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ETIOLOGY AND TRANSMISSION OF ENDOSEPSIS (INTERNAL ROT) OF THE FRUIT OF THE FIG[1]

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FIGS, CAPRIFIGS, AND CAPRIFICATION

A comprehensive discussion of the diseases of the fruit of the fig should take into consideration its structural peculiarities. Eisen(10), (11) discusses exhaustively the morphology and structure of this fruit. Condit(5) gives a brief discussion of the fig fruit and its structure.

The fig is a nearly closed, more or less hollow receptacle, the inner walls of which are lined by the flowers when immature and by the fruit when ripe. It is not, therefore, a fruit in the strict botanical sense of the word but an aggregation of fruits lining the cavity of a hollow receptacle, technically a synconium, with an opening at the center of the flattened, distal end, which is closed during the early stages by a system of overlapping bracts. As the fig begins to ripen and soften these bracts or scales loosen and an opening is formed, the diameter of which varies from 2 to 10 mm., according to the variety of fig. This opening is usually referred to as the "eye" of the fig. In the ripe fig the wall of the receptacle to which the flowers are attached is called "the meat," and the aggregation of mature florets "the pulp." Figure 1 shows the internal appearance of the receptacle when split longitudinally. The flowers of the fig vary with the variety, the sex, and the crop.

[1] A thesis submitted in partial fulfillment of requirements for the degree of Doctor of Philosophy, University of California, 1926.

[2] Junior Plant Pathologist. Resigned June 30, 1926.

The fig is a dioecious, insect-pollinated plant, the staminate and pistillate flowers being borne on different trees. The male tree is known as the caprifig and in its receptacles we find chiefly two types of flowers. The staminate flowers are arranged, in most varieties, around the eye and the gall flowers (modified female flowers) occupy the rest of the cavity. The gall flowers have a very short style, particularly suited to the needs of the pollinating insect as will be explained later. The caprifig bears a succession of fruit known as the "mamme" or overwintering crop, the "profichi" or spring crop, which is the important one in relation to the edible figs, and the "mammoni" or fall crop. This succession of crops provides the proper habitat for the insects throughout the year. The caprifigs are not edible. They are usually rather small and non-succulent, the size varying with the variety. The names of the varieties of caprifigs most commonly grown in California and which were used in the experiments to be outlined later are: Roeding No. 1, Roeding No. 2, Roeding No. 3, Roeding No. 4, Markarian No. 1, Markarian No. 2, the Stanford, the Milco, and a number of seedling male trees.

The female tree bears also a succession of crops, one on the wood of last year's growth and a second on the new wood. These are the edible figs. In most cases the second crop only is of commercial importance. The receptacle of the edible fig is lined by a single kind of flowers, the pistillate, which resemble the gall flowers of the caprifig except that the style is much longer. The edible fig varieties are of two types, the parthenocarpic, or varieties which develop fruit without pollination, and the varieties that require pollination (caprification). The important varieties of the first category, grown in California, are the White Adriatic, the Black Mission, and the Kadota (Dottato), while the varieties which require pollination are chiefly those called Calimyrna, Stanford, and San Pedro. The first two were introduced into California from Asia Minor, the first being the commercially famous "Lob Injir" of Smyrna.

The process of pollinating the pistillate flowers of the fig is known as caprification. It is effected through the agency of a small hymenopterous insect, *Blastophaga psenes* L. (*B. grossorum* Grav.), which according to Cotte and Reynier(8) parasitizes the gall flowers of the male or caprifig. Eisen(11), Vallese(43), Rixford(31), Condit(5), and most recently Grandi(15), (16), have given extensive accounts of caprification and its agent. A brief account of the process is given here. It consists in suspending caprifigs on the branches of the female tree at the time of the issuing of the insects. The female

blastophaga enters the female fig receptacle for the purpose of oviposition and in so doing carries pollen from the caprifig into the edible fig. Oviposition, however, is not effected, because, as has been already mentioned, the styles of the female flowers are much longer than the styles of the gall flowers where the blastophaga oviposits. The insect

