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# SPECIES OF STIGMINA AND STIGMELLA OCCURRING ON PLATANUS[1,2,3]

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## INTRODUCTION

THE FOUR OR FIVE species of *Platanus*, or plane trees, commonly known as "sycamores" in the United States, occur widely throughout North America, Europe, and Asia. These trees are notable for their picturesque beauty in their natural habitats and are widely planted for use as ornamentals on lawns and along streets. Their foliage, however, is subject to attack by various fungi, among which are species of *Stigmina* and *Stigmella*. These closely related conidial fungi possess oval to oblong brown spores, whose distinguishing characteristics are the transverse septa in those of *Stigmina* sp. and the muriform septa in those of *Stigmella* sp. These fungi have been reported to cause the production of lesions on the leaves of *P. orientalis* L., *P. occidentalis* L., and *P. racemosa* Nutt. The diseases which they cause are not of major importance, for they do not seriously threaten the destruction of the trees. In certain localities, however, and in certain seasons, the leaf spots are conspicuously abundant, and affected trees are prematurely defoliated.

Most plant pathologists and mycologists who have collected specimens of *Stigmina* and *Stigmella* on *Platanus orientalis, P. occidentalis,* and *P. racemosa* have regarded the pathogens as one and the same species, that is, *Stigmina Platani* (Fckl.) Sacc. But, when this study was begun in December, 1935, at the University of California Citrus Experiment Station, it soon became apparent that the identity and nomenclature of these fungi were in a confused state. For this reason, a comparative study of *Stigmina* and *Stigmella* on *Platanus* was undertaken.

## ORGANISMS INVOLVED

Three related but distinct fungi have been shown to be involved in these diseases: *Stigmella Platani-racemosae* Dearn. and Barth. *apud* Dearn. on *Platanus racemosa,* in California; *Stigmina Platani* (Fckl.)

[1] Received for publication October 28, 1940.

[2] Paper no. 438, University of California Citrus Experiment Station, Riverside, California.

[3] The major part of this paper is based on a thesis by the senior author submitted in partial fulfillment of the requirements for the degree of Master of Science, University of California, 1937. (Typewritten.) Copy on file in the Library of the University of California, Berkeley. The present paper includes further research undertaken, after the thesis was completed, in coöperation with the junior author.

[4] Graduate student at the Citrus Experiment Station, 1935 to 1937.

[5] Associate in the Experiment Station; retired July 1, 1941.

Sacc. on *P. orientalis,* in Europe; and a species of *Mycosphaerella* on *P. occidentalis,* in the southeastern and southern central United States.

The third fungus was described as a new species by Wolf (*24*)[6] and named *Mycosphaerella Stigmina-Platani,* in the belief that it was the perithecial stage of *Stigmina Platani.* This *Mycosphaerella* has an unnamed polymorphic conidial stage, some of whose conidia are typical of the genus *Stigmina,* others of *Cercospora,* and still others intermediate in shape between these two types. Polymorphism in the conidial stage is indicated by Wolf (*24,* fig. 8). The conidial stage of *Stigmina Platani* does not show polymorphism. The evidence from pathogenicity, appearance of the disease, physiological characteristics, and morphology of the conidial stage, discussed later in the paper, indicates that the conidia of the *Mycosphaerella* on *Platanus occidentalis* are distinct from those of *Stigmina Platani* and that the *Mycosphaerella* described by Wolf (*24*) is apparently not the perithecial stage of *Stigmina Platani.*

It seems advisable, therefore, to reject the name *Mycosphaerella Stigmina-Platani* Wolf as untenable, according to the *International Rules of Botanical Nomenclature* (*9*), which states:

> Art. 64. A name of a taxonomic group must be rejected if the characters of that group were derived from two or more entirely discordant elements, especially if those elements were erroneously supposed to form part of the same individual.

In its place, the name *Mycosphaerella polymorpha* is proposed, with the following description:

> *Mycosphaerella polymorpha* n. n.
> *Mycosphaerella Stigmina-Platani* Wolf (*24,* p. 60–61) *nomum confusum.*

Perithecia in vernale in putrescentibus foliis efformantia, hypophylla per totum folium dense dispersa, punctiformia, nigra, erumpenti-immersa, sphaeroidea, 65–85 $\mu$ diam.; ascis sacciformibus, fasciculatis, octosporis, aparaphysatis, 55–70 × 9–11 $\mu$; sporidiis biseriatis, loculis inaequalibus, loculo superiore crassiore, hyalinis, rectis vel curvulis, 8–19 × 4–7 $\mu$.

Spermogoniis autumno efformantibus, numerosis, hypophyllis, innatoprominulis, paginis inferioribus ex toto vel in maculis exaridis occupantibus, ovatis vel globosis, nigris, 55–65 $\mu$; spermatiis bacilliformibus, 2–3 × 1 $\mu$, hyalinis.

Hab. in foliis dejectis *Platani occidentalis.*

Status conidicus: Statum conidicum *Stigmina polymorpha* n. n. sistit. Caespitulis hypophyllis, atris, primo maculicolis deinde subeffusis; conidiis polymorphicis, stigminoideis vel cercosporoideis, 14–70 × 3–11 $\mu$, intense olivaceis, 1–8-septatis, non-constrictis; conidiophoris fasciculatis, fuscidulis. Hab. stato conidico non modo in pagina inferiore *Platani occidentalis* parasitico sed in foliis vivis *Platani racemosae, P. Wrightii,* atque *P. acerifoliae,* in Amer. bor.

There remains, as will be indicated in this report, the possibility that the pathogen *Mycosphaerella polymorpha* on *Platanus occidentalis* has

[6] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

been confused with *M. platanifolia* (Cke.) Wolf. *M. platanifolia* was first described from leaves of *P. occidentalis* by Cooke (*3*) as *Sphaerella platanifolia,* and its conidial stage is stated by Wolf (*24* and *25*) to be *Cercospora platanicola* Ellis and Everhart (*5*).

Whether the organism identified by Saccardo (*20*) as *Stigmina Visianica* Sacc. is correctly named, has been questioned by Bubák (*2,* p. 219), who says:

> Bei einem amerikanischen Exemplare dieses Pilzes (Claremont bei Los Angelos in Süd-Kalifornien, *Platanus racemosa,* leg. Baker) finde ich sehr oft auch Sporen mit einer Längswand, so dass die Unterschiede zwischen *Stigmella* und *Stigmina* nicht allzu fest sind.
>
> Die zweite europäische Art *Stigmina Visianica* Sacc. ist von *St. Platani* nicht verschieden. Schon Lindau . . . . weist auf diesen Umstand hin. Ich finde die Sporen bei beiden genannten Arten sehr variabel und gleich gross. Demnach ist *Stigmina Visianica* Sacc. nur ein Synonym zu *Stigmina Platani* (Fuckel) Saccardo.

A specimen from the Farlow Herbarium, bearing the notation "*Stigmella Visianica* Sacc.? if it is different from *Stigmina Platani* f. Ellis. Pine Hills, Illinois, September 1884," was examined by the writers and found to be similar to the fungus on *Platanus occidentalis,* herein identified as *Mycosphaerella polymorpha.*

## HISTORY AND DISTRIBUTION OF THE FUNGI

The name *Stigmina Platani* was employed by Saccardo in 1878 (*18*) and in 1880 (*19*) for an organism called, as early as 1815, *Puccinia Platani* Bivona. Later, Saccardo (*20*) used the name *Stigmina Platani* also to replace that of *Stigmella Platani* Fuckel, employed by Thümen (*22*) for the same organism. Specimens of this fungus had been sent from Greece to Thümen and transmitted by him to Fuckel, who, in 1873, named the fungus *Stigmella Platani.* That it occurs elsewhere in Europe is evidenced by the fact that Saccardo (*20*) records it from Germany, Bubák (*2*) from Tirol (Austria) and Istria (Italy), and Nattrass (*13*) from Cyprus.

The fungus now known as *Stigmella Platani-racemosae* was stated by Harkness (*7*), who collected it on *Platanus racemosa* near Niles, in Alameda County, California, to have been present in California as long ago as 1885; and its presence in this state was mentioned by McClatchie (*12,* p. 376) in 1897. Both Harkness and McClatchie called it *Stigmina Platani,* however, in the belief that it was identical with the organism occurring in Europe on *P. orientalis.* Apostolides (*1*), in 1929, studied this disease in California and also identified the causal fungus as *Stigmina Platani.* In the same year, Dearness and Bartholomew (Dearness, *4*) described this pathogen as *Stigmella Platani-racemosae,* recognizing

that the leaf-spot fungus on *P. racemosa* was distinct from *Stigmina Platani* and basing their description upon specimens collected at Riverside, California. Specimens in the Claremont College herbarium (Baker's collection no. 3956), identified as *Stigmina Platani* on *P. occidentalis,* were examined by the writers and found, also, to be *Stigmella Platani-racemosae* on *P. racemosa.*

To date, *Stigmella Platani-racemosae* is not known to occur outside of California. Through correspondence with interested collectors, the writers have determined that the fungus is present in the following counties in southern California: San Diego, Los Angeles, Orange, Riverside, San Bernardino, Santa Barbara, and Ventura. It has not been collected in the northern part of the state except by Harkness (*7*).

