Potato-dextrose	% increase due to temp.
G6	M= 5.0813±.0345 O= .5117±.0244 C=10.0702±.4803	6.2100±.0440 .6531±.0309 10.5169±.5013	22.2	6.7256±.0446 .6522±.0315 9.8459±.4696	7.6
G20	M= 5.0337±.0203 O= .301 ±.0143 C= 5.9999±.2861	5.7911±.0328 .4872±.0232 8.4129±.4013	13.0	6.4718±.0495 .735 ±.0350 11.3569±.5417	10.5
G81	M= 4.9588±.0337 O= .5005±.0238 C=10.0931±.4814	6.0256±.0413 .6132±.0292 10.1765±.4854	21.5	6.4225±.0515 .7637±.0364 11.8910±.5672	6.5
G81M	M= 4.9980±.0255 O= .3794±.0185 C= 7.5910±.3621	5.9185±.0443 .658 ±.0313 11.1176±.5303	18.4	6.4911±.0445 .6601±.0314 10.1693±.4850	7.9

Table 9b. Mean Size of Spores of PH. F.

Potato-dextrose, Room Temp.

M=12.6392±.0864
O= 1.281 ±.0611
C=10.1351±.5409

PH. F. were oblong with rounded ends. Fusiform spores were common and a few could be found that were very nearly globose, especially on potato-dextrose cultures. Ovate spores were not uncommon. In fact practically all gradations in shape between sub-globose and oblong were present, with the latter, as was pointed out above, in the great majority.

Number of guttulae in the spores varied from none to seven. In general, spores which developed on potato-dextrose agar were in the majority pluriguttulate, but no definite correlation could be established between environmental factors and guttulae because of the great variation which occured under all conditions.

IV. DISCUSSION OF RESULTS AND REVIEW OF LITERATURE.

The results of the experimental part of this investigation indicate that the morphological characters of the clones under consideration are significantly affected by the nature of the substratum and temperature, and that reproduction -- in some of the clones -- is in addition influenced by the factor of light.

In addition, it was shown that certain characters such as size and shape of pycnidia are extremely variable even under as nearly identical environmental conditions as could be reproduced.

A review of some of the important work which has been done in this field of study is opportune at this time together with a comparison of the results obtained by the writer with that of other investigators.

Practically every paper on the physiology of fungi deals partly with the effect of certain environmental conditions on growth. Many of these experiments have been performed at room temperature and though they may in certain cases indicate to a degree the difference in growth between two individuals, they cannot be an accurate index of this character because of the difference in response to changes in temperature which exist between individuals. This point has been brought out in the previous chapter under "Thermal Relations" and need not be dealt with here any longer. Stevens (58) found that the temperature at which agar was made had a bearing upon its nutritive value and in this way

affected growth relationships of various Helminthosporia. The quantity of available medium was also a factor which influenced growth as was temperature. Chaudhuri (16) agrees with Stevens in reporting that thickness of the medium affects the growth of Verticillium albo-atrum. Brown (7) working with a number of fungi found that "in the early stages, the rate of growth is small and that it then rises to a maximum which may, or may not, be maintained". He reports (7) that, in general, the rate of growth increases with the concentration of the nutrient up to a certain extent and with the rise of temperature up to a certain limit. Most other investigations as well as the one herein reported agree in that a rise in temperature is accompanied by an increase in rapidity of growth and that the nature and thickness of the substratum, are also factors which influence the latter.

The formation of reproductive bodies is another character which is, in many fungi, influenced by environmental conditions. The researches of Klebs (34) with Saprolegnia are too well known to need elaboration. Previous to his work, Raciborski had reported (48) that concentrated solutions check the formation of zygospores in Basidiobolus ranarum.

Light seems to play an important part in the formation of fruiting bodies in certain fungi, but does not seem to be necessary in others. Ever since the classic researches of Brefeld (78) numerous investigators have undertaken experiments seeking to discover the photic relationships of fungi. Thus, among others, Buller (11)

has shown that light is necessary for the development of the pileus of Polyporus squamosus and Lentinus lepideus; Peltier (45) reported that darkness "favored non-production of conidia and the formation of a large number of the striated pseudo-sclerotia, while light favored the greater development of conidia" in Botrytis cinerea. Light was found to be essential for the production of pycnidia in Plenodomus fuscomaculans by Coons (20). It could be replaced, however, by certain oxidizing agents. Leonian (37) reports that absence of light at room temperature prevented the formation of pycnidia in Hendersonia sp. and Sphaerographium fraxini and reduced the number of pycnidia formed by twelve other fungi among which was Phoma urens. Six fungi remained indifferent to the light factor. The same author found that a temperature of 30°C generally induced a better pycnidium formation in spite of the absence of light.

The interelationship of these various environmental factors should, therefore, be taken into consideration for a single factor may cause a response from an individual under certain conditions, but have no effect in the presence or absence of some other factor. In these Sphaeropsidales with which Leonian worked, pycnidium development and rich hyphal growth were found to be associated.

Hedgcock (28), in 1906 found that spore formation in Cephalothecium, Mucor and Hormodendron was uniform over the whole surface of the mycelium when they were grown under red or orange light, or in total darkness. Cultures grown under blue light or ordinary daylight showed marked zonation.

Green light gave the same effect as blue, but to a lesser extent. It was concluded that blue rays of light inhibit spore formation. On the other hand, Peuser (46) has recently reported that color of light influences fructification in Fusarium nivale and that the blue rays may be the most important ones in stimulating the production of spores.

N. E. Stevens (67) was unable to demonstrate in the laboratory that Endothia parasitica was in any way affected by the light factor. Caldis and Coons (14) report that light is not an important factor in the growth of Cladosporium fulvum. Sawyer (56) states that in Empusa, "vegetative growth and the reproduction and germination of conidia take place as well in total darkness as in light".

The writer has shown that three of the clones treated in this paper did not produce any fruiting bodies on corn-meal agar at room temperature when light was excluded and that two others produced about twice as many pycnidia in the light than they did in darkness. One clone remained indifferent to the presence or absence of the light factor under the conditions stated above.

Another factor which seems to be connected with reproduction in fungi is that of aeration as Lakon (36) has shown for certain Coprini. Matsumoto (40) has reported that sclerotial formation is suppressed by inadequate aeration, in certain forms of Rhizoctonia solani. Microscopic characters such as pycnidial and spore dimensions and shapes which are used for identification purposes have been shown in many fungi

to be subject to modification by the environmental factors. Some of the important papers which contain evidence to bear out this fact are reviewed briefly below: Ravn (49) states that Helminthosporium conidia developing under different conditions vary in size. Their length depends upon the conditions under which they develop. Neger (42) has shown that the nature of the substratum is a factor in the determination of the size of conidia in certain Erysiphaceae. Kauffman (33), in his work with Saprolegnia states that variations were found to be so extensive that nearly all characters used in classification of the group were affected. Jonge and Dorst (32) have shown that hairiness of pycnidia is largely dependent upon environmental conditions.

Stevens and Hall (66) found that density of spores in the inoculum, composition of medium, and light, caused great variations in macroscopical and microscopical characters. These variations were in certain instances so great as to change the taxonomic position of the fungus in question from one order to another.

The composition of the medium was shown to have a considerable effect on the size of spores. Certain morphological characters of Diplodia gossypii have been shown by Tabenhaus (69) to be so greatly influenced by environmental conditions so as to show characters of five supposed genera. Spore size of certain species of Alternaria and Macrosporium was found to vary with the medium used by Elliot(24). In general there was a reduction in the size of spores on synthetic agars over that on bean agars. Temperature was

also found to be a factor influencing the size of spores, lower temperatures favoring an increase in size. Moreau (41) has shown that the size of spores in Sporodinia grandis, is greatly influenced by the concentration of the medium.

The length of spores in Septoria lycopersici is found by Norton (43), to be considerably affected by different hosts. The spores become longer on potato and Solanum carolinense, but shorter upon Datura tatula than they were upon the original host. Brierley (3) has shown that the nature of the substratum influences the size of the conidia in Botrytis cinerea. Beach (1), found that environmental conditions altered the size of spores in Septoria tritici and Septoria verbascicola and concludes that "If all the species of Septoria have a corresponding relation to the environment, and if all have a broad range between the maximum and minimum spore length, as in S. tritici and S. verbascicola, it is obvious that many of the measurements now given in specific descriptions are far from accurate". On the other hand the same author finds that there was no alteration in spore length when these same fungi were transfered from one host to another under the same environmental conditions.

In so far as value of disease characters is concerned Beach (1) summarizes the situation very well as follows: "The variable nature of disease characters, as manifested by the host, has been well demonstrated. These variations are dependent upon the species, the age, and the part of the host as well as upon environmental conditions. On this account these characters lose much of their value in

taxonomy....."

Mutto and Pollacci have shown, according to Brierley (4) that the morphological characters of the spores in species of Coniothyrium and Phyllosticta vary with differences in conditions. Brooks and Searle (6) have demonstrated that an individual of Phoma destructiva (Plowr.) C. O. Jamieson, varied with environmental conditions as to pycnidial size and shape, spore size and shape, and presence of guttulae in the spores. Wormald (77) finds that the nature of the substratum greatly influences the size of conidia in Monilia cinerea. He states: "The dimensions of conidia of the two species are generally quoted as a distinguishing character. The great variation in the size of conidia of M. cinerea according to conditions under which they are developed renders this distinction somewhat unreliable unless the environmental factors are taken into consideration".