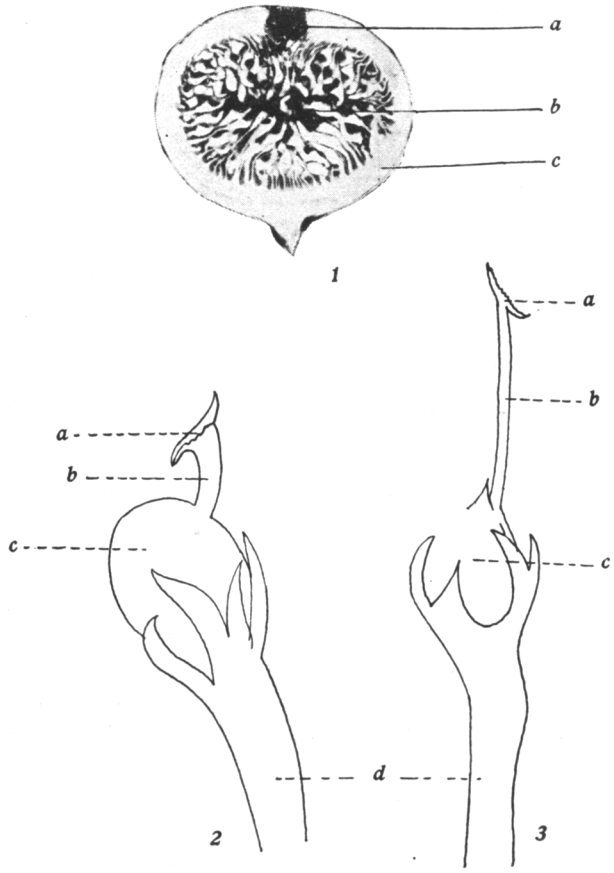

Fig. 1. Parts of the fig. (1) Longitudinal section of a Calimyrna fig. *a*, Osteolum or eye; *b*, pulp; *c*, meat. (2) Enlarged gall flower of caprifig. *a*, Stigma; *b*, style; *c*, gall; *d*, stalk. (3) Enlarged female flower of edible fig. *a*, Stigma; *b*, style; *c*, seed; *d*, stalk.

usually perishes in this vain attempt, but not without an effect, as the introduction of the pollen fertilizes the flowers of the varieties requiring such stimulation and causes the fruit to set and mature and form fertile seed. Without such a stimulation the fruit of the Calimyrna, San Pedro, and Stanford varieties drop when about one-

fourth grown, while those of the parthenocarpic varieties continue growth to maturity, but the seeds which they contain are not fertile (phenospermic).

In the gall flowers of the caprifig, the blastophaga inserts its ovipositor through the style and deposits a stalked egg in the ovule. The egg hatches and the larva lives inside the gall where it also pupates. The adult males issue first from their galls, fertilize the adult females while the latter are still in their galls and thus open the way for them to emerge later. The males, as a rule, never leave the figs. The females come out through the eye of the fig and while doing so rub against the staminate flowers which surround the orifice and shed their pollen at that time. The female insect may then carry the pollen into a fig of an edible variety or she may enter another caprifig, perhaps on the tree bearing the one from which she just emerged. In the latter case the pollen does not generally function as there are usually no receptive female flowers in the caprifigs. The term "caprification" is also applied to the setting of the male figs which results from the visit of the blastophaga.

DISEASES OF FIGS

It is only quite recently that the diseases of the fig have attracted the attention of investigators in California. The fig has been reputed to be particularly free from disease in most of the treatises on this fruit (Eisen[11], Roeding[32]). Foliage, twig, trunk, and root diseases have been reported from other states and from Europe, but, except a die-back of the twigs reported by Condit and Stevens(6) and Phillips(23), no disease of the tree has been known in California. Decline of the tree due to neglect, soil conditions, and nematodes has been discussed by Condit(4). Of the diseases of the fruit, a number occur in the South Atlantic and Gulf States and cause considerable damage. Edgerton(9) from Louisiana, Matz(21) from Florida, Stevens and Hall(41) from South Carolina, Potts(28) from Texas, and Gould(14) report a number of diseases of the fruit, the most important of which are an anthracnose caused by *Glomerella cingulata* (Stonem.) S. and v. S. (*Colletotrichum carica* Stev. and Hall) and a soft rot caused by *Rhizopus nigricans* Ehr. The first of these troubles has never been reported from California. *Rhizopus* has several times been isolated from rotted figs in the San Joaquin Valley.

Souring was the first disease of importance reported from California. It was attributed by Pierce(25) to an unidentified yeast but

no further work was done. Considerable time has been devoted to the study of this disease in connection with the present work; these findings will form the subject of another paper. A black smut due to *Sterigmatocystis* was reported by Hodgson(19) in 1918. An investigation of this disease was undertaken by Smith and Phillips(40) and a preliminary report was published in 1922. A more detailed account of this investigation was published by Phillips, Smith, and Smith(24) in 1925.