The fungus *Mycosphaerella polymorpha* is of widespread occurrence on *Platanus occidentalis* in the southeastern and southern central United States, especially in the valleys of the lower Ohio and lower Mississippi rivers. Among the early records of the occurrence of this fungus (under different names) is that of Jennings (*10*) in 1890, in Texas; Tracy and Earle (*23,* p. 116) in 1895, in Mississippi; Patterson (*14,* p. 31) in 1902, in Illinois. Later, Hoffer (*8*) and Pipal (*15*) recorded its occurrence in Indiana. And in 1925, Martin (*11,* p. 380) reported that the conidial stage of this organism on *P. occidentalis* had been collected in Arkansas, Georgia, Illinois, Indiana, Iowa, Louisiana, Mississippi, Missouri, North Carolina, Oklahoma, Texas, and West Virginia.

The writers have examined specimens of *Mycosphaerella polymorpha* from Arkansas, Illinois, Mississippi, Missouri, North Carolina, and Oklahoma, and have found them all to be specifically identical and distinct from *Stigmina Platani* from the Old World and also from *Stigmella Platani-racemosae* from California.

## MATERIALS USED

The materials used in these studies were from many different sources. Herbarium specimens of leaves were generously loaned by Claremont College, Claremont, California, and by Dr. D. S. Welch, of Cornell University. Herbarium specimens and information were provided by the Farlow Library and Herbarium, Harvard University; by the University of California Herbarium, Berkeley; and by the University of California Citrus Experiment Station, Riverside. Freshly pressed leaves of *Platanus occidentalis* affected by *Mycosphaerella polymorpha* were received from Dr. Frederick A. Wolf of Duke University; and leaves of *P. orientalis* affected by *Stigmina Platani* were received from Dr. R. M. Nattrass, Mycologist, Nicosia, Cyprus. Leaves of *P. racemosa* affected by *Stigmella Platani-racemosae* were collected by the writers in California.

The species of *Platanus* used in the inoculation experiments were: (1) *P. orientalis,* trees grown from seed sent by Professor P. Th. Anagnostopoulos, Superior School of Agriculture, Athens, Greece; (2) *P. Wrightii* S. Wats., trees grown from seed and cuttings from Dr. R. B. Streets, University of Arizona; (3) *P. occidentalis,* trees grown from cuttings from Dr. Carroll W. Dodge of the Missouri Botanical Garden, and trees from local nurseries; (4) *P. acerifolia* Wild (hybrid), trees from local nurseries; and (5) *P. racemosa,* trees from local nurseries and trees growing on the Citrus Experiment Station campus.

## APPEARANCE OF THE DISEASES

Both macroscopic and microscopic differences may be employed in distinguishing these diseases.

*Macroscopic Appearance.*—Leaf-spot disease on *Platanus racemosa,* caused by *Stigmella Platani-racemosae* (fig. 1, *A–C*), is manifested by the presence of small, effuse, black-colored areas, 1 to 3 mm in diameter, on the lower surfaces of the leaf blades and on the stipules. These areas generally increase in diameter to about ½ cm, but the entire lower leaf surface may become blackened because of numerous secondary infections. The blackening is produced by the abundance of conidiophores and conidia. If the lesions are widely scattered, the spots may gradually enlarge to 1 cm in diameter. The leaf tissues immediately above the fungus (fig. 1, *A*) are at first yellow, but later become brown and necrotic. The margins of the spots are usually definite, irregular, and surrounded by green tissue.

Lesions on *Platanus orientalis* caused by *Stigmina Platani* (fig. 1, *F*), as observed on herbarium material from Cyprus, are very similar in general appearance to the spots on *P. racemosa* caused by *Stigmella Platani-racemosae.* The two diseases can best be distinguished by comparative microscopic examination of the conidiophores and conidia (see "Microscopic Appearance," below.)

Lesions produced by the conidial stage of *Mycosphaerella polymorpha* are at first pale-green, indefinitely limited areas, if viewed from the upper leaf surface. Thin, weftlike gray stippled areas (fig. 1, *D*) cover the corresponding areas on the lower leaf surface. When the disease has progressed to the extent that a large proportion of the upper leaf surface is pale green, the entire lower leaf surface may be invested with an effuse gray coating (fig. 1, *E*) of conidia and conidiophores. At this stage, which may have developed by midsummer, the trees will appear blighted, and defoliation will have begun.

*Microscopic Appearance.*—Sections of lesions caused by *Stigmella Platani-racemosae* on *Platanus racemosa* show the fungus to be localized

at first in the stomatal chambers (fig. 2, *I*). Later, hyphae are produced that ramify between the cells; these may extend throughout the tissues to the upper epidermis. Mycelia and haustoria were not found within the cells when sought in paraffin sections or in freezing microtome sec-

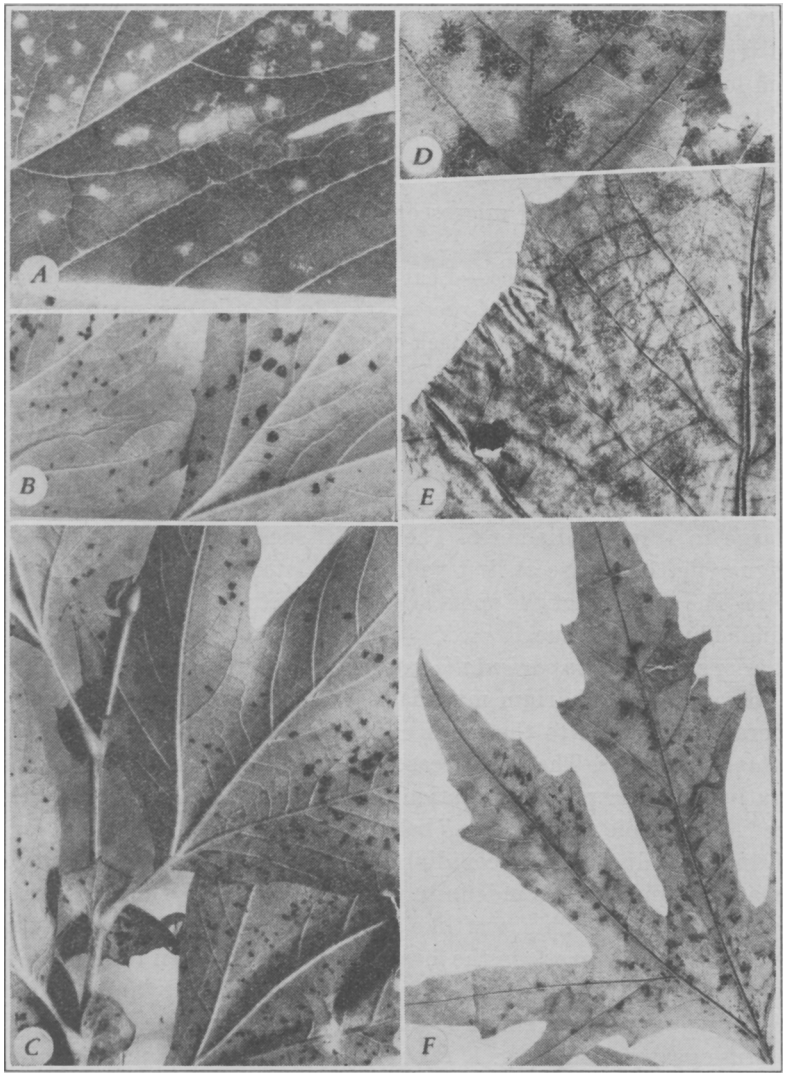



Fig. 1.—Natural infection on leaves of *Platanus* spp.: *A–C, Stigmella Platani-racemosae* on *P. racemosa—A,* showing as spots on upper leaf surface; *B,* on lower leaf surface; *C,* on lower leaf surface and on stipules. *D, E, Mycosphaerella polymorpha* on lower leaf surfaces of *P. occidentalis. F, Stigmina Platani* on lower leaf surface of *P. orientalis* (from Cyprus).

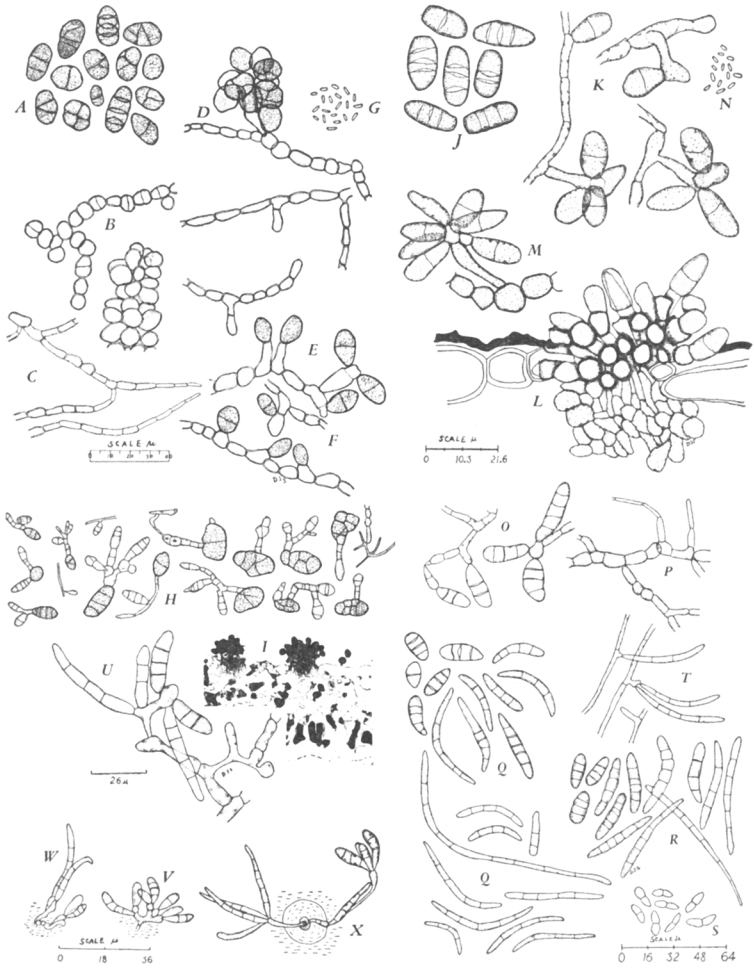



Fig. 2.—Camera-lucida drawings of spores and mycelium of species of *Stigmina* and *Stigmella* on *Platanus* spp.