Brooks and Kidd (5) report that the spores of Cladosporium herbarum which develop at - 5.5°C are of the character as those developing at ordinary temperatures. The conidiophores, however, developing at low temperatures are sometimes entirely unbranched thus differing from those produced at normal temperatures.

The morphology of certain strains of Colletotrichum gloeosporioides was affected by the different media on which it was grown by Burger (12). This worker found that spore size was considerably affected by the different media. Koch and Rumbold (35) reported that pycnidia and conidia of Phoma insidiosa varied in size, shape and content on different

parts of the host and on culture media.

Small (57) reports that the presence or absence of setae is not a constant character in Colletotrichum coffeanum and that this character appears to be governed by physiological conditions. Thom and Church (70) found that a strain of Aspergillus tamari grown on Czapek's solution agar showed vescicles about 35 u in diameter, primary sterigmata 8-14 X 3-5 u and conidia 5-6 u long. Respective measurements for the same strain on unsterilized clean corn were 100 u, 25-35 X 5-9 u, and 8-9 u. Van der Bijl (74) found that pycnidia of a Phyllosticta species which measured 80-106 u on the host would measure up to 133 u in diameter when produced on beer-wort agar.

Stevens (58) showed that the morphological characters of Helminthosporium are influenced by environmental factors. He states "Very slight changes in environment may produce marked alterations in colony characters--growth rate, color, aerial mycelium, clumping of mycelium, and even in the shape, septation, and size of the conidia". Dosdall (22), and Dosdall and Christensen (23), and Christensen(17), showed that different conditions of growth influence the length of spores and other morphological characters in Helminthosporium sativum.

The results of a number of investigators on the effect of environmental factors on the morphology of the Fusaria are important. Burkholder (13) finds that certain morphological and physiological changes take place in Fusarium martii phaseoli when this is grown in pure culture for a long

time. Both macroscopic and microscopic characters tend to revert toward the original by passing the fungus through a bean plant and reisolating. Because the fungus is a hemiparasite, Burkholder concludes that "That these changes also take place in nature seems reasonable to expect.....The question then naturally arises whether these variations might not eventually become permanent. The fact that there is a large number of closely related species and varieties in the genus Fusarium would lead to this belief".

Brown and Horne (9) and Horne and Mitter (30) have illustrated that the septation and length of Fusarium spores are greatly affected by the concentration of the medium used. The first two authors have shown that a rise in temperature tends to lower the degree of septation of the spores. Changing the C:N ratio is, according to these authors, by far the most effective method of changing the character of the spores of Fusarium. They find that "A high C:N ratio tends to increase, a low C:N ratio tends to diminish the average septation". Brown and Horne further report that "according to the cultural treatment, the spores of any strain may show hyaline, granular, or vacuolated spores".

Finally, the results of the Japanese investigators should be mentioned in connection with this ~~discussion.~~ review. Togashi (72) reports for an Alternaria on Gomphrena, that "The shape and size of the conidia of our fungus vary conspicuously with the environmental factors. After a rainy night the conidia measured in the following day are remarkably

longer than those observed on normal days, although the difference in width is not notable. "The mean value of the length of the conidia on the natural host is more than five times that in the culture media, and the conidia formed in the latter are usually verrucose".

Investigations by Sueda (68) on Piricularia oryzae show that "The size of conidia is variable according to different moisture and temperature: under warm and moist conditions they are large and narrow, while under reverse conditions, small and broad".

Togashi (73) finds that "In Loeucostoma the size of pycnospores is variable according to various seasons: their mean size as well as coefficient of variability being larger in August, September and October than in December. Furthermore, in both fungi, asci and ascospores are largest in late winter and early spring; when mature, they are very variable in their dimensions".

To summarize: the results of this investigation show that practically all characters which have been used in the descriptions included in Chapter II, to differentiate between 45 "species" of Phoma, Phyllosticta and Macrophoma which have been described from Vitis and Parthenocissus are more or less definitely affected by the nature of the substratum and by the temperature at which they develop. Some of them are, in addition, extremely variable in the same individual under a given set of conditions.

It has been shown, that the results reported herein are in accord with those of numerous other investigators obtained from the study of many different fungi both in pure culture and under natural conditions. Unfortunately all inoculation experiments undertaken in connection with this work failed, and for this reason the data were totally obtained from studies in pure culture.

The significance of variability in morphologic characters in fungi in connection with systematic work has been pointed out by many investigators who's publications have been discussed or quoted above. Before this section is ended Hansford's work with the Fusaria of Jamaica ( ) must be mentioned. He says: "Not only are these differences between the so-called 'species' so minute, but at the same time, each organism shows such a great range of variability in its morphological characters that the differences used to separate the various 'species' are almost negligible by comparison."

V. DISCUSSION ON CLASSIFICATION

From the conclusions which have been drawn in the previous chapter from the results of the experimental part of this work and from the results of numerous other investigators, the difficulty of deciding the limits of our taxonomic groups in these fungi, under our present system of classification is evident.

The main purpose of uniting individuals into groups is to facilitate identification. Under our present system of classification, our taxonomic groups are concepts without definite limits. The fact that mycologists regard individuals which differ in some degree from a description, as members of the species which the description typifies, is ample proof of that fact.

Let us take the case of Guignardia bidwellii (Ellis) Viala et Ravaz. Reddick (50) gives Phoma uvicola, Phoma ustulatum, Phyllosticta labruscae and Phyllosticta vitea (Phyllosticta viticola Thum.) as names applied to the pycnidial stage of the fungus. The limits of the spore measurements as set by these descriptions are 7-8μ, 7-8μ, 9-11 X 6-7μ, and 8-9 X 4μ respectively. Reddick himself found that the pycnospores of G. bidwellii which he studied, measured 8.5-11.5 X 6.5-8.5μ, yet he considered them to be true to type, so to speak. The writer fails to see any greater differences in any character between the descriptions listed as synonymous and between them and the descriptions of Phoma uvarum (B. et C.) Sacc., Phyllosticta neurospilea Sacc. et Berl., and Phoma

viniferae Cooke. Moreover if it is reasonable to include this form which Reddick studied and which will require us to change the upper limits of spore length and width by .5μ and 1.5μ respectively, why is it not as reasonable to include Phoma cordifolia, Phoma nidulans, and Phoma negrianum which will require us to change our spore limits one or two more microns without affecting to any appreciable extent the descriptions of the other characters which are supposed to be valuable in limiting "species"?

Let us turn for a moment to Macrophoma reniformis. The researches of Jaczewski (31) point out that, in all probability, many of these forms which resemble Macrophoma reniformis are nothing more than phenotypic expressions of the same fungus under different conditions. Thus, he concludes that Macrophoma flaccida (Syn. Phoma flaccida) and Macrophoma reniformis (Syn. Phoma reniformis) are synonymous, and uses Phoma reniformis to designate both forms.

Prillieux and Delacroix (47) observed that one and the same pycnidium contained spores, some of which answered the description of Macrophoma flaccida and others, that of Macrophoma reniformis, and concluded, therefore, that the two forms ought to be refered to by the same name.

Macrophoma reniformis Viala et Ravaz is the imperfect stage of Guignardia baccae (Cav.) Jacz. (Syn. Guignardia reniformis Prillieux et Delacroix) which differs from Guignardia bidwellii (Ellis) Viala et Ravaz, in that its asci and spores are longer. (G. bidwellii, asci:70-90 X 10-12μ; spores:12-16 X 4.5-6μ. G. baccae, asci: 80-100 X 9-12μ;

spores:16-20 X 6-7u). Jaczewski further believes that Macrophoma reniformis is in all probability identical with Macrophoma rimiseda Sacc.

The findings of these workers together with the spore measurements presented in Chapter III, indicate that the variation in spore measurements of the pycnidial stage of Guignardia baccae is quite large. Here, as in the case of Guignardia bidwellii, there are described forms which agree very closely with the descriptions of Macrophoma reniformis, Macrophoma flaccida, and Macrophoma rimiseda. We have but to widen our species concept a little with regards to spore size, in order to include Macrophoma longispora, Macrophoma farlowiana, Macrophoma sicula, Macrophoma hederacea, Phoma vitea, Phoma cookei, Phyllosticta frankiana, etc.

Thus as we widen little by little our species concepts of Guignardia bidwellii and Guignardia baccae, the differences between the imperfect stages of the two groups tend to disappear. It may or may not be significant in this connection, that the measurements of the asci and spores of the two perfect stages as given by Jaczewski, overlap.

The similarity between Macrophoma lenticularis Cavara, and Phyllosticta viticola Sacc. et Speg., prompted Jaczewski to undertake inoculation experiments. Thus, he inoculated young leaves of the grape with pycnidiospores of Phyllosticta vitis Sacc., which he evidently considers synonymous with Phyllosticta viticola Sacc. et Speg., and obtained lenticular pycnidia with spores measuring 6.8-8 X 3-4μ. He then proceeded to inoculate grape berries with

these spores and as a result he obtained pycnidia the spores of which agreed with those described for Macrophoma lenticularis Cavara. He, therefore, considers these four names as synonyms.

When we examine the literature dealing with G. bidwellii, we find that the measurements given, are seldom the same. That is not hard to explain; no two investigators, probably, have ever examined the same individual or group of individuals under identical conditions. Let us also remember when we compare our specimens with the original descriptions or even with the type specimens, that both may be mixtures. We have no assurance that a leaf spot on Vitis which bears pycnidia, has been caused by a monosporous infection. Our descriptions in many cases are as valueless as experimental results obtained with unknown mixtures of organisms! We are, thus, comparing the measurements of an unknown mixture of organisms, which has developed under one set of environmental conditions, with the description of another unknown mixture which has developed under a different set of conditions. If the two happen to coincide we call them the same; if they are slightly different; we "stretch" the limits of one or the other and still call them the same; if we think they are considerably different, we create a new species of Phyllosticta and add it to the great number which already exist. Thus, to this mixture of unknowns, we add the element of personal error.