ENDOSEPSIS (INTERNAL ROT) OF THE FIG

Synonymy

The term 'souring' has been used indiscriminately in the past for almost every deterioration of the fruit of the fig. In careful observations, however, it is quite easily seen that symptoms vary considerably, and that there must be a number of agencies responsible for the large percentage of culls detracting from the profit of fig growing. The subject of this paper is a specific fruit-spoilage disease of the fig which has hitherto, except for a short note by the writer(2), remained undescribed and undifferentiated. At times a number of names have been used vaguely by growers to distinguish this trouble from what they consider typical souring, and the names 'pink rot,' 'brown rot,' 'soft rot,' 'stem-end' or 'eye-end rot' are frequently heard as referring to symptoms of disease observed in spoiled Calimyrna figs. All these names, although suggestive, seem either to be confusing or to apply only to certain phases of the disease. The names 'brown rot' and 'soft rot' are confusing because the first is applied to the well known disease of the stone fruits which does not affect the fig, and the second is applied to a rot of the fig in the Gulf States caused by *Rhizopus nigricans* Ehr. The names 'pink rot,' 'stem-end rot' and 'eye-end rot' refer to spots occasionally seen on the fruit, but as, in many cases, these spots are nothing but the external symptoms of a generalized disintegration of the meat and pulp of the fruit, they are not very descriptive. The name 'endosepsis (internal rot) is proposed here as more appropriate and less confusing.

Symptoms

The disease manifests itself internally as soon as the figs begin to ripen. In severe cases, even before the pulp sweetens and just as soon as the stalks and the sepals of the individual florets swell

and begin to color, brown streaks may be seen running down the flower stalks almost to the meat. As the fig ripens such streaks develop into spots, yellow-brown in color, and may involve a number of flowers. In most cases these colored spots are first found in the pulp near the eye of the fig, but any other part or parts of the pulp may develop this symptom according to the locus of infection. In very early stages, just as the fig begins to sweeten, these spots stand out very clearly against the bright-colored healthy pulp. Plate 1 shows six figs, five of which are at the same stage of maturity and alike in external appearance, firm, and bright green, just at the stage when figs are picked for canning or for fresh shipment. From their external appearance all five could be taken for sound ripe figs. Fruit *a* represents a healthy fig at this stage of maturity. The pulp should be bright amber to pink, with a small amount of sweet juice in the cavity. Fruits *b, c, d,* and *e* show the endosepsis symptoms at different stages of development. In fruit *b* the brown includes almost the entire mass of flowers in the vicinity of the eye, in the third fruit the browning includes three-quarters of the pulp, in the fourth all but a few flowers at the stem end are involved, and in the fifth the entire pulp is disintegrated. Such pulp is slightly watery and is easily pulled away from the meat. Until this last stage is reached, and even later, there is almost no external sign of this diseased condition of the pulp.

When the fig softens and begins to dry, a water-soaking of the skin appears in indefinite areas, mostly around the eye in a circular spot, or extending down the sides to the neck of the fig. This water-soaking gradually assumes a bright pink or purple color and the epidermis of the fig may easily be rubbed off on such water-soaked spots (pl. 2). The fig may dry in this condition and fruit *f* in plate 1 and the fruits in plate 3 represent such dried figs. These pink spots should not be confused with the pink spots produced by *Aspergillus niger* van Tiegh., the black-smut organism of the fig, as described by Phillips, Smith, and Smith(24). In the case of the internal rot the spots are not very wet and the margins are not shrunk and have not the tendency to be easily detached from the rest of the skin as in the black-smut spots. In the internal rot they appear as normal skin except in color. It is not always, however, that such colored spots appear on the decaying figs. Under favorable weather conditions the figs dry before they reach the stage of external symptoms, and undoubtedly even before the entire pulp is decayed. In many cases only a small water-soaked ring appears around the eye and a drop of liquid is exuded, varying in color from clear to caramel. This

exudate is never in sufficiently large quantities to drip and soil the foliage or solidify in long hanging drops, as is the case in souring. Many figs dry and pass for good fruit when the pulp is in reality full of the rot fungus. The interior is destroyed only in part and the flavor is not greatly affected. Such figs appear practically normal on the outside, although the inside is a little dry and "seedy" (see pls. 4 and 5) and the flavor slightly peculiar.