*Stigmella Platani-racemosae* from *Platanus racemosa*, *A–I*: *A*, conidia; *B*, mycelium thick, dark and massed; *C*, mycelium hyaline and slender; *D*, attachment of spores to mycelium; *E*, *F*, mycelium, conidia, and conidiophores, showing spore formation; *G*, spermatia from pure culture; *H*, spore germination in drops of water on slides (germinating spores gave rise to additional spores, but attempt at repetition of these results was unsuccessful); *I*, section showing conidia and conidiophores growing out of stomatal chambers (× 305).

*Stigmina Platani* from *Platanus orientalis*, *J–N*: *J*, spores from type species; *K*, spores and mycelium produced on Czapek's-agar film on slide; *L*, section of living material; *M*, spores, showing attachment; *N*, spermatia from pure culture growing in leaf juice on filter paper.

*Mycosphaerella polymorpha* from *Platanus occidentalis*, *O–X*: *O*, *Stigmina*-type spores from culture from single-spore isolation of *Cercospora* type, growing in leaf juice on slide, after 21 days; *P*, mycelium from same culture; *Q*, *Cercospora*- and *Stigmina*-type spores from an infection on *P. racemosa* by an isolate from a single *Cercospora* spore, after 56 days; *R*, dark-colored, subhyaline spores of *Stigmina* and *Cercospora* types from culture from single-spore isolation of *Stigmina* type on Czapek's agar; *S*, ascospores from a dead leaf; *T*, *Cercospora*-type spores from Czapek's-agar film on slide, after 21 days; *U*, sporulation, on slide, of growth from a single *Cercospora* spore, showing variation in spores attached to a conidiophore; *V–X*, spores from artificial inoculation on leaf of *P. acerifolia* (the culture used in this inoculation was from a single *Cercospora*-type spore from a single-spore-culture inoculation that had fruited only *Cercospora*-type spores)—*V*, showing variation in spores attached to a conidiophore; *W*, different types of spores attached to single hypha; *X*, mycelial growth from base of a leaf hair and two types of spores.

tions stained with the differential stains, safranin light-green, triple stain, or iron-alum—hemotoxylin, applied according to methods outlined by Rawlins (*16*). Conidia are produced singly on conidiophores from fascicles projecting through the stomata. Affected leaf tissue shows fewer chloroplasts than normal tissue.

A study of freehand sections of leaf spots caused by *Stigmina Platani*

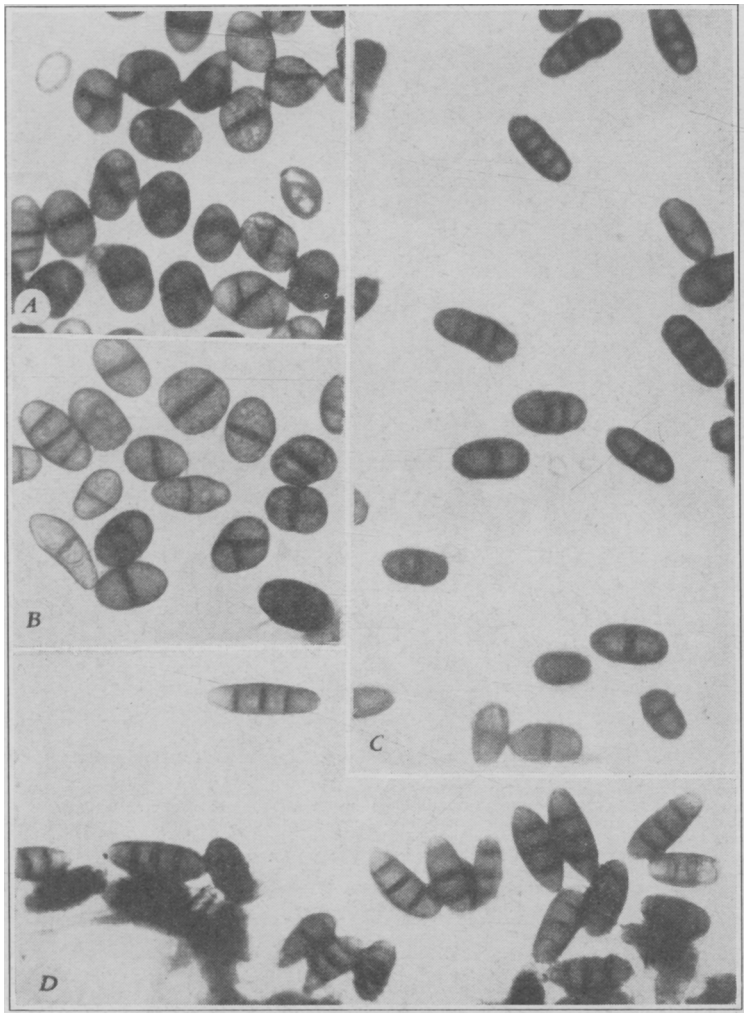



Fig. 3.—Photomicrographs of conidia of the three species of fungi: *A, B, Stigmella Platani-racemosae* from natural infection on *Platanus racemosa*, Riverside, California; *C, Stigmina Platani* from *P. orientalis* from Nicosia, Cyprus; *D, Stigmina* sp. (*Mycosphaerella polymorpha*) from Fungi Columbiana no. 2885 on *P. occidentalis* collected at Rogers, Arkansas. (All × 667.)

on *Platanus orientalis* (fig. 2, *L*) and by *Mycosphaerella polymorpha* on *P. occidentalis* indicated that the relations of the pathogens to the diseased tissues in these species are similar to those found in *P. racemosa.*

The conidia of the three fungi, while somewhat similar in appearance, are sufficiently distinct to suggest three different species. The *Stigmina* type of conidia are oblong, dark-colored, septate bodies (fig. 3, *C*). The *Stigmella* type differed in having numerous irregular septations (fig.

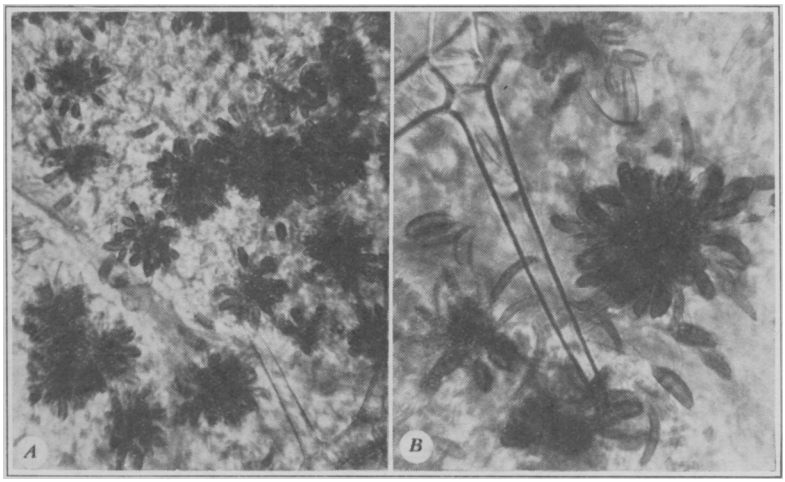



Fig. 4.—Fruiting of fungus on leaf of *Platanus racemosa,* from artificial inoculation with conidial spores of *Mycosphaerella polymorpha* taken from a diseased leaf of *P. occidentalis* from North Carolina: *A, Stigmina-* and *Cercospora*-type conidia shown in the same spore clusters (×149); *B,* fruiting of fungus (×305).

3, *A, B*). The conidia of *Mycosphaerella polymorpha* were variable in size and shape, as illustrated in figure 4 and in figure 2, *Q, R, T,* but often the *Stigmina* type (fig. 3, *D*) were the only ones to be found.

## MORPHOLOGICAL COMPARISON OF THE THREE FUNGI

Conidial spores of the three fungi, *Stigmella Platani-racemosae, Stigmina Platani,* and *Mycosphaerella polymorpha,* taken directly from their respective primary hosts, show wide variation in size and septation (table 1 and fig. 2). While individual spores cannot always be identified with certainty as belonging to one or the other of these three species, conidia of each species, en masse, in spite of variations in septation and size, are sufficiently distinctive to make identification possible.