The results can plainly be seen in Table . The forty-five forms have been listed here in a series of increasing spore lengths. These, range from 2.5μ to 35μ. It will

Table 10. Species of Phoma, Phyllosticta and Macrophoma From Vitis and Parthenocissus, Arranged in a Series of Increasing Length of Spores in microns.

Phyllosticta bizzozeriana	2.5	- 3.5	X 1.5		
Phoma vitis	3.0	- 3.5	X 1.0	- 2.0	
Phyllosticta turmalis	3.5	- 5.0	X		
Phoma parvula	3	- 4	X 3		
Phoma ampelogena	4		X 3		
Phyllosticta pilispora	4	- 5.5	X .5	- 1.5	
Phyllosticta confluens	5		X		
Phyllosticta spermoides	5		X		
Phoma succedanea	5		X 2.5		
Phyllosticta viticola	5		X 2.5		
Phoma cicinnoides	5.6		X 2.5	- 3	
Phoma globigera	5	- 6	X 5	- 6	
Phoma negrianum	5	- 7	X 3	- 3.5	
Phoma ampelinum	5	- 9	X 2.5	- 3.5	
Phyllosticta vitis	6		X 3		
Phyllosticta badhami	6	- 7	X 2		
Phoma viniferae	7		X 4		
G. bidwellii (Phoma uvicola)	7	- 8	X		
G. bidwellii (Phoma ustulatum)	7	- 8	X		
Phoma cordifolia	7	- 9	X 2.5	- 3	
Phoma uvarum	7	-10	X 5		
Phoma ampelocarpa	7.5	-	X 2.5		
Phoma lenticularis	7.5	- 8.5	X 3	- 3.5	
Phoma pallens	7.5		X 5		
Phoma nidulans	8	-10	X 2.5		
Phyllosticta vitea	8	- 9	X 4		
Phyllosticta neurospilea	8	-10	X 5	- 6	
Phoma descissens	9	-10	X 3		
G. bidwellii (Phyllosticta labruscae)	9	-11	X 6	- 7	
Phyllosticta leveillei	10		X		
Phyllosticta microspila	10		X 3		
Phoma baccae	12		X		
Phyllosticta frankiana	12	-17	X 3.5	- 4	
Phoma cookei	13		X 4.5		
Macrophoma sicula	13	-20	X 5	- 8	
Macrophoma hederacea	15		X 3	- 3.5	
Macrophoma flaccida	16	-19	X 6		
Macrophoma longispora	20		X 4	- 4.5	
Macrophoma rimiseda	18	-21	X 4.5	- 6.5	
Macrophoma farlowiana	21		X 5.4		
Macrophoma acinorum	20	-28	X 6	- 7.5	
Macrophoma reniformis	22		X 6		
Macrophoma viticola	22	-24	X 5	- 6.5	
Macrophoma peckiana	30		X10	- 12	
Macrophoma ampelopsidis	30	-35	X12		

be noticed that the series is quite complete and that the measurements of many of the "species" overlap. The same is true of the width measurements of the spores, which range from .5μ to 12μ.

If the descriptions of all these forms be examined carefully, it will be noticed, as was pointed out in Chapter II, that of all the fungous characters which are used to separate these groups, size and shape of spores are considered as most important. Shape of pycnidium, size of pycnidium, type of ostiole and presence or absence of guttulae in the spore are mentioned in most cases in the descriptions, but the majority of the groups resemble each other very closely as far as these latter characters are concerned.

If, indeed, the main purpose of taxonomy is to facilitate identification, we are continuously defeating our own purpose as Table 10 plainly shows.

But, there seems to be still another aspect to the problem. The Deuteromycetes, constitute parts of the life-histories of Ascomycetes. We may, then, have two individuals of Phoma which appear exactly alike on a host plant, but which may belong to two ascomycetous individuals which are so different, as to be asigned to different species or genera by the systematist. As long as the perfect stages of these individuals remain unknown, we have no means of telling what their phylogenetic relationships are. From that standpoint, then, we may be wrong if we call them by one name and equally wrong if we give them two names. If the purpose of taxonomy is to arrange individuals ac-

cording to their phylogenetic relationships, our present system of classification again defeats its own purpose. A natural system can only be based on a knowledge of the genetic constitution of the individual, and its phenotypic expression under known environmental conditions.

As the taxonomic situation becomes more and more complicated in fungi, many investigators are turning to physiologic characters for help. Thus, Coons and Strong (21) attempt to coordinate morphologic characters with physiologic ones, such as the response to growth-inhibiting substances, in order to get at a better understanding of the classification of Fusaria. Gregor (27) advises that physiological characters be taken into consideration in fungus taxonomy, and Leonian (38) states that "the specific unit can be fashioned only from the combination of both morphological and physiological characters of not one but of dozens of 'type species'".

The writer seriously doubts if the use of physiological characters will be of much help in the taxonomy of the Deuteromycetes under our present system of classification. In all probability, after these characters have been studied in a number of individuals, we would have the same overlapping as we have at present with the morphological characters. We would then come to fully realize in the words of Hochreutiner (29) "qu'il n'y a dans la nature ni familles, ni genres, ni especes, ni meme de lignees, si l'on ajoute une valeur systematique a ce terme. Il n'y a que des individus qui se resemblent plus ou moins". We would, then, either have to name and describe every single in-

dividual which we studied, or continue moving in a sphere of uncertainty which would be much more chaotic than the present one.

All attempts to put the system of classification of the Deuteromycetes on a more natural-relationship basis are bound to be unsatisfactory until more knowledge has been obtained on the genetic affiliations of these organisms. Much experimental work in this field is needed in this connection and it will be a long time before enough data have accumulated to make a natural system of classification possible.

In the meantime, we must recognize the absolute necessity of a purely artificial system as a convenient tool for the study of the Deuteromycetes. Such a system must be one in which the limits of our taxonomic groups are defined for purposes of convenience and must completely disregard seemingly natural division lines which do not serve our purpose.

Such an attitude, together with the fullest appreciation of the fact that environmental conditions do influence the fungous characters which we use in taxonomy will greatly diminish the number of "species" which cannot be distinguished from each other and which serve no scientific or practical purpose.

Because of the limited amount of material available for study, the writer does not feel justified to ~~either~~ unite any of the "species" listed in Table 10 . From the study of the descriptions and from the experimental results of this and other investigations which have been cited on variations in fungi due to environment, however, it seems that certain of

these forms which are indistinguishable from each other can be conveniently grouped as follows:

Guignardia bidwellii (Ellis) Viala et Ravaz

Phoma uvicola B. et C.

Phyllosticta labruscae Thum.

Phyllosticta vitea Sacc.

Phoma ustulatum B. et C.

Phoma ampelogena Sacc.

Phoma parvula P. Brun.

Phyllosticta vitis Sacc.

Phyllosticta viticola Sacc. et Speg.

Phoma lenticularis Cavara

Phoma cookei Pir.

Macrophoma longispora (Thum.) Berl. et Vogl.

Macrophoma rimiseda (Sacc.) Berl. et Vogl.

Macrophoma viticola (Cooke) Berl. et Vogl.

Macrophoma flaccida (Viala et Ravaz) Cav.

Macrophoma reniformis (Viala et Ravaz) Cav.

Macrophoma acinorum Pass.

Phoma farlowiana Viala et Sauv.

The opinions expressed in this Chapter have been forced upon the writer from a study of taxonomic literature, and the results obtained by him and previous workers in this field.

VIII. VITA

Constantine John Alexopoulos was born in Chicago, Illinois, on March 17, 1907.

When only a few years old he went with his family to Athens, Greece, where he received his grade school education in the Athenian public schools and began his high school education at the Second Gymnasium of Piraeus, Greece. At the close of 1919 he returned to Chicago where he completed his high school training at Lane Technical High School, graduating in June 1923. In the fall of the same year, he entered the College of Agriculture of the University of Illinois and received his B. S. degree in June 1927. He was at that time appointed Scholar in Horticulture and entered the Graduate School of the University of Illinois in September of the same year, receiving his M. S. degree in June 1928.

He was research assistant to Prof. F. L. Stevens in Botany at the University of Illinois from 1928 to 1929, and assistant in Botany from 1929 to 1930. During the fall of 1930 he visited California and in February 1931 he returned to the University of Illinois to become research assistant to Prof. B. T. Palm in Plant Pathology.

He worked with Prof. B. T. Palm, as his assistant during the summer of 1931 at the Rocky Mountain Biological Station. He was Fellow in Botany, University of Illinois, 1931 to 1932.

He served as Plant Inspector for the Illinois State Department of Agriculture during the summer of 1929 and for the Illinois State Natural History Survey during the summer of 1930 in connection with the Peach Yellows investigations.