Finally, in certain orchards where the rate of drying has been slow on account of close planting, late irrigation, or climatic conditions, the disease becomes generalized in the fruit and the rotting of the pulp is so rapid as to separate the latter from the meat. The pulp slips easily from the meat and the fig appears sagging, wet, dripping, extremely soft and deformed. The pink spots may or may not develop on the sides, or end, depending on whether or not the parasite has actually invaded the meat.

The effects on the eating qualities of the fig produced by the disease vary considerably, depending largely on the bacterial flora present. Ordinarily, figs affected with endosepsis are lacking in odor and flavor rather than possessing disagreeable ones. The taste is rather flat, watery, lacking the proper sweetness and the characteristic fig flavor, with the seeds very prominent. There is no odor. In some cases, however, there is a very disgusting, putrid, somewhat bitter taste, very characteristic but impossible of description, and an odor which suggests that of spoiled tomatoes. There seem to be no ill effects from the eating of diseased figs.

Distribution and Economic Importance

Internal rot is distributed throughout the fig belt of California and wherever the Calimyrna is grown. No district seems to be entirely free from the disease and although it is confused with souring and the estimates of injury may be somewhat high, it is the opinion of the writer that this disease is very largely responsible for the size of the cull pile. Its severity depends largely on the climatic conditions, and the relation between these conditions and the disease will be discussed later on. However, it is due to this disease that successful growing of Calimyrna figs for the dry-fig market in certain regions and in certain years has become problematic. A large percentage of the dry product is either entirely unmarketable on account of the external pink discoloration, or the quality is lowered because of the internal deterioration of the pulp.

The loss given by the growers at different meetings in 1924 and 1925, and often probably greatly underestimated, varied from 20 per cent to 90 per cent of the dry crop. Specifically, the growers in one county in 1925 suffered from 20 to 50 per cent deductions for culls at a cannery on account of the internal rot. These shipments were made up of carefully selected fresh figs. An estimate of the percentage of dry figs showing *external* symptoms of the rot, as they were drying in the yard of one orchard, yielded a conservative figure of 70 per cent. The number of figs that had dropped to the ground from the several trees of another orchard was counted and the percentage of the figs showing external symptoms was found to average 30 per cent. At a meeting of fig growers held in 1924, the estimated percentage of loss from internal rot ranged from 33 to 71 per cent. If it is taken into consideration that the rot does not show actively until the fig has passed the canning stage, at which it must be firm, and that growers pick for canning only the best figs in the orchard, this percentage is alarmingly high. Many other counts and estimates were made at different times and the percentage of injury from this disease was always high.

Influence of Environment

It has previously been mentioned that climatic factors determine the severity of the disease. Such factors exercise no influence other than determining the rate of ripening and drying of the fig. This, in turn, determines whether the internal rot will become generalized in the pulp and also affects the appearance of external symptoms. Since, as will be shown later, infection takes place at a very early stage and the active fungous growths do not begin until the fig commences to ripen, the rate of ripening will determine the extent of damage to the fruit. That this is actually the case is shown by the percentages of disease observed in different localities and in different years. Sections with later and cooler seasons show always a larger percentage of damage than those which are warmer and dryer in late summer. This was especially well marked in 1925.

The amount of winter rainfall does not seem to be a factor, as in 1924 there was an alarming percentage of disease in all the sections, although the winter of this year was one of the dryest ever known in California. On the other hand, 1925 was a year of normal rainfall, but some sections showed a very small percentage of external symptoms of fig rot. The determining climatic influences are there-

fore the ones prevailing during July, August, and September. Quick ripening, rise in sugar content and concentration stops the growth of the fungus in the pulp and the fig dries without much loss in quality.