Conidia of *Stigmella Platani-racemosae* (figs. 2, *A,* and 3, *A, B*) are ovate to oblong, 10–22 × 7–13 $\mu$. Septations range from one to three cross

TABLE 1

COMPARATIVE SIZE AND SEPTATION OF SPORES OF SPECIES OF *Stigmella* AND *Stigmina* FOUND ON *Platanus* SPP.

| Species and source | Predominant type of spore* | Spores measured | Average | | | Minimum and maximum | | | Irregularly septate cells† |
|---|---|---|---|---|---|---|---|---|---|
| | | | Cells | Length | Width | Cells | Length | Width | |
| | | *number* | *number* | *microns* | *microns* | *number* | *microns* | *microns* | *number* |
| *Stigmella Platani-racemosae* from California | *Stigmina* | 35 | 3.48 | 16.84 | 10.64 | 1-5 | 10-22 | 7-13 | 9 |
| *Stigmina Platani* from Cyprus | *Stigmina* | 50 | 2.94 | 18.77 | 8.75 | 2-4 | 14-24 | 7-11 | 1 |
| *Stigmina* sp.‡ from North Carolina | *Stigmina* | 51 | 4.40 | 22.24 | 8.91 | 3-9 | 14-45 | 7-11 | 2 |
| *Stigmina* sp.‡ from North Carolina | *Cercospora* | 52 | 5.23 | 47.78 | 4.54 | 3-7 | 17-70 | 3- 6 | 0 |
| *Stigmina Visianica*‡ from Illinois | *Stigmina* | 35 | 5.23 | 24.21 | 8.55 | 2-6 | 13-34 | 7-10 | 1 |
| *Stigmina Visianica*‡ from Illinois | Intermediate | 6 | 5.83 | 34.00 | 7.46 | 4-7 | 26-43 | 6- 9 | 0 |

* *Stigmina*-type spores are oblong, dark-colored, and septated (figs. 2, *O*, and 3, *D*); *Cercospora*-type spores are crescent-shaped, septated, and nearly hyaline (fig. 2, *T*); the "intermediate" type are those intermediate in shape between the *Stigmina* and *Cercospora* types.

† Irregularly septate spores are those that have a cross wall and other septa at an angle to the cross wall (fig. 2, *A*)—a muriform type of septation characteristic of *Stigmella*.

‡ Now considered to be a conidial stage of *Mycosphaerella polymorpha*.

septa or from one to four diagonal or irregular septations. The abundant, irregularly septate spores distinguish this fungus from the fungi on *Platanus orientalis* (figs. 2, *J*, and 3, *C*) and on *P. occidentalis* (figs. 2, *O–T*, and 3, *D*).

Conidia of *Stigmina Platani* from *Platanus orientalis* are narrower, and some are longer than those of *Stigmella Platani-racemosae,* as indicated by the dimensions, 14–24 × 7–11 μ, given in table 1. These spores rarely show the irregular septa characteristic of *Stigmella.*

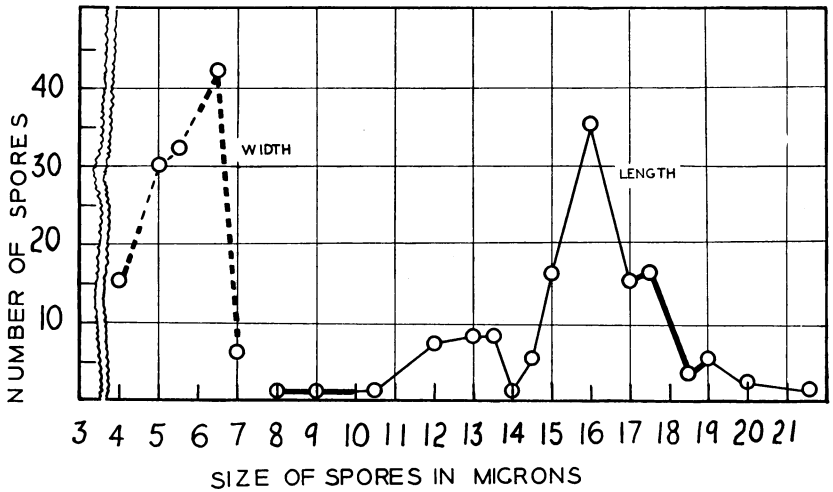



**Fig. 5.—Frequency-distribution curves of measurements of 125 ascospores of *Mycosphaerella polymorpha.*** Heavy lines indicate measurements corresponding with those of Wolf's (*24*) description of what he called *M. platanifolia* and *M. Stigmina-Platani.*

The *Stigmina*-type spores of *Mycosphaerella polymorpha* (figs. 2, *O,* and 3, *D*) are more elongate and have more acute ends than those of the two other species. They are oval, dark, thick-walled, and have been found to measure 14–45 × 7–11 μ (table 1). *Cercospora*-type spores are still more elongate, are light brown to dark brown in color, thinner-walled, and usually curved or crescent-shaped (fig. 2, *T*); they measure around 17–70 × 3–6 μ, although spores 120 μ in length have been found. Between these two types there are other spores intermediate in shape and size.

One hundred and twenty-five ascospores of *Mycosphaerella,* ejected from leaves into drops of distilled water suspended on the inside cover of a petri dish, were measured under an oil immersion; they ranged in size from 8–19 × 4–7 μ. (Ascospores from the same leaves were ejected onto agar media for culture studies; see p. 219.)

A frequency-distribution curve (fig. 5) shows a number of spores of an intermediate size that apparently bridges the gap between the spore

sizes of *Mycosphaerella polymorpha* and *M. platanifolia* (Cke.) Wolf (*24*). Wolf[7] states, however, that two population curves are shown here (fig. 5), and that measurements of a larger number of spores should confirm this.

## PATHOGENICITY

Inoculations of *Platanus* spp. with fungi of *Stigmella* and *Stigmina* spp. were made by the following procedures. (1) Conidia from the leaves or from pure cultures of the fungi were suspended in water. The fungus suspension was applied to the leaves and young shoots by means of either a camel's-hair brush or an atomizer. In nearly all of these tests, a paraffin-paper or cellophane bag or a bell jar was used as a covering to maintain moisture conditions favorable for infection. This protection also prevented fortuitous dissemination and spread of the fungi used in the inoculations. The paper bags (fig. 6, *C*) were not sealed but had their edges twice-folded and kept in place by means of paper clips. In preliminary experiments, it was found that injuries, such as perforations made with a pin, were not necessary for infection, for infections seldom occurred in the loci of injuries but were common between them. (2) Infected leaves were pinned to normal leaves, which were then covered with paraffin-paper or cellophane bags. This method was very satisfactory in moist weather. (3) Spores and bits of mycelium from single-spore cultures of each of the three organisms were placed on leaves enclosed in cellophane bags. Each of the three causal organisms was later recovered in pure culture from the artificially inoculated leaves. Results of the tests are given in table 2.

The fungus *Stigmella Platani-racemosae* caused infection and spread rapidly both on *Platanus Wrightii* (fig. 6, *E* and *F*) and on *P. racemosa* (fig. 6, *G–I*). So far as can be determined, this fungus has not previously been reported on *P. Wrightii*. It failed repeatedly, however, to produce infection when inoculated on *P. orientalis, P. occidentalis,* and *P. acerifolia* (hybrid trees).

Inoculations with *Stigmina Platani* (Cyprus strain) from *Platanus orientalis* resulted in infection on *P. orientalis* (fig. 6, *A*), but were ineffective on *P. racemosa, P. Wrightii, P. occidentalis,* and *P. acerifolia.*

Ascospores and conidia of both *Stigmina* and *Cercospora* types of *Mycosphaerella polymorpha* from *Platanus occidentalis* produced infection on *P. occidentalis* (fig. 6, *D*), *P. racemosa, P. Wrightii,* and *P. acerifolia,* but no infections developed on *P. orientalis.* All inoculations with cultures from single conidia of the *Stigmina* type of *Mycosphaerella polymorpha* produced some spots on which the *Cercospora*-type conidia were

[7] Wolf, F. W. In letter to the senior author dated October 17, 1938.