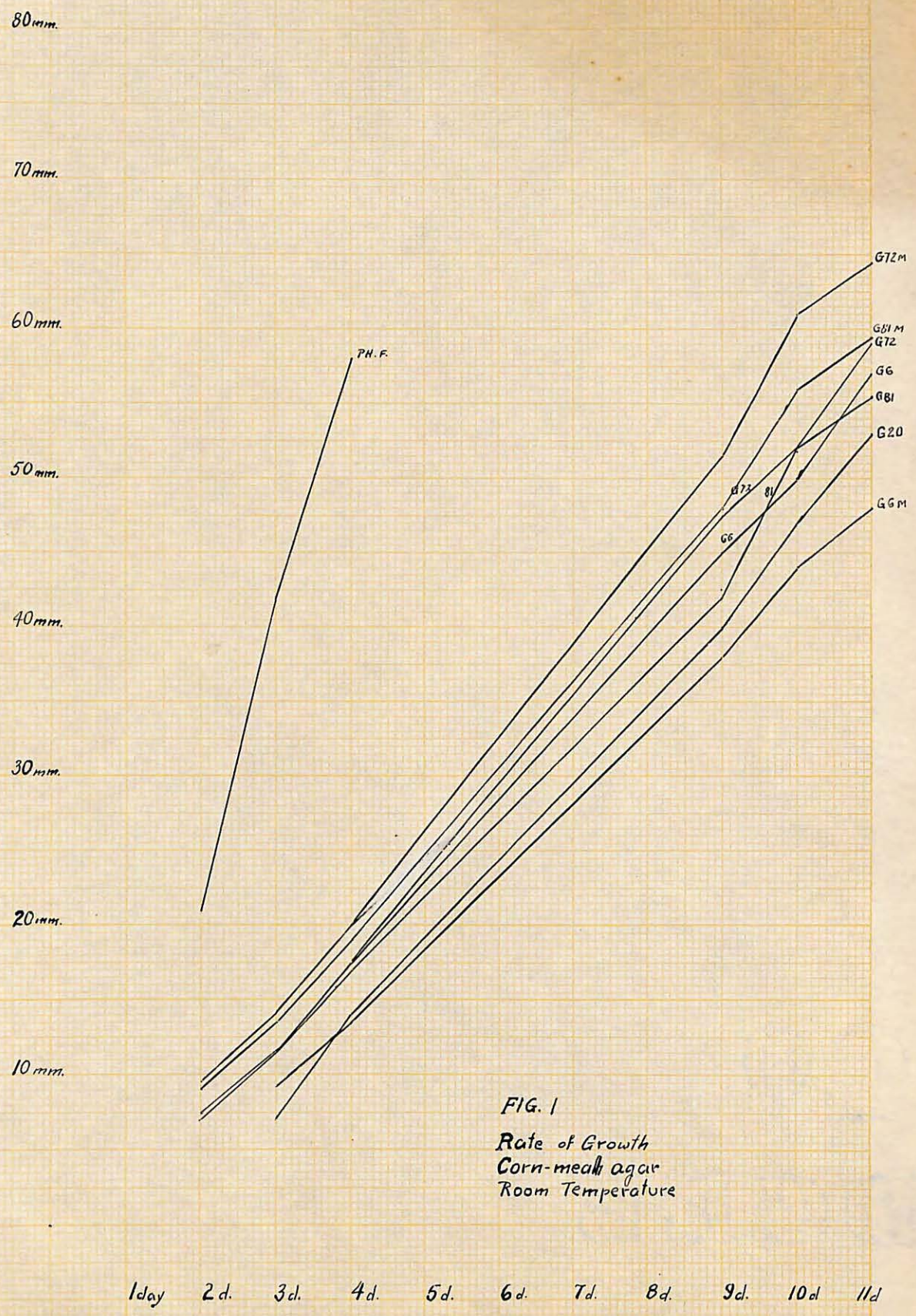

FIG. 1

Rate of Growth
Corn-meal agar
Room Temperature

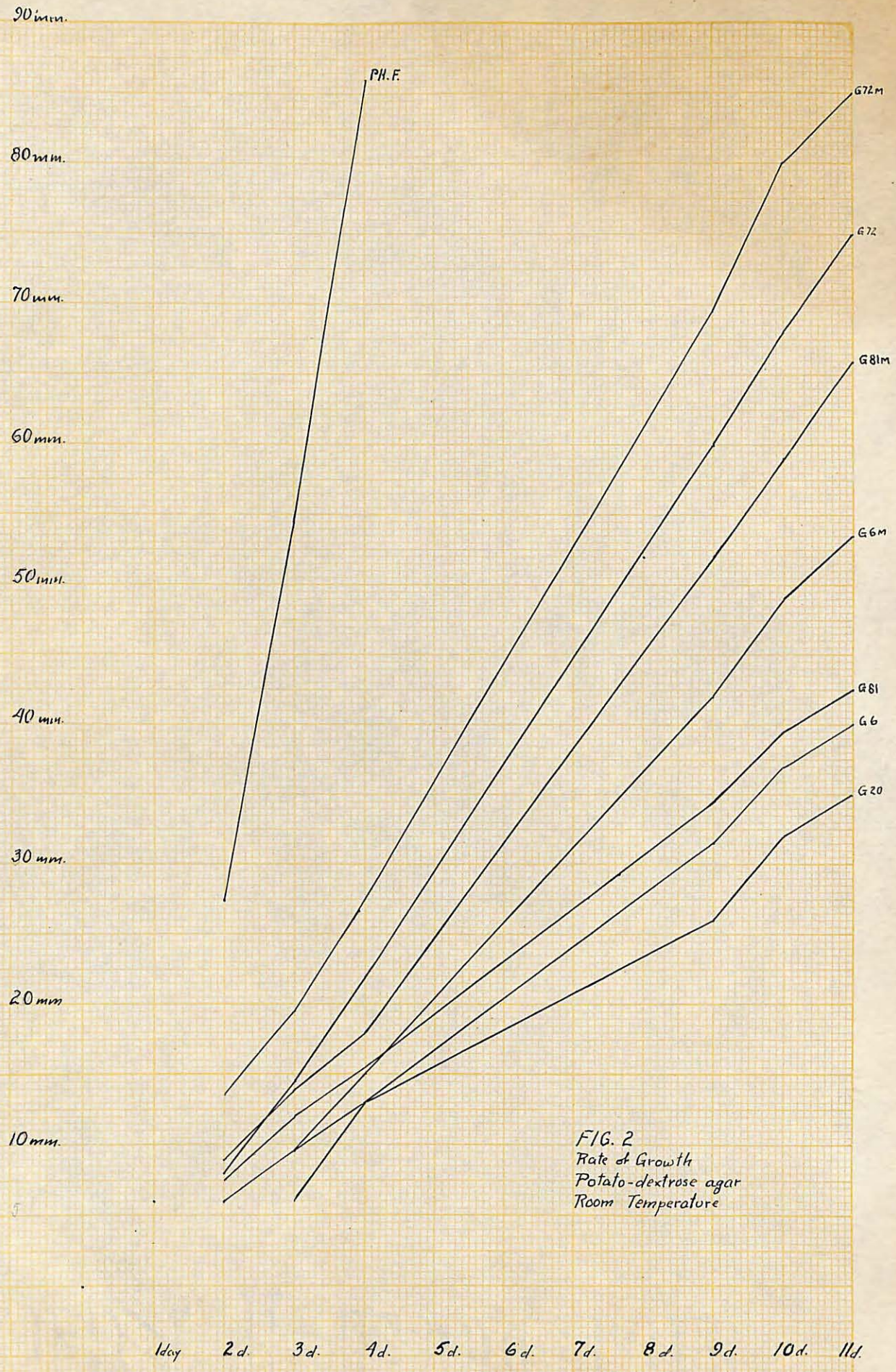

FIG. 2
Rate of Growth
Potato-dextrose agar
Room Temperature

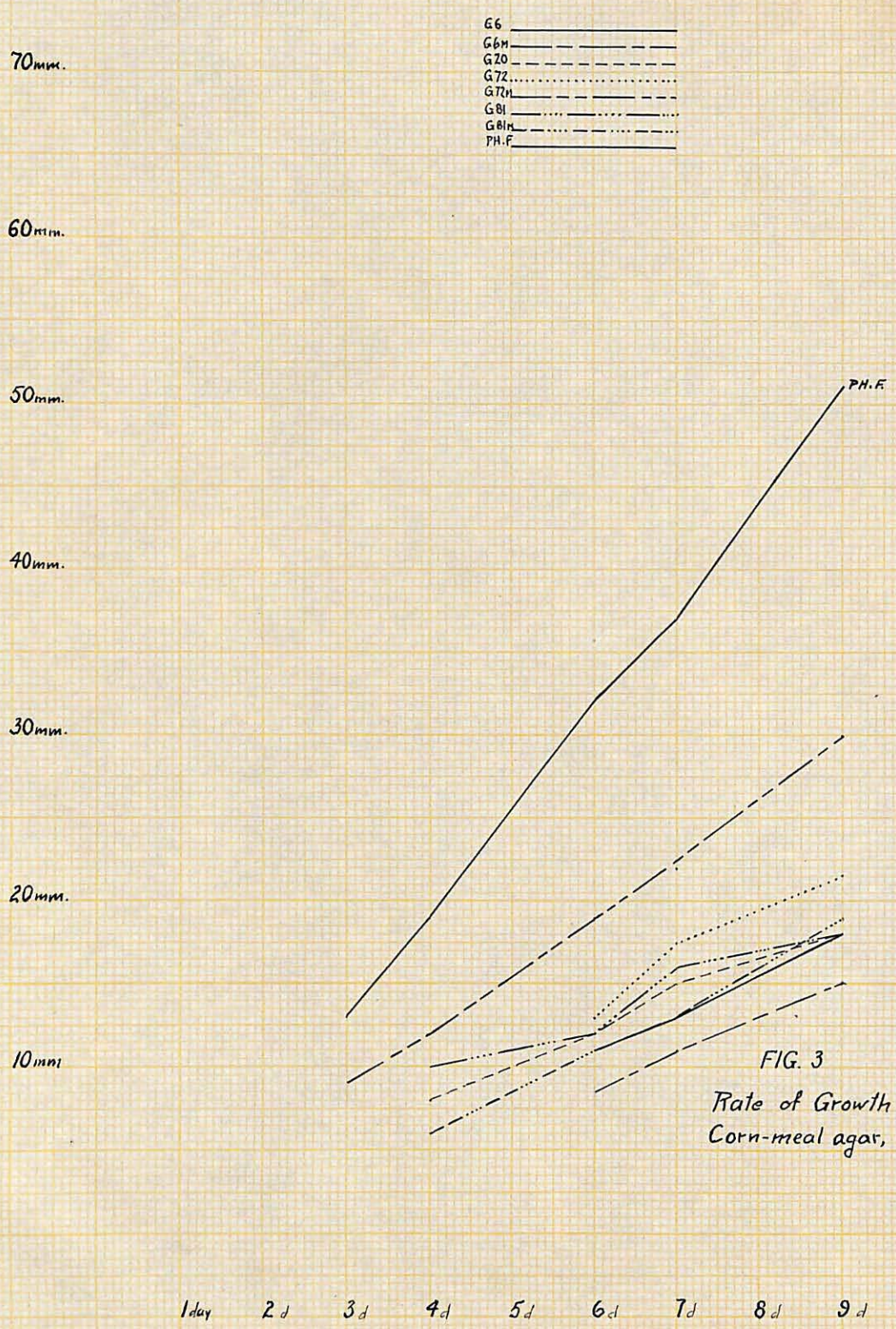