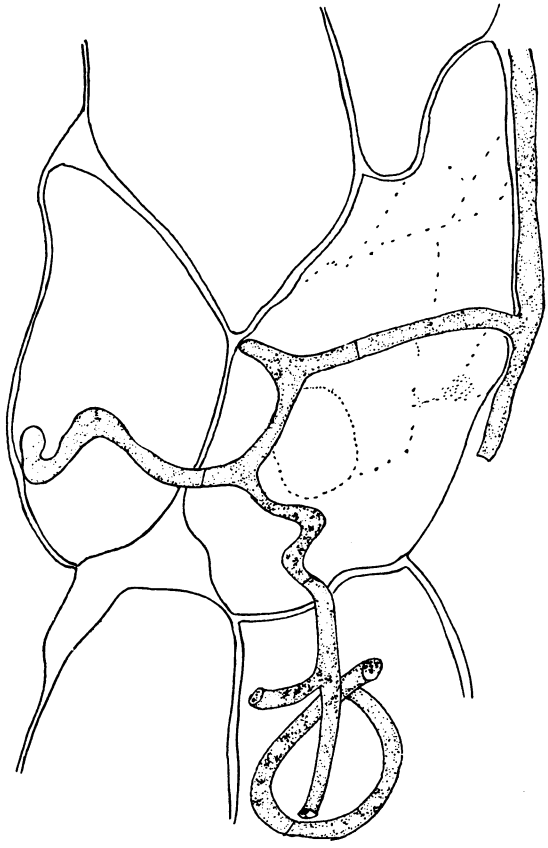

Fig. 2. Camera lucida drawing from stained preparations showing the penetration of the cells of the fig by the hyphae of *Fusarium moniliforme* Sheld. var. *fici* n. var. (× 1000.)

Etiology

Isolations.—The disease is caused by the fungus *Fusarium moniliforme* Sheld. var. *fici* n. var.

In a preliminary report(2) on this disease in the Calimyrna fig it has been stated that the diseased tissue is found permeated by a hyaline, septate mycelium of a fungus which can be very easily isolated and which grows luxuriantly on a variety of culture media.

The fungus does not usually produce aerial mycelium, either on the surface or in the cavity of the fig, but if a small portion of the rotting pulp is crushed under a cover glass and examined with the microscope it will be found permeated by a network of hyphae. The hyphae are more easily demonstrated by removing a small portion of water-soaked skin, dehydrating quickly in 85 per cent alcohol, staining in 2 per cent solution of Magdala red in 85 per cent alcohol, washing with absolute alcohol, clearing in xylol and examining under the low power of the microscope. The hyphae are colored red, while the host tissue is colorless or slightly pink (fig. 2).

If diseased figs are placed in a moist chamber they are soon covered by a woolly-white or pinkish, aerial growth. Figs, however, especially when ripe, form an ideal substratum for almost any saprophytic fungus; moist chamber studies, therefore, should not be relied upon for isolating the pathogen. Plate cultures from the pulp of a diseased fig which has not been exposed to the atmosphere always yield a typical growth or rather a group growth, which consists almost exclusively of the fungus mentioned and of a cream-colored and a bright red bacterium (pl. 6). This association has been found to be remarkably constant. It may be said also that both bacteria have been obtained from figs irrespective of disease symptoms, and in all stages of maturity, but not previous to caprification. These facts indicated a flora in the receptacle of the fig and an investigation was started to determine the flora of the edible fig in all the stages of development.

Phillips, Smith, and Smith(24) in 1925, in a study of fig smut, report that the pulp of Adriatic figs in early stages of maturity (stages 1, 2, and 3) before the fruit has been entered by insects, is entirely sterile. The classification of stages of maturity established as a basis for reference by Smith and Phillips in 1922(40) has been used in this work. Descriptions of the different types follow. For pictures of the different stages the reader is referred to Phillips, Smith, and Smith(24).

1. Fruit not quite full grown, still green and hard.
2. Full grown, eye scales beginning to loosen.
3. Eye fairly well opened, fruit still green and firm.
4. Slightly yielding to pressure, pulp succulent but still firm.
5. Fig ripe as for picking for fresh shipment. No shriveling, pulp opaque.
6. Skin slightly shriveled, pulp somewhat translucent.

7. Distinct shriveling, contents still red, not sticky.
8. Much shriveled and skin beginning to discolor; pulp mahogany color, slightly sticky.
9. Skin brown but flexible, pulp brown, translucent, sticky; stage of completed normal drying.

A repetition of the work of Smith and Phillips(40) regarding the flora of the Adriatic in stages 1 to 3 yielded identical results. The study was extended to figs of other varieties and the results indicated clearly that all parthenocarpic varieties, up to the time that they reach full growth and begin ripening as indicated by the loosening of the scales, coloring of the pulp and softening of the skin and pulp, are sterile. Varieties studied other than the Adriatic were the Black Mission (California Black), Brown Turkey, White Ischia, Kadota (Dottato), San Pedro White (first crop only), Cordelia, and miscellaneous other figs of unknown varieties. Both the first and the second crops were examined.