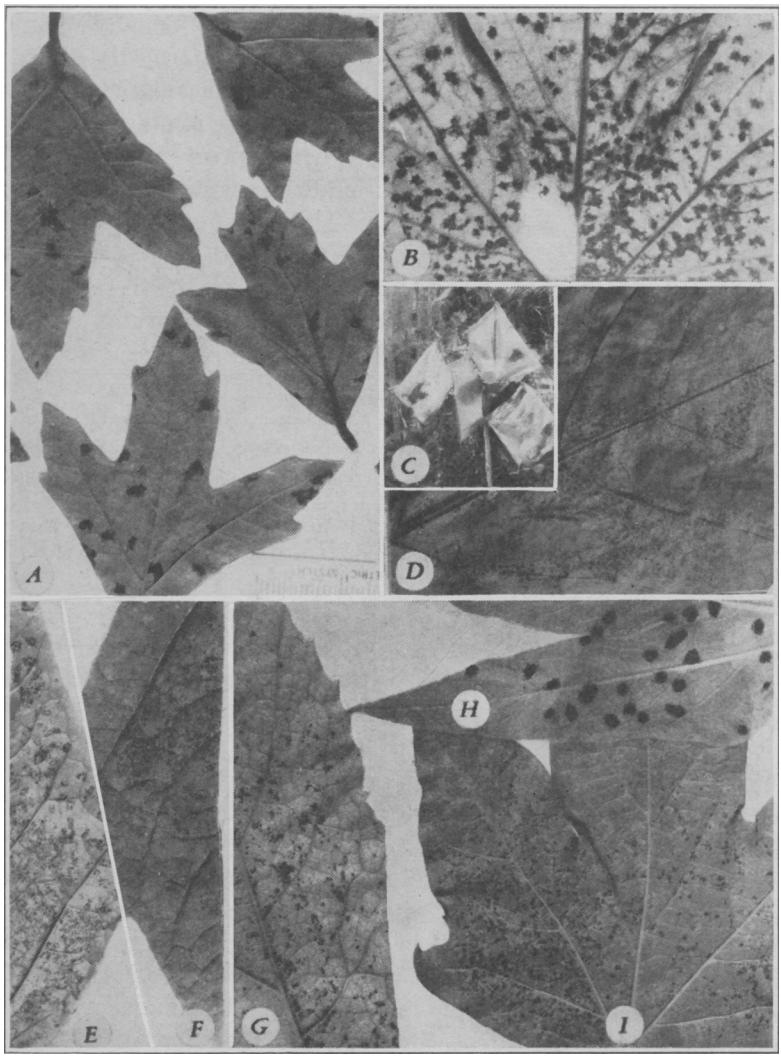



Fig. 6.—Artificial inoculations on species of *Platanus: A, Stigmina Platani* on young leaves of *P. orientalis; B, Mycosphaerella polymorpha* (from natural infection) on *P. racemosa; C,* inoculated leaves protected by paraffin-paper bags; *D, Mycosphaerella polymorpha* (culture from a single *Stigmina*-type spore isolated from *P. occidentalis* from North Carolina) on authentic leaf of *P. occidentalis,* where *Stigmina-* and *Cercospora*-type spores fruited in abundance; *E–I, Stigmella Platani-racemosae—E* and *F,* on upper and lower surfaces, respectively, of young leaf of *P. Wrightii,* after one month; *G,* on succulent leaf of *P. racemosa,* after one month; *H,* on normal leaf after three months; and *I,* after one month.

found. Most inoculations with *Cercospora*-type conidia produced some spots in which the *Stigmina*-type conidia were found. Usually a mixture of the two forms of conidia, together with conidia intermediate in form, was found on lesions resulting from single-spore inoculations. In some cases the fascicles bore *Stigmina*-type conidia only; in others, conidia of the *Cercospora*-type only; and in others, both types were formed on one and the same fascicle. Variations in conidia from single-spore cultures are shown in figure 2, *U–X* (p. 211).

TABLE 2

RESULTS OF INOCULATIONS OF *Platanus* SPP. WITH FUNGI OF *Stigmella* AND *Stigmina* SPP.

| Host | Single-spore inoculations | | | | | |
|---|---|---|---|---|---|---|
| | *Stigmella Platani-racemosae* from *Platanus racemosa* | | *Stigmina* sp.* from *Platanus occidentalis* | | *Stigmina Platani* from *Platanus orientalis* | |
| | Positive results | Negative results | Positive results | Negative results | Positive results | Negative results |
| *Platanus acerifolia* | 0 | 5 | 8 | 0 | 0 | 8 |
| *P. occidentalis* | 0 | 9 | 4 | 3 | 0 | 10 |
| *P. orientalis* | 0 | 7 | 0 | 5 | 3 | 1 |
| *P. racemosa* | 10 | 0 | 10 | 5 | 0 | 8 |
| *P. Wrightii* | 7 | 0 | 5 | 3 | 0 | 4 |

* Conidial stage of *Mycosphaerella polymorpha*.

The minimum time from inoculation to the beginning of sporulation was about three weeks. In the cooler spring months, the period of incubation was from four to five weeks.

When the surface of leaves atomized with conidia of *Stigmella Platani-racemosae* was stained with safranin, germ tubes entering the stomata could occasionally be found. When surface growth on very young spots was removed, the substomatal areas were observed to be darkened. The absence of darkening elsewhere indicated that the fungus growth was confined to the stomatal areas. Stained paraffin sections of very young spots also showed the fungus localized in the stomatal cavities, with no growth elsewhere (fig. 2, I, p. 211). Infection was often abundant on the lower surface of uninjured leaves of *Platanus racemosa* that had been atomized with a spore suspension. These microscopic observations indicate that the pathogen penetrates by way of the stomata. Similar results were obtained with *Stigmina Platani* and *Mycosphaerella polymorpha;* so that each fungus appears to have the same mode of entrance into the leaves and the same type of subsequent growth within the substomatal tissues.

If susceptibility to infection is an index of relationship, artificial inoculations indicate that *Platanus racemosa* and *P. Wrightii* are closely related and that *P. occidentalis* is more closely related to the American species of *Platanus* than to the European, *P. orientalis*.

## CULTURE STUDIES

*Methods and Media.*—The fungi were isolated in single-spore cultures by streaking suspensions of conidia on the surface of agar plates, where they could be observed in position under the high power of a binocular microscope; then by proper manipulation, single conidia could be picked up with a sharp needle and transferred to media in test tubes. Cultures from ascospores were obtained on agar in petri dishes inverted over moistened dead leaves, from the surface of which the perithecia protruded and discharged the spores onto the surface of the agar.

The media used, in the order of decreasing suitability for sporulation, were as follows: (1) *Platanus racemosa* leaf juice sterilized by filtration, (2) a special medium described by Smith and Smith (*21*) and made by the aseptic addition of an equal amount of the leaf juice to 4 per cent Czapek's agar, (3) Czapek's agar, (4) leaf-extract agar containing 3 per cent sucrose, (5) glucose potato agar, and (6) carrot plugs.

Filtered leaf juice was used both on slides supported on U-shaped glass rods in petri dishes and in Van Tieghem cells, for comparison of the sporulation of isolates from different types of spores. Living leaves were used for certain tests.

*Results.*—By these procedures, single-spore cultures of *Mycosphaerella polymorpha* were isolated as follows: 147 from *Cercospora*-type conidia, 165 from *Stigmina*-type conidia, and 76 from ascospores. Results of the tests are presented in table 3.

In other tests (not reported in table 3), 8 transfers from ascospore cultures of *Mycosphaerella polymorpha* onto Czapek's agar yielded 5 cultures that produced both *Stigmina*- and *Cercospora*-type spores, 1 culture that produced only *Cercospora*-type spores, and 2 that did not sporulate. Three isolates of *Cercospora*-type spores, when grown on Czapek's agar, yielded 2 cultures that produced only *Stigmina*-type spores and 1 that produced both *Stigmina*- and *Cercospora*-type spores.

Single-spore isolations of *Mycosphaerella polymorpha,* whether from conidia or from ascospores, produced on Czapek's agar two general types of colonies (fig. 7) about equal in number. One type was flat, light mouse gray to mouse gray (*17*); the other type was elevated and smaller in diameter than the first type under the same growing conditions. The color of the latter type was a similar gray, some colonies having white areas, however, and black margins (fig. 7). With age, a light-yellow

color sometimes appeared. When viewed from beneath, both types of colonies appeared dark olive-gray to olivaceous black (*17*). Colonies produced on glucose potato agar were usually much elevated and gray to black in color. On a basis of minor differences, the colonies could be

TABLE 3

SPORULATION OF *Mycosphaerella polymorpha* FROM SINGLE-SPORE ISOLATIONS IN CULTURE MEDIA AND ON LEAVES OF *Platanus racemosa*

| Type of spore isolated and medium inoculated | Total inoculations | Infections from different types of sporulation | | |
|---|---|---|---|---|
| | | *Cercospora*-type | *Stigmina*-type | Mixed, *Cercospora* and *Stigmina** |
| | *number* | *number* | *number* | *number* |
| *Cercospora:* | | | | |
| Living leaves (March, 1938) | 1 | 1 | 0 | 0 |
| Living leaves (April, 1938) | 1 | 1 | 0 | 0 |
| Living leaves (May, 1938) | 4 | 0 | 1 | 3C |
| Living leaves (June, 1938) | 9 | 3 | 1 | 5C |
| Living leaves (September, 1938) | 12 | 0 | 1 | 5C and 6S |
| Czapek's agar | 16†‡ | 7 | 0 | 8C |
| Leaf juice on slides | 3 | 2 | 0 | 1C |
| Leaf juice in Van Tieghem cells | 6 | 0 | 0 | 6C |
| *Stigmina:* | | | | |
| Living leaves (September, 1938): | 11 | 0 | 0 | 6C and 5S |
| Czapek's agar | 16† | 10 | 0 | 6C |
| Leaf juice in Van Tieghem cells | 6 | 0 | 0 | 6C |
| Ascospore: | | | | |
| Living leaves (May, 1938) | 15 | 10 | 0 | 5C |
| Czapek's agar | 74‡ | 72 | 0 | 0 |
| Leaf juice on slides | 8‡ | 1 | 0 | 5C |
| Leaf juice in Van Tieghem cells | 4‡ | 2 | 0 | 0 |

* The letters "C" and "S" indicate the type of spore (*Cercospora* or *Stigmina*, respectively) predominating.

† Isolates selected at random.

‡ Some of these inoculations failed to produce spores.

grouped into no less than fourteen different types. While occasional differences were noted in the size of spores produced by the different isolates, these variations could not be correlated with the kind of spore from which the culture originated.

Spermatia (fig. 2, *G* and *N*) of each of the three organisms developed both in cultures and on infected leaves. In cultures of *Mycosphaerella polymorpha,* dense mycelial masses developed containing these small bacilluslike bodies. But when these were streaked on nutrient media, no evidence of spermatial germination was obtained.