FIG. 3
Rate of Growth
Corn-meal agar, 10°C.

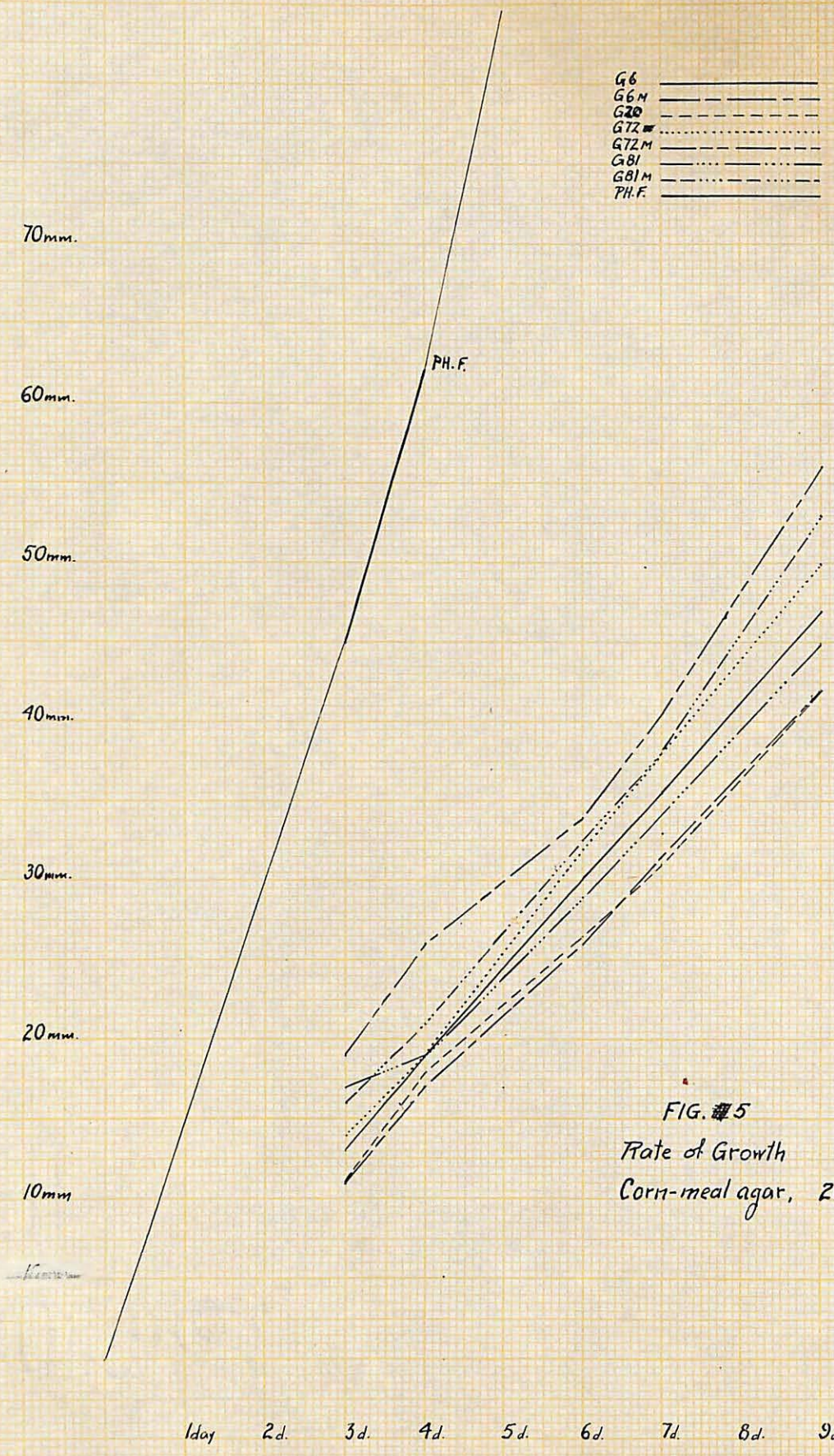

FIG. #5
Rate of Growth
Corn-meal agar, 20°C.

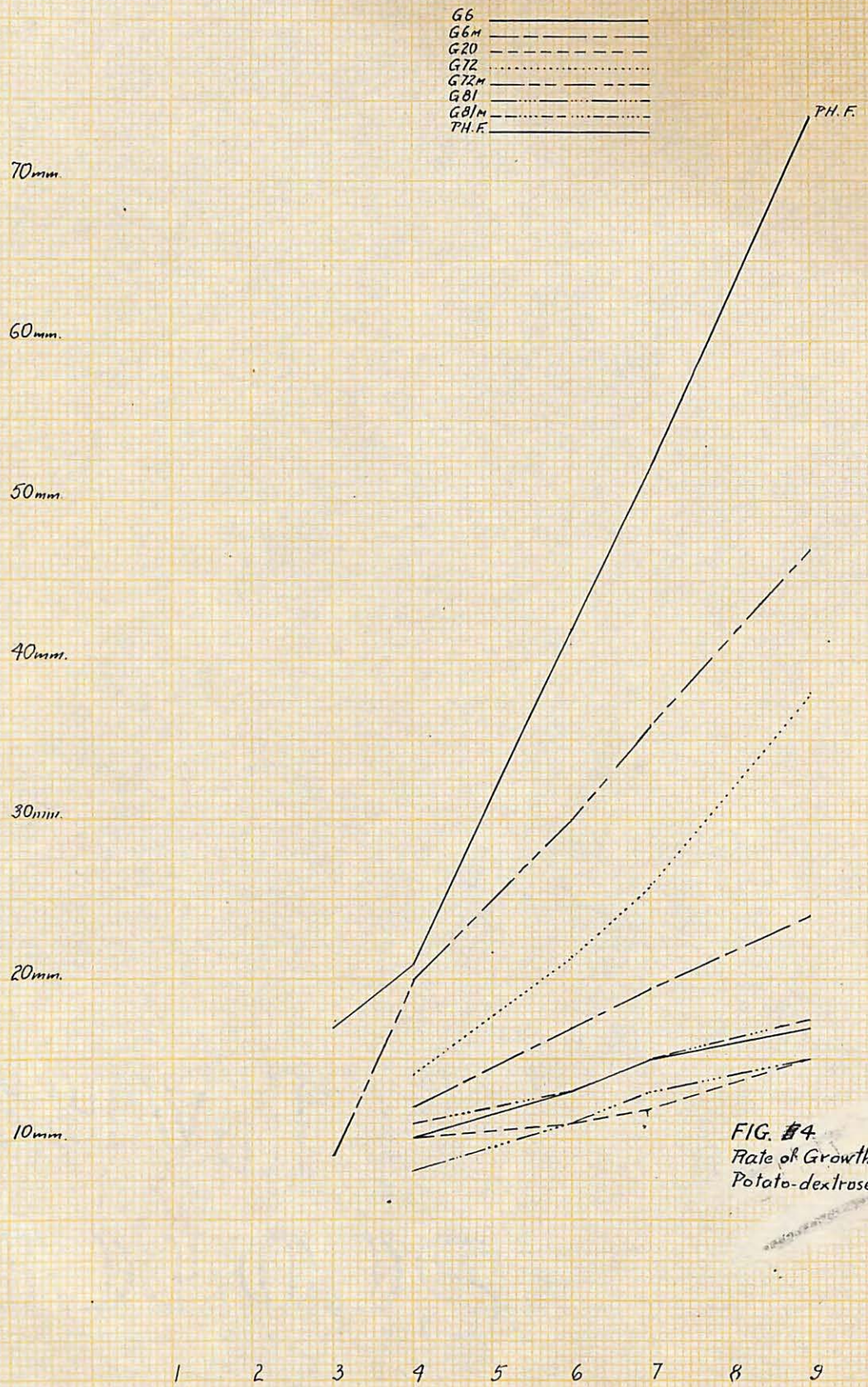

FIG. #4
Rate of Growth
Potato-dextrose 10°C.