The method used was the standard poured-plate method with nutrient-dextrose agar, fig-infusion agar, or Czapek-dextrose-synthetic agar. The fig was washed with alcoholic $HgCl_2$ 1:1000, then split open with a sterile knife without touching the pulp, and a portion of the latter was scooped out and placed on the plate. A control plate was poured after every twenty-five inoculated plates. The Petri dishes were stacked and placed on a small platform in the center of a panful of water. A bell jar was then put over the stack of dishes with the mouth of the bell jar immersed in the pan of water. A U-tube was slipped under the wall of the bell jar so that one arm opened into the chamber and the other communicated with the outside, thus equalizing the air pressure. These precautions were taken to guard against excessive drying of the agar during the summer months in the interior valleys of California and to guard also against insects crawling in and contaminating the plates. The plates were thus kept reasonably free from contamination for a long time as indicated by the control plates remaining sterile. The plates were examined macroscopically ten days after pouring, and microscopically after another ten days. Figs were collected from different localities in the San Joaquin and Sacramento Valleys and the results of these investigations from 274 figs of different varieties are summarized in table 1.

Fifty-six Calimyrna first-crop figs not caprified were also found sterile. The second crop of the Calimyrna variety when not caprified was also sterile as shown by pouring plates both from figs that

dropped because of lack of caprification and also from figs in which caprification was prevented by bagging the twigs with manila paper hat bags a week before the blastophaga began issuing. Fifty-four figs were thus examined and were found to be sterile. When, however, the figs of any of the above mentioned varieties were caprified it was found that immediately after caprification their flora consisted of one or more of the three organisms mentioned in connection with isolations from diseased Calimyrnas.

TABLE 1

VARIETIES, LOCALITIES, AND NUMBERS OF PARTHENOCARPIC FIGS EXAMINED IN DETERMINING THE FLORA OF THE FIG RECEPTACLE

	Crop	Sacramento	Modesto	Fresno	Condition
Adriatic	First	4	10	53	All sterile
	Second	4	15	68	All sterile
Mission	First	3	.....	16	All sterile
	Second	.....	.....	18	All sterile
Kadota	First	.....	.....	20	All sterile
	Second	.....	.....	18	All sterile
San Pedro	First	.....	.....	18	All sterile
White Ishia	First	.....	.....	4	All sterile
Brown Turkey	First	.....	.....	4	All sterile
Cordelia	.....	10	.....	4	All sterile
Miscellaneous	.....	5	.....	.....	All sterile

Plate 7 shows the flora typically obtained from parthenocarpic varieties and from Calimyrna figs. The plates of the right hand series were poured from unripe, half-grown, fruits of Adriatic, Mission, and Kadota figs, respectively. The plates of the left hand series were poured from caprified Calimyrnas of the same stage of ripeness as that of the parthenocarpic figs used in the first series. It may be seen that there was no growth at all on the first series, while the fungus and the two bacterial organisms grew on the plates of the second series. Those discussed are typical of the many hundreds of plates poured during the course of this work. Plate 8 shows two Petri dishes, the upper poured with material from a caprified Adriatic fig, the lower with material from a non-caprified Adriatic. An abundance of growth is seen on the first plate, while the second one is sterile.

A great number of strains of the pathogen were isolated from diseased Calimyrna figs from several localities. The collection now includes over 250 strains which show more or less similar cultural characteristics. The majority of these strains are identical, the only difference being the origin or place of isolation. A great many of them exhibit variations of major or minor importance. The variations concern the habit of growth, size of spores, type of spores produced, color of mycelium, color of substratum, type of growth on different media, and lack of one or more of the following structures: Sporodochia, sclerotia, pionnotes, and catenulation of spores. Most of them, however, agree in a general way and evidently belong to the same group species, while three of them are quite different.

Eleven of the strains exhibiting the greatest differences were selected and studied intensively for over two years on a great number of media, including the ones recommended by Sherbakoff(36), the Fusarium Conference(47), Morris and Nutting(22), and others used for the first time in this study. Most of the differences were found constant. Certain strains, however, developed characteristic structures which were helpful in identification. The chief difficulty encountered in these studies was the stubbornly persistent failure of certain strains to produce the macroconidial type of spore characteristic of the genus *Fusarium* while their other characteristics clearly indicated such a relationship.

Morphology.—Strain 93 is considered as the type form of the new variety and has the following morphological characters. Microconidia in false heads or in chains formed on white to dark maroon purple[3] colored, aerial mycelium; ovoid-fusoid, 4.7–10.6 × 2.4μ.