Of the 50 single-spore cultures of *Stigmella Platani-racemosae* made in these tests, 1 isolate produced a flat, spreading colony, when grown on

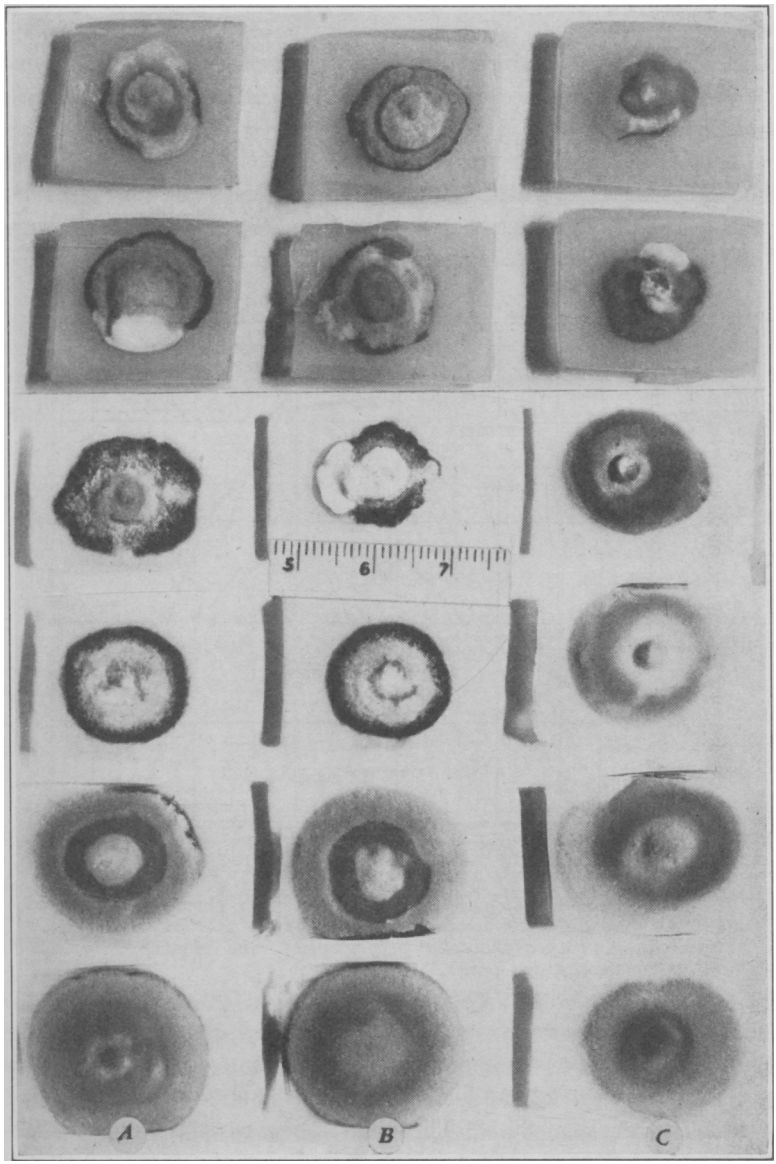



Fig. 7.—Colonies grown on Czapek's agar from single-spore culture isolations (different spore types) of *Mycosphaerella polymorpha*. The vertical rows show colony variation: *A*, colonies from *Cercospora*-type spores; *B*, from *Stigmina*-type spores; *C*, from ascospores. The horizontal rows show colonies of similar types of growth.

glucose potato agar at room temperature; the other 49 produced colonies that were elevated.

The colonies of *Stigmina Platani* closely resembled those of *Stigmella Platani-racemosae* but were less variable than those of *Mycosphaerella polymorpha.*

## TEMPERATURE RELATIONS

The effects of temperature on these three species of fungi were determined by the use of constant-temperature chambers having a range of 10°–35.5° C. A few tests were also made in a refrigerator at 5°. The

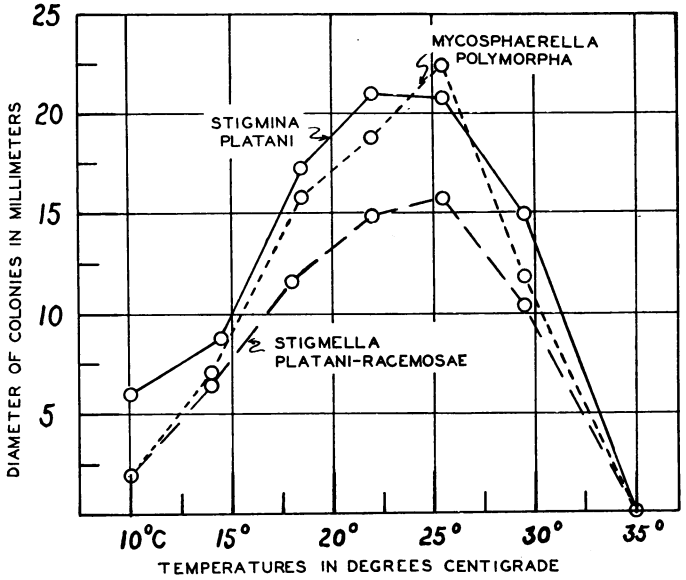



Fig. 8.—Growth response of colonies grown on glucose potato agar (pH 4.7) at different temperatures: *Stigmina Platani,* six weeks; *Stigmina* sp. (*Mycosphaerella polymorpha*), one month; *Stigmella Platani-racemosae,* one month.

diameter of colonies grown on glucose potato agar was used as an indicator of mycelial growth response. The optimum for all three organisms (fig. 8) was between 22° and 26°. They showed slight growth below 10°, but failed to grow at 35.5°. The growth curves of *Stigmella Platani-racemosae* and *Stigmina Platani* are flatter near the optimum than that of *Mycosphaerella polymorpha. Stigmella Platani-racemosae* gave the best sporulation between 14° and 19°; the other two fungi did not sporulate within this range during the time of the experiment.

The temperature at which the maximum germination of conidia of *Stigmella Platani-racemosae* and of *Mycosphaerella polymorpha* (fig.

9) took place (conidia of *Stigmina Platani* were not tested) agrees with that of the optimum temperature for growth of the mycelia of these two fungi. At 25° C, there was 75 per cent germination within 28½ hours, and 90 per cent within 47 hours. The count was based upon 50 spores selected at random. The germ tubes varied in length with the temperature; at 25° they reached an average length of 15.5 $\mu$ in 47 hours.

The thermal death points of the mycelia and spores of the three fungi were tested with cultures and conidia taken directly from the leaves of their respective hosts. Mycelium obtained from nonsporulating colonies

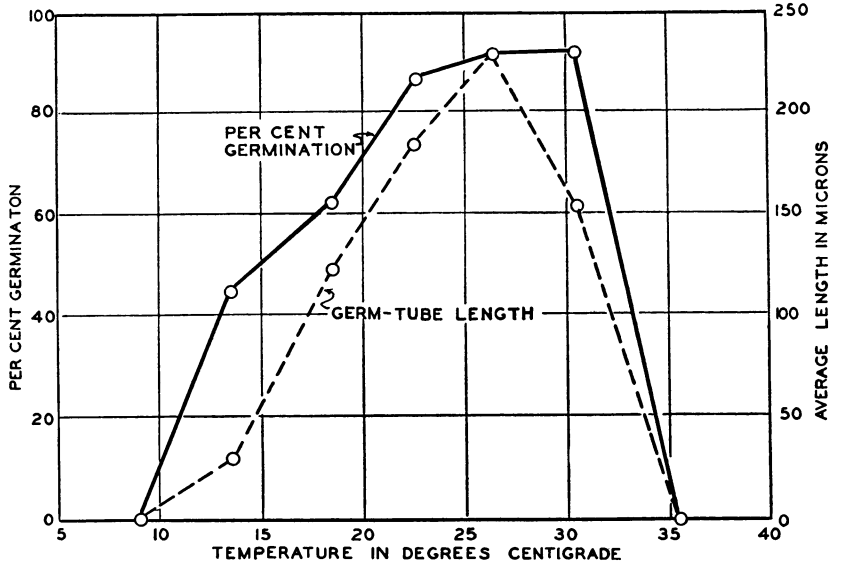



Fig. 9.—Spore germination and germ-tube growth of *Stigmina* sp. (*Mycosphaerella polymorpha*) on glucose-potato-agar film on slides for 48 hours at different temperatures.

was broken into bits by means of a sterile rod. Concentrations of the suspensions of mycelia or conidia were adjusted by the addition of sterile water. These suspensions were put into sterile capillary tubes, 4 to 5 inches long, after which the tubes were sealed by means of a microburner. The temperature of the bath, which consisted of a large pan of water, was modified by means of a microburner. After exposure for a 10-minute period in the bath, the capillary tubes were surface-sterilized in mercury bichloride and washed in sterile water; the contents were then ejected upon glucose-potato-agar plates. Observations of the resultant growth were made at intervals of from one day to two weeks. The results of these tests are shown in table 4.

The thermal death point of mycelia and spores of both *Stigmella Platani-racemosae* from *Platanus racemosa* and *Stigmina Platani* from *P. orientalis* was found to lie between 45° and 46.5° C. The mycelium and spores of the *Stigmina* stage of *Mycosphaerella polymorpha* had a thermal death point between 47° and 48°, approximately 2° higher than that of the other two fungi.