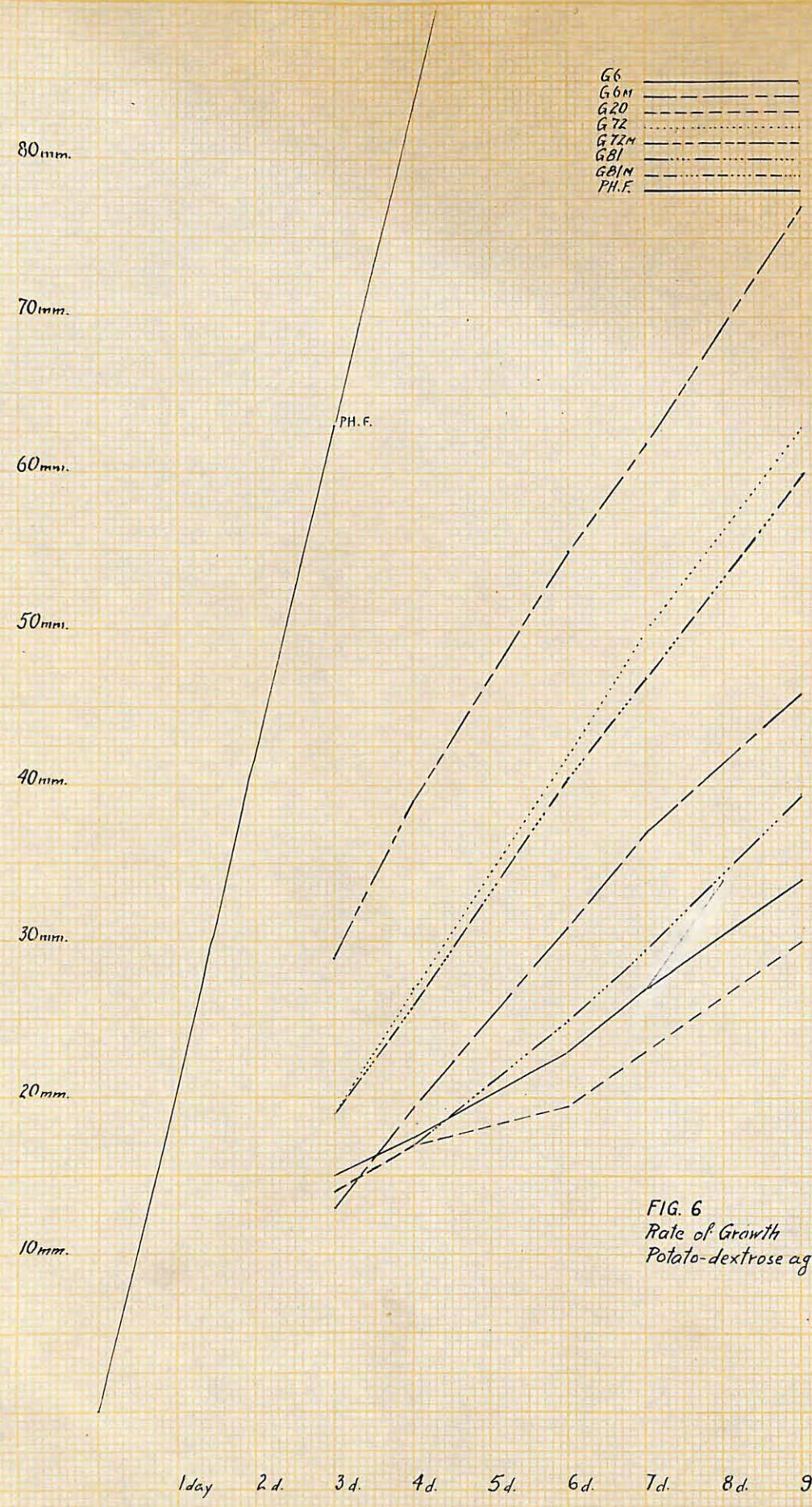

FIG. 6
Rate of Growth
Potato-dextrose agar 20°C.

Plate 1

Colonies of G6, and G6M at the end of nine days of growth at room temperature, on corn-meal agar (left) and potato-dextrose agar (right).

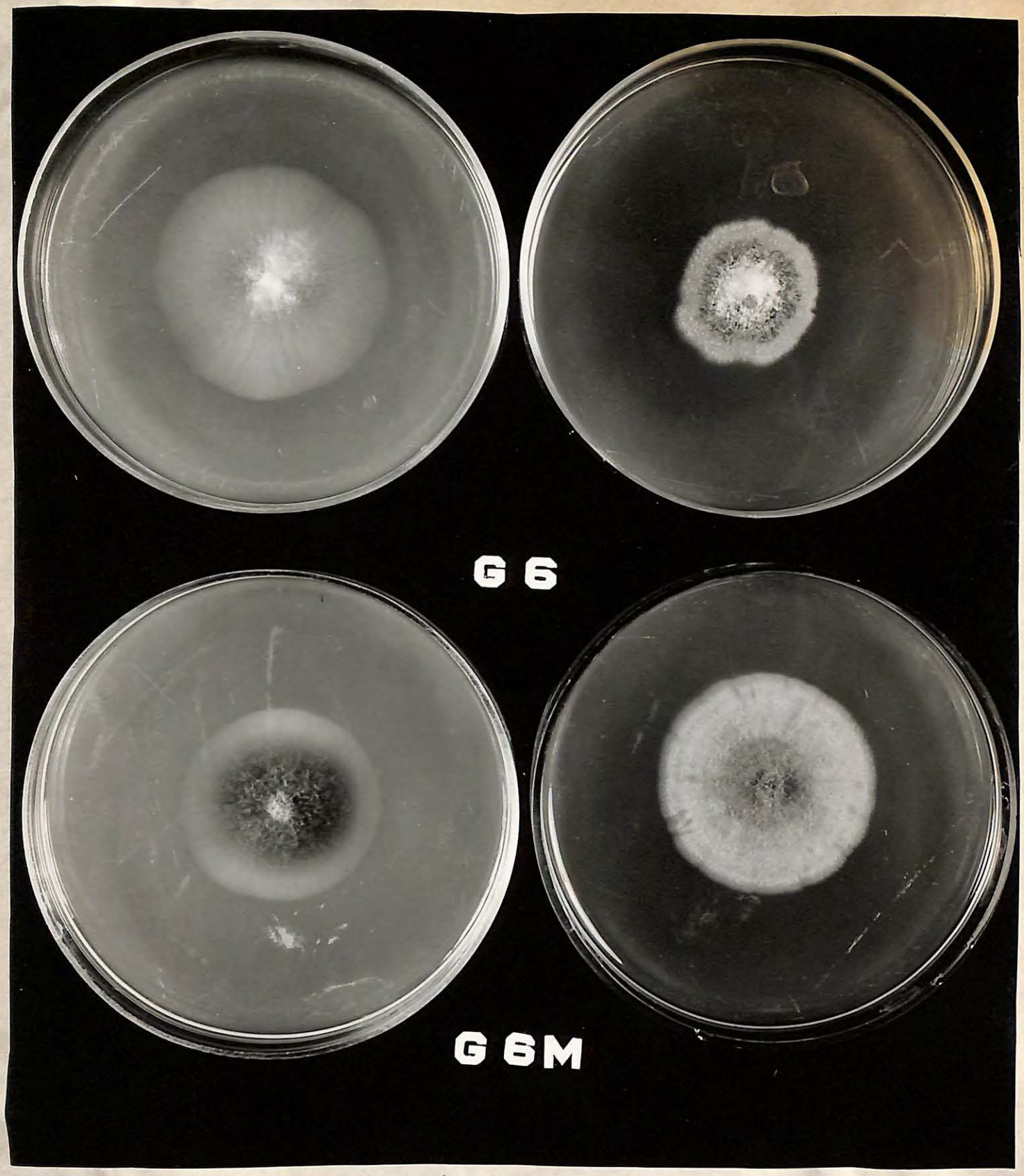

Plate 1

Plate 2

Colonies of G72 and G72M at the end of nine days of growth at room temperature, on corn-meal agar (left) and potato-dextrose agar (right).