Macroconidia delicate, slender, sickle-shaped, attenuate, subpedicellate, occur only in sporodochia on steamed corn meal and on woody stems, salmon-buff becoming wood brown with age. Sporodochia effuse, in columns, or in threads .5 to 1 mm. thick, twisting in loops or spirals 4 to 5 mm. long. Macroconidia mostly 3-septate (74 per cent) 19.9–49.4 × 2.3–7.4μ, 4-septate 39.9–49.3 × 3.52 to 4.1μ, 5-septate (rare) 43.5–51.7 × 3.5–4.7μ. Aerial mycelium dense fine or loose woolly, color varying from white to dark maroon purple, occasionally white with Payne's-grey-colored spots. Substratum light ochraceous buff becoming Vandyke red, Hessian brown or Perilla purple. Conidiophores simple on the sides of hyphae or di- and trichotomously branched to dendroid, alternately or oppositely arranged. Swollen cells (pseudochlamydospores) arranged in chains or singly are frequently found.

[3] The colors are after Robert Ridgway's color standards and nomenclature.

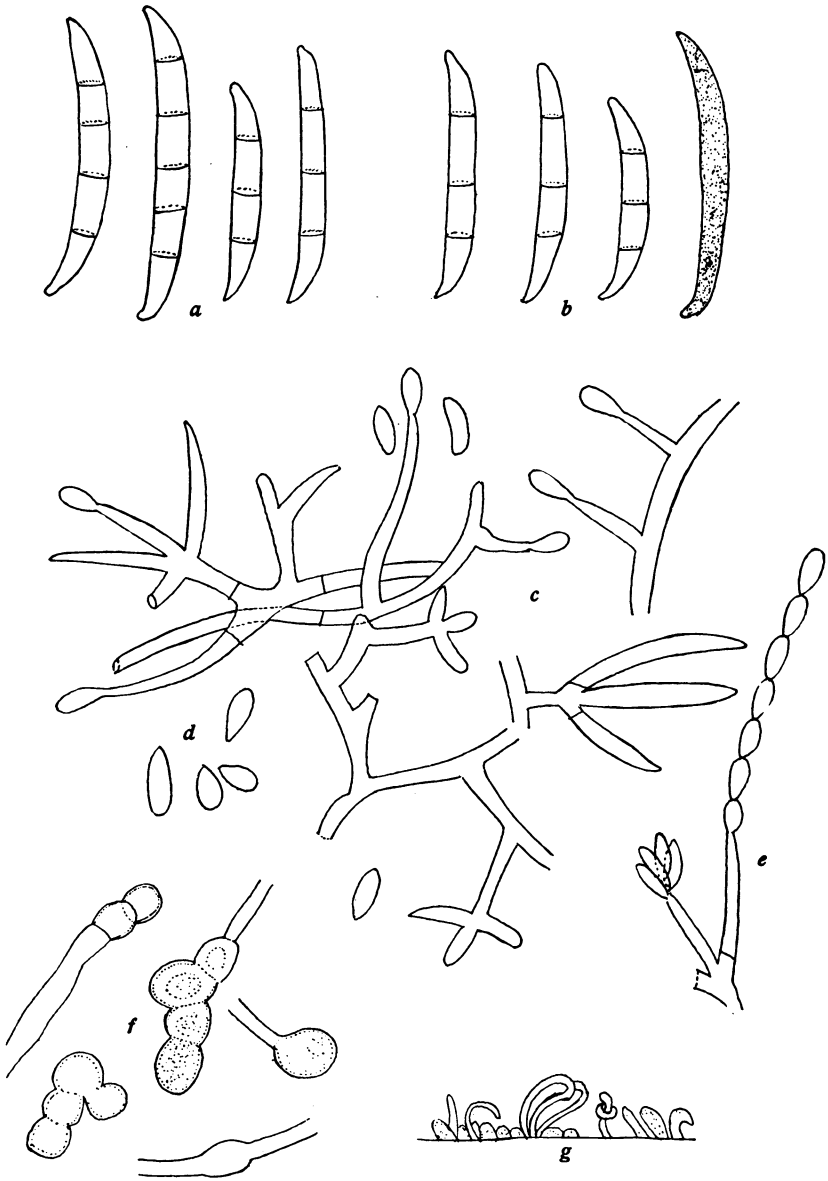

Fig. 3. *Fusarium moniliforme* var. *fici* n. var. *a*, Macroconidia from sporodochium on autoclaved cornmeal (× 782). *b*, Macroconidia from sporodochium on blackberry stem (× 782). *c*, Conidiophores and microconidia. *d*, Microconidia. *e*, Microconidia in chains and loose heads. *f*, Pseudochlamydospores (× 782). *g*, Sporodochia on autoclaved cornmeal.