*Mycosphaerella* was cultured in sterile leaf juice in Van Tieghem cells to test the influence of temperature on sporulation. Five cultures were

TABLE 4

DETERMINATION OF THERMAL DEATH POINT OF MYCELIUM AND SPORES OF THE THREE FUNGI

| Fungus growth treated | Effect of 10-minute treatments at different temperatures* | | | | | | |
|---|---|---|---|---|---|---|---|
| | 44° C | 45° C | 46° C | 46.5° C | 47° C | 48° C | 49° C |
| *Stigmella Platani-racemosae* from *Platanus racemosa:* | | | | | | | |
| Spores | + | + | + | − | − | − | |
| Mycelium | + | + | | − | | − | |
| *Stigmina* stage of *Mycosphaerella polymorpha* from *P. occidentalis:* | | | | | | | |
| Spores | + | + | + | | + | − | − |
| Mycelium | + | + | + | | + | − | − |
| *Stigmina Platani* from *P. orientalis:* | | | | | | | |
| Spores | + | + | − | | − | | − |
| Mycelium | + | + | | − | | − | |

* In the columns + = continued fungus growth indicated; − = no growth and probable death of the organism; blank spaces indicate that results at these temperatures were not recorded. For description of treatments see text (p. 223).

maintained at 15° C, 5 at 19°, 16 at room temperature (21°–33°), 5 at 26.5°, and 5 at 30.5°. Of the 16 cultures grown at room temperature, 6 originating from *Stigmina* spores produced a mixture of *Stigmina-* and *Cercospora*-type conidia; 6 originating from *Cercospora*-type spores also produced conidia of both kinds; 2 of the remaining 4 cultures, which originated from ascospores, produced *Cercospora*-type conidia only, and 2 remained sterile. Sporulation did not occur in any of the other cultures with the exception of one of those maintained at a temperature of 26.5°, which produced *Cercospora*-type conidia two weeks after inoculation.

*Cercospora*-type conidia were often found in cultures from the conidia (both *Cercospora-* and *Stigmina*-type) and ascospores of *Mycosphaerella polymorpha,* but were never found in cultures of *Stigmella Platani-racemosae* or of *Stigmina Platani.*

Results of inoculations with cultures from single spores on leaves of *Platanus racemosa* would seem to indicate that temperature may be a factor in determining the type of spore that will develop on the infected

leaf tissue (table 5). The production of the *Cercospora*-type was favored by the higher temperatures, and the *Stigmina*-type developed best at the lower temperatures.

## HYDROGEN-ION RELATIONS

The hydrogen-ion relations of *Stigmella Platani-racemosae* and of *Stigmina Platani* were tested in carrot dextrose broth (fig. 10), on Czapek's agar, and on carrot dextrose agar. *Mycosphaerella polymorpha*

TABLE 5

SPORULATION FROM SINGLE-SPORE INOCULATIONS ON LEAVES OF *Platanus racemosa* WITH SPORES OF *Mycosphaerella polymorpha* UNDER DIFFERENT TEMPERATURE CONDITIONS; NOVEMBER—DECEMBER, 1938

| Type of spore isolated and culture no. | Lathhouse, temperature variable* | | Cool greenhouse, temperature 21°–27° C | | Warm greenhouse, temperature 27°–32° C | |
|---|---|---|---|---|---|---|
| | Inoculations | Sporulation† | Inoculations | Sporulation† | Inoculations | Sporulation† |
| | *number* | | *number* | | *number* | |
| Ascospore: | | | | | | |
| No. 5 | 1 | S only | 1 | None | 1 | None |
| No. 11 | 1 | S and C equal | 1 | None | 1 | None |
| No. 17 | 1 | S only | 1 | None | 1 | None |
| No. 22 | 2 | S only | 2 | None | 2 | C only |
| No. 23 | 2 | None | 2 | None | 2 | C only |
| No. 34 | 2 | Mixed, S predominant | 1 | S and C equal | 1 | C only |
| *Stigmina*-type: | | | | | | |
| No. 227 | 1 | S only | 1 | None | 1 | None |
| No. 239 | 1 | None | 1 | None | 1 | Mixed, C predominant |
| *Cercospora*-type | | | | | | |
| No. 115‡ | 1 | None | 1 | Mixed, C predominant | 1 | None |
| No. 118 | 1 | None | 1 | None | 1 | None |
| No. 130 | 1 | None | 1 | None | 1 | Mixed, C predominant |
| No. 136 | 1 | None | 1 | Mixed, C predominant | 1 | None |

* No temperature records were taken for the lathhouse, but outside temperatures as recorded at the Citrus Experiment Station for November were: minimum, 2° C; maximum, 29°; mean minimum, 4.3°; mean maximum, 24.6°. For December the minimum was 2°; maximum, 32°; mean minimum, 6.5°; mean maximum, 21.9°.

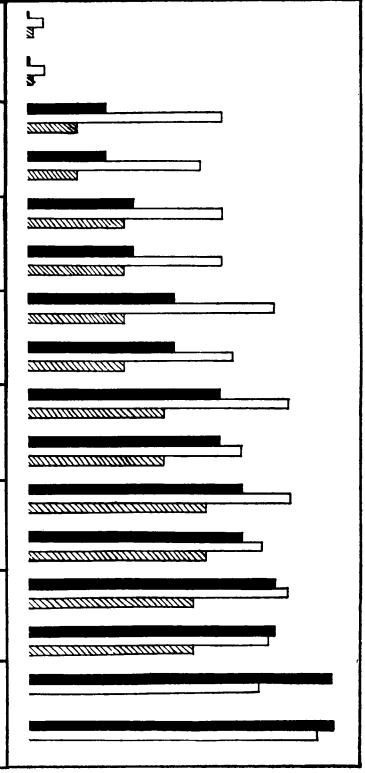
† The letters "S" and "C" indicate the type of sporulation, *Stigmina* or *Cercospora*, respectively.

‡ Organism isolated from inoculation by a previous single-spore culture.

was not tested. The fungi were grown in the liquid medium for three and one-half months and on the solid media for six weeks, at their optimum temperature of 25° C. In the cultures on Czapek's agar and on carrot dextrose agar, the minimum pH value was 2.0, the optimum 5.0, and the maximum between 7.0 and 8.0. Growth was slight at the alkaline end of the range. Growth of the fungi caused increased alkalinity in liquid cul-

tures, whereas the control liquids were more acid at the end than at the beginning of the experiment (fig. 10). There was little change in the pH value of the media where the initial concentration was pH 7.0.

| INITIAL PH VALUE | ORGANISM | FUNGUS GROWTH IN GRAMS |
|---|---|---|
| 2.08 | STIGMELLA | 0.333 |
| | STIGMINA | 0.257 |
| 3.64 | STIGMELLA | 0.302 |
| | STIGMINA | 0.392 |
| 4.22 | STIGMELLA | 0.253 |
| | STIGMINA | 0.440 |
| 5.05 | STIGMELLA | 0.450 |
| | STIGMINA | 0.416 |
| 6.02 | STIGMELLA | 0.390 |
| | STIGMINA | 0.328 |
| 6.48 | STIGMELLA | 0.362 |
| | STIGMINA | 0.364 |
| 7.15 | STIGMELLA | 0.245 |
| | STIGMINA | 0.230 |
| 8.30 | STIGMELLA | 0.302 |
| | STIGMINA | 0.220 |




INITIAL PH
FINAL PH OF CONTROL
FINAL PH WITH ORGANISM

Fig. 10.—Hydrogen-ion relations of *Stigmella Platani-racemosae* and of *Stigmina Platani* grown for three and one-half months on the surface of 100 cc of carrot dextrose broth in 200-cc flasks at 25° C. Control liquids contained no fungus inoculation.

Germination of spores and germ-tube length of *Stigmella Platani-racemosae* at different pH values, when tested in carrot-dextrose-agar drops on slides for 28 hours, showed maximum germination of approximately 85 per cent at pH 5.0. The germ-tube length, however, was great-

est at pH 4.5. At pH 5.0, the average germ-tube length was decreased by about one half of that at pH 4.5. The juice of leaf blades of *Platanus racemosa* had a pH value of 4.8 in April, as determined by the quinhydrone glass electrode; that of *P. acerifolia* gave a reading of pH 5.1 in September; there is, therefore, a close correlation between the pH of the leaf juice and that which is optimum for the pathogen.

## CONTROL

The writers have conducted no experiments for control of the leaf spot on *Platanus racemosa.* The other two leaf spots are not found in California. Should control be found necessary, treatment with bordeaux mixture, as suggested by Felt and Rankin (*6*) in the control of *Gnomonia veneta* (Sacc. and Speg.) Kleb., which causes leaf and twig blight of plane tree, or sycamore, should be satisfactory. These writers recommend that the trees be sprayed with bordeaux mixture after the buds burst and before the leaves are half grown, and that a second application be given one week later. A third and fourth spraying at intervals of two weeks are suggested if the season is rainy. It is anticipated that burning the fallen leaves would lessen the severity of the initial infections and might well be a very important control measure.

## DISCUSSION AND SUMMARY

On the basis of morphological and physiological differences and of host specificity, it is shown that three distinct species of fungi are involved in leaf spots on *Platanus* (plane tree). Each produces a different symptom complex and has a distinct geographical host range.

*Stigmina Platani* (Fckl.) Sacc. on *Platanus orientalis* L., from Europe, failed to infect other species of *Platanus.*

*Stigmella Platani-racemosae* Dearn. and Barth. is pathogenic to *Platanus racemosa* Nutt. in California. In pathogenicity tests it proved to be capable of infecting *P. Wrightii* S. Wats., not previously known to be susceptible. Other species of *Platanus* proved to be immune.