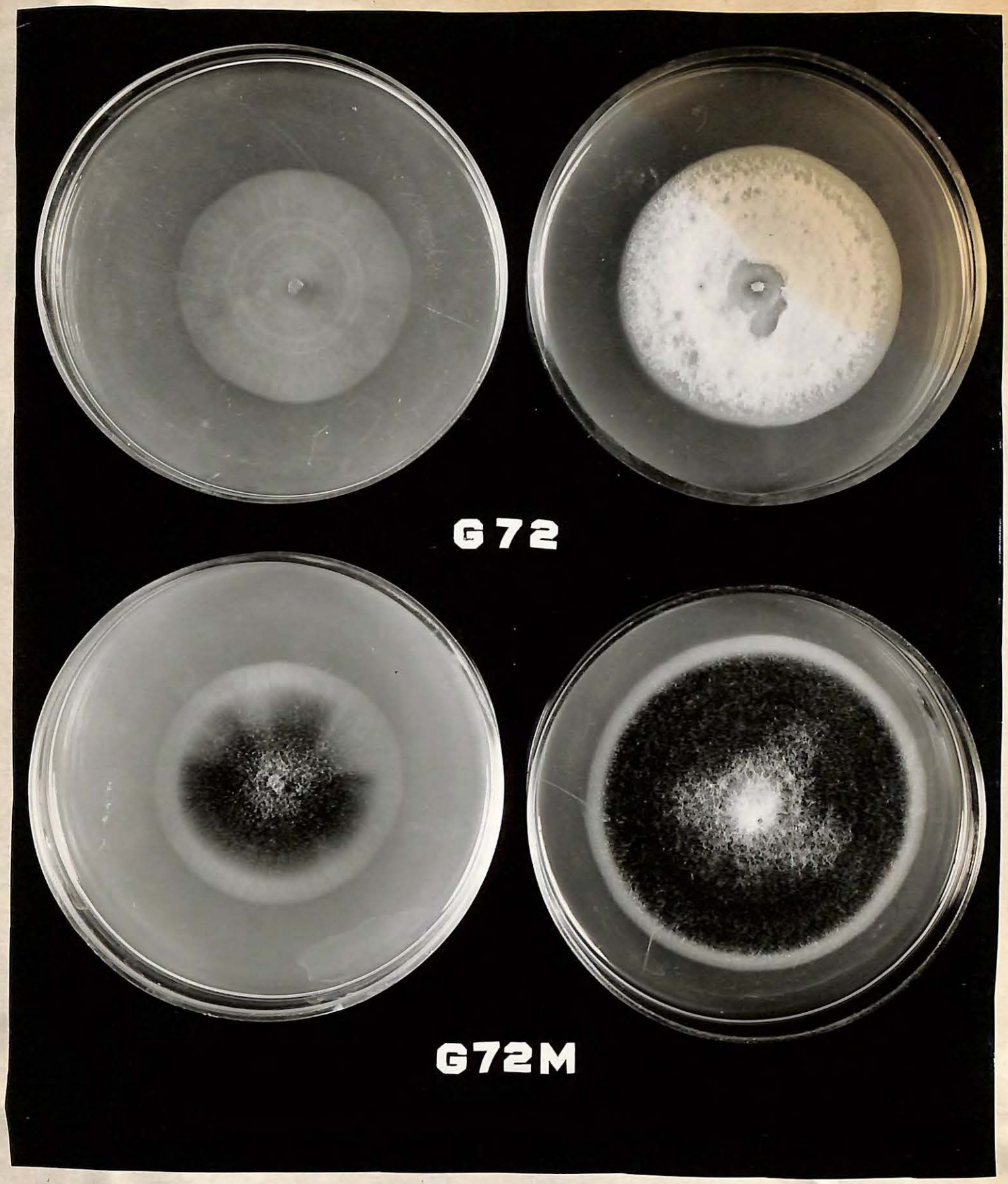

Plate 2

Plate 3

Colonies of G81 and G81M at the end of nine days of growth at room temperature on corn-meal agar (left) and potato-dextrose agar (right).

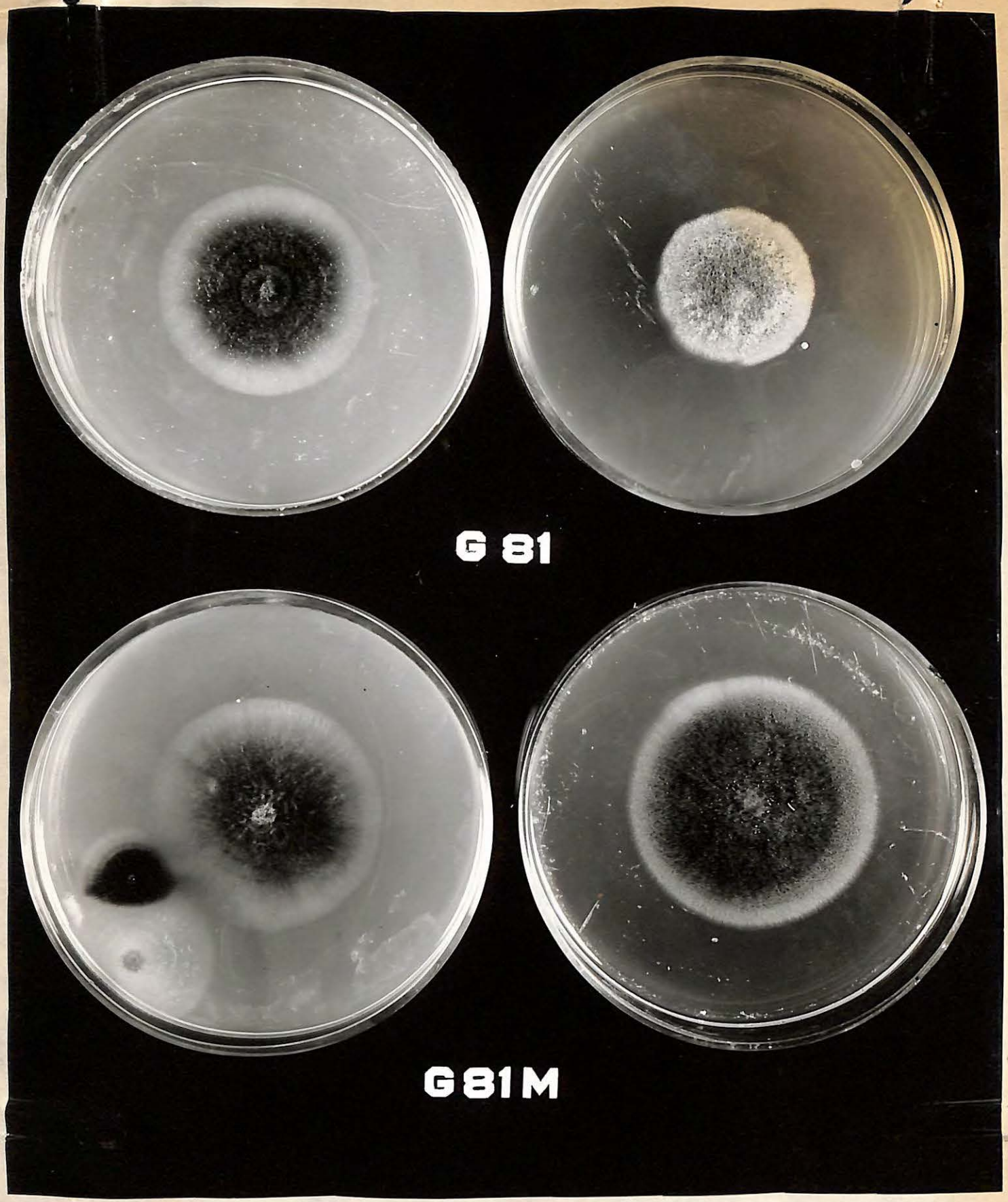

Plate 3

Plate 4

Colonies of G20 and PH. F. at the end of nine days of growth at room temperature on corn-meal agar (left) and potato-dextrose agar (right).

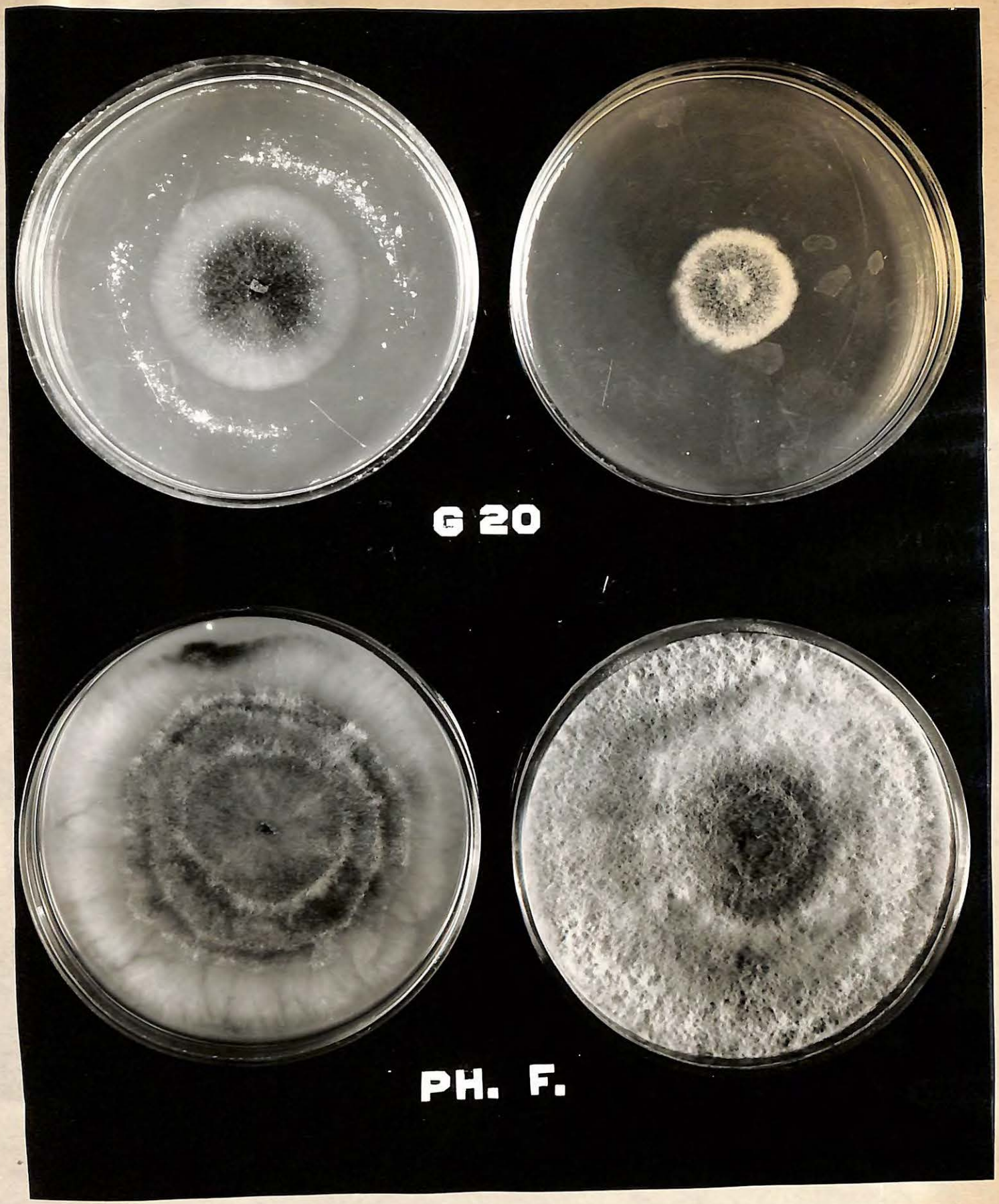

Plate 4

Plate 5

Effect of light on pycnidial production by G81.