Sclerotia are rarely produced on steamed rice and on hard oat agar. This characteristic is more pronounced in some of the strains, while in others it is entirely absent. The sclerotia are very small (1 mm. in diameter) and greenish black in color on rice, but larger and lighter in color on oat meal.

What is apparently the perfect stage of the fungus has been observed only once, on a plate from a Milco variety mamme (capri) fig collected from a tree at Ripon, California. Nothing unusual was noticed at the first observation of the plate, ten days after pouring. The flora on the plate was typical of hundreds of plates. But on a reëxamination of the plate sixty days after pouring, it was found that a semicircular row of black bodies had formed at the point of contact between two colonies of *F. moniliforme fici* originating from fragments of gall flowers. On a preliminary examination of this plate the following observation was recorded: "White fungus at a corner from flowers, the rest sterile." This refers to colony *b* (plate 9), which started from small fig fragments. Colony *a* is either a contamination or a later development from the large fig fragments in the plate. The black bodies referred to above proved to be perithecia. Scattered perithecia were found on the intermixed hyphae. The perithecia are black, osteolate, gregareous or solitary, containing ascospores which are greenish, thick-walled, usually 1-septate, measuring $10.15 \times 5.1\mu$ (12.3–8.2μ). Germination of the ascospores is rather low. Isolation of the two parent colonies was made but repeated attempts on different media to induce the production of perithecia failed.

Nomenclature.—In a preliminary report (2) on this disease in the Calimyrna fig the organism constantly isolated from diseased figs was tentatively identified as *Oospora verticillioides* Sacc. and described as "having a hyaline, frequently branching septate mycelium . . . fruiting abundantly, producing catenulate, short or long, tapering or slightly curved, unicellular conidia on single or branched conidiophores borne on the sides of the hyphae." In a recent paper by Wineland (45) an ascigerous stage was ascribed to *F. moniliforme* Sheldon and its synonymy critically analyzed. There is considerable evidence given to indicate that *Oospora verticillioides* Sacc. is a synonym of *F. moniliforme* Sheldon, although there is no direct proof of such a relation.

Chen (3), in studying the internal fungous parasites of agricultural seeds, has isolated from seed corn a fungus which according to his description closely resembles the fungus isolated from figs. Chen

did not find macroconidia of *Fusarium* in his cultures, therefore the fungus was identified by him as *Oospora verticillioides*. He states that "should *Fusarium* spores develop, the parasite would have to be referred to *Fusarium moniliforme* Sheldon." Fusarium spores were found in these cultures later by Wineland(45). Both *Oospora verticillioides* and *Fusarium moniliforme* have repeatedly been reported as parasites or saprophytes on corn, the first from Europe by Deckenbach in Bessarabia and by Cuboni, Tiraboschi, and others in Italy; and the second in America by Sheldon, Valleau, and many others. The relation of these fungi and references to the literature were recently given in detail by Wineland(45). The experience of investigators working with *Fusarium moniliforme* tends to show that macroconidia are not always produced, and this has been my experience. In the studies on the distribution of the fig disease throughout California, I poured more than four thousand plates from caprifigs and parthenocarpic and caprified figs, and had occasion to study several thousands of colonies of the fungus causing the rot. Macroconidia were never seen in any significant number in aerial mycelium, nor were sporodochia, pionnotes, or related structures ever observed on these plates. The media used in this study were mostly nutrient-dextrose agar, Czapek-dextrose-synthetic agar, fig-infusion agar, and potato-decoction agar. Macroconidia were, however, obtained in a special study and only from certain of the strains of the fungus. Macroconidia were also observed on the surface of Czapek-dextrose-agar and fig-infusion-agar dilution plates soon after the germination of the microconidium. These macroconidia, however, were soon overrun by aerial mycelium and after this only microconidia were produced on these media. Strains producing no macroconidia would have to be referred to *Oospora verticillioides,* at least tentatively, until macroconidia are obtained. The production of macroconidia in culture would place such fungi in the genus *Fusarium.*

In order to be sure that no mistakes were made in the identification of the fungus, a strain of *Fusarium moniliforme