The fungus called, in this paper, *Mycosphaerella polymorpha* and found on *Platanus occidentalis* L., occurs in the southeastern and southern central United States. It was found to be pathogenic also on *P. racemosa, P. Wrightii,* and *P. acerifolia,* but not on *P. orientalis.* This organism produces polymorphic conidia that range in shape from those typical of *Stigmina* on the one hand, to those typical of *Cercospora* on the other. Conidia of each type and also of intermediate types may be borne on the same conidiophoral fascicle. This conidial stage has hitherto not been named, for the reason that it has been erroneously identified as *Stigmina Platani.*

The proper denomination of these three fungi could only be accomplished if their perfect stages could be developed under artificial conditions or were found to exist in the natural state on decaying leaves. The writers attempted unsuccessfully to induce the development of the perithecial stage of *Stigmella Platani-racemosae* under California conditions. Furthermore, at the request of the writers, leaves infected with *Stigmina Platani* were maintained in Cyprus under natural conditions, to permit the development of the perfect stage, but to no avail. Each of these organisms probably possesses a perithecial stage. Evidence for this is found in the fact that each possesses a spermatial stage, as previously noted (see "Culture Studies," p. 220). In the light of our knowledge of other ascomycetes, the production of spermatia may properly be interpreted as indicative of the presence of perithecia in the developmental cycle. The fact that these organisms can survive from year to year as conidial stages shows that the perfect stage is not essential to survival; but this is not proof of the nonexistence of a perfect stage.

The perithecial stages of *Mycosphaerella polymorpha* and *M. platanifolia* indicate that these species may be identical. The measurements for freshly discharged, hence mature, ascospores of *M. Stigmina-Platani* (*M. polymorpha*), given by Wolf (*24*, p. 58), are 17–19 × 6–7 $\mu$; and for those of *M. platanifolia,* 8–10 × 4–4.5 $\mu$. In the present study, the range of measurements of ascospores discharged from perithecia borne in leaves of *Platanus occidentalis* was 8–19 × 4–7 $\mu$, as previously stated (p. 215), and included spores 12.0, 13.5, 14.5, and 16 $\mu$ long and 4.8, 5.4, and 5.6 $\mu$ wide. These measurements indicate that there are spores intermediate in size between those published by Wolf for the two species of *Mycosphaerella.*

Wolf (*24*, p. 59) mentions two types of colonies: one type isolated from either the conidia or the ascospores of *Mycosphaerella Stigmina-Platani* (*M. polymorpha*) and the other type from either the conidia of *Cercospora platanicola* or the ascospores of *M. platanifolia.* But he states further[8] that the two types of colonies found in the present studies (see "Culture Studies," p. 219) and shown in figure 7 (p. 221) appear to have the same characteristics as those found in his studies.

Differences in appearance of colonies within one and the same species, however, are now known to be characteristic of an increasingly large number of fungi. The evidence in hand at present, therefore, as to the possible identity of *Mycosphaerella polymorpha* and *M. platanifolia* must be regarded as insufficient, and the solution of the problem must be left for future study.

[8] Wolf, F. A. In letter to the junior author dated August 10, 1940.

Unfortunately, Wolf (*24*) used the specific name *Stigmina-Platani* for the *Mycosphaerella* on *Platanus occidentalis,* whereas the present studies establish the fact that the conidial fungus *Stigmina Platani* is specifically distinct and occurs only on *P. orientalis.* This error, if preserved, would add to the nomenclatorial confusion, especially if the perithecial stage of *Stigmina Platani,* when discovered, should happen to be found to belong to *Mycosphaerella,* as this genus is now delimited. It has been deemed advisable, therefore, to reject the name *Mycosphaerella Stigmina-Platani* Wolf, and the new name *Mycosphaerella polymorpha* is proposed in its place.

*Stigmina Visianica* Sacc. appears to be identical with the conidial stage of *Mycosphaerella polymorpha.*

Each species has been isolated and grown in single-spore culture. Sporulation in culture was best induced by growth on *Platanus* leaf juice sterilized by filtration and on Czapek's agar.

Temperatures within the range of 22° to 26° C were found to be optimal for growth of each of the three species.

Germination of spores and mycelial growth occurred best in media having an acidity of approximately pH 5.0.

Removal of fallen leaves and spraying of the trees may be anticipated to be effective control measures.

## ACKNOWLEDGMENTS

The writers wish to express appreciation to Professor William T. Horne, under whose supervision the work was conducted, and to others, whose generous coöperation made these studies possible (see "Materials Used," p. 208). Grateful acknowledgment is made to Dr. H. S. Fawcett for assistance in preparation of the manuscript. Valuable information, criticisms, and suggestions were also given by Dr. F. A. Wolf, Dr. L. J. Klotz, Dr. D. E. Bliss, and Dr. C. L. Shear. Dr. Wolf was especially helpful in the problem of nomenclature. The photomicrographs were taken by Dr. L. J. Klotz.

## LITERATURE CITED

1. APOSTOLIDES, C. A.
1929. A leaf spot of sycamore caused by *Stigmina platani* (Fuckel) Sacc. Phytopathology **19**:667–71.

2. BUBÁK, FR.
1914. Ein Beitrag zur Pilzflora von Tirol und Istrien. Ann. Mycol. **12**(2):205–20.

3. COOKE, M. C.
1883. On *Sphaerella* and its allies. Jour. Bot. **21**:67–71, 106–20, 136–39.

4. DEARNESS, JOHN.
1929. New and noteworthy fungi. VI. Hyphomycetes. Mycologia **21**(6):326–32.

5. ELLIS, J. B., and B. M. EVERHART.
1887. Additions to *Cercospora, Gloeosporium,* and *Cylindrosporium.* Jour. Mycol. **3**(2):13–22.

6. FELT, E. P., and W. HOWARD RANKIN.
1932. Insects and diseases of ornamental trees and shrubs. 507 p. The Macmillan Company, New York, N. Y.

7. HARKNESS, W. H.
1885. Fungi of the Pacific Coast. California Acad. Sci. Bul. **1**(3):159–76. (See specifically p. 164.)

8. HOFFER, G. N.
1914. The more important fungi attacking forest trees in Indiana. Indiana State Bd. Forestry Ann. Rept. **14**:84–97.

9. INTERNATIONAL BOTANICAL CONGRESS.
1935. International rules of botanical nomenclature. Adopted by the International Botanical Congresses of Vienna, 1905, Brussels, 1910, and Cambridge, 1930. 3d ed. 151 p. Gustav Fischer, Jena.

10. JENNINGS, H. S.
1890. Some parasitic fungi of Texas. Texas Agr. Exp. Sta. Bul. **9**:23–29. (See specifically p. 28.)

11. MARTIN, G. HAMILTON.
1925. Diseases of forest and shade trees, ornamental and miscellaneous plants in the United States in 1923. Plant Disease Reporter Sup. [Issued by the U. S. Dept. Agr. Bur. Plant Indus.] **37**:349–446. (Mimeo.)

12. MCCLATCHIE, A. J.
1897. Seedless plants of southern California. South. California Acad. Sci. Proc. **1**:337–98.

13. NATTRASS, R. M.
1933. Annual Report of the Mycologist for the year 1932. [Cyprus.] Dept. Agr. Ann. Rept. **1932**:44–49.

14. PATTERSON, FLORA W.
1902. A collection of economic and other fungi prepared for distribution. U. S. Dept. Agr. Bur. Plant Indus. Bul. **8**:7–31.

15. PIPAL, F. J.
1916. A list of plant diseases of economic importance in Indiana, with bibliography. Indiana Acad. Sci. Proc. **1915**:379–413. (See specifically p. 394.)

16. RAWLINS, T. E.
1933. Phytopathological and botanical research methods. 156 p. John Wiley and Sons, Inc., New York, N. Y.

17. RIDGWAY, R.
1912. Color standards and color nomenclature. 43 p. 53 color plates. Published by the author, Washington, D. C.
18. SACCARDO, P. A.
1878. Fungi Veneti novi v. critici. Seriei VIII, Appendicula. *In:* Michelia: commentarium Mycologicum 1:351–55. Typis Seminarii, Patavii.
19. SACCARDO, P. A.
1880. Conspectus generum fungorum Italiae inferiorum, nempe ad Sphaeropsideas, Melanconieas, et Hyphomyceteas pertinentium systemate sporologico dispositorum. *In:* Michelia: commentarium Mycologicum 2:1–38. Typis Seminarii, Patavii.
20. SACCARDO, P. A.
1886. Sylloge Fungorum IV. Parta VII. (Sylloge Hyphomycetum) p. 394.
21. SMITH, DONALD J., and C. O. SMITH.
1939. The use of special media for sporulation of fungi. Phytopathology 29:821.
22. THÜMEN, F.
1871. Mycologische Notizen von Griechenland. Bot. Ztg. 29:27.
23. TRACY, S. M., and F. S. EARLE.
1895. Mississippi fungi. Mississippi Agr. Exp. Sta. Bul. 34:80–122.
24. WOLF, F. A.
1938. Life histories of two leaf-inhabiting fungi on sycamore. Mycologia 30(1):54–63.
25. WOLF, F. A.
1938. Correction. (*Cercospora platanifolia* Ellis and Ev. to *C. platanicola* Ellis and Ev.) Mycologia 30(2):243.