The colony ~~above~~ marked A was kept continuously in the dark while the one below was grown under natural, light conditions.

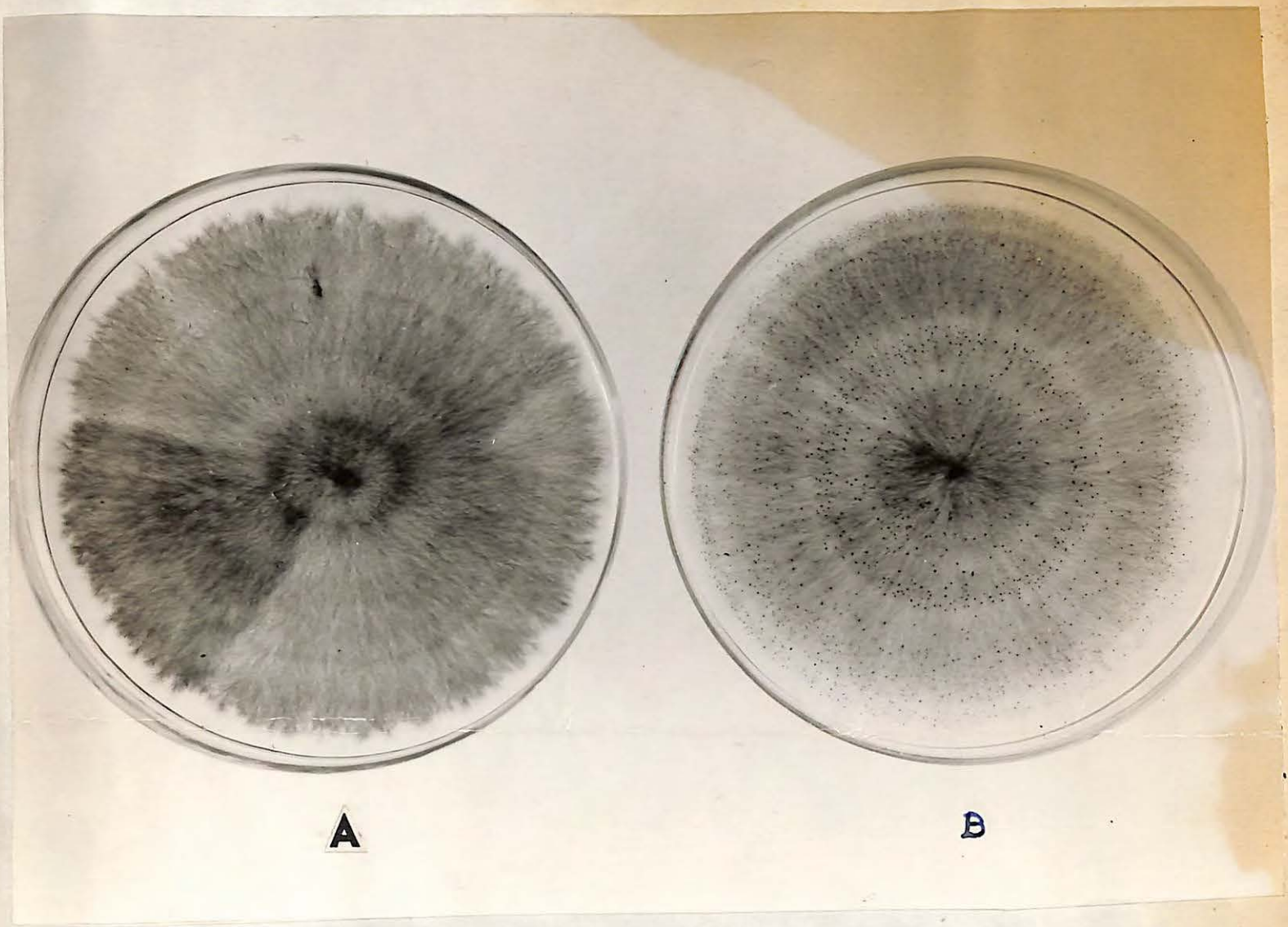

Plate 5
~~Fig. 1~~ .- Clone G81. A. Culture kept in the dark for fourteen days did not develop pycnidia. B. Culture of the same age as that in A showing abundance of pycnidia developed under normal light conditions.

Plate 6

Effect of ultra-violet irradiation on G72. The half of the colony marked + was irradiated for one minute. Pycnidial production is greatly increased and a brown pigment develops in the mycelium as a result of irradiation.

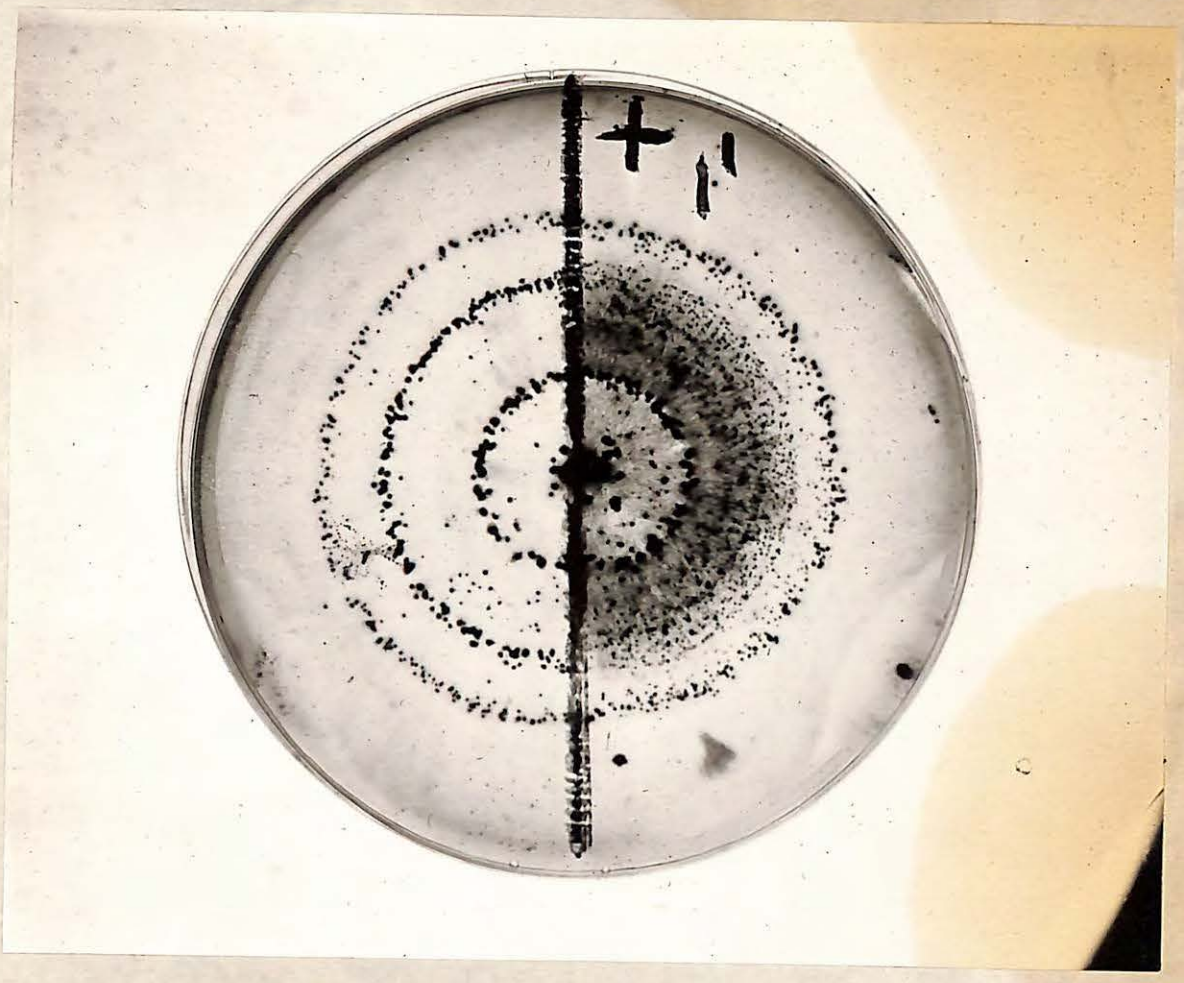

Plate 6

PLATE 7

1. Mycelium of G6
2. Mycelium of G6M
3. Mycelium of PH. F.
4. Mycelium of G72
5. Mycelium of G72M
6. Mycelium of G81
7. Mycelium of G81M

X 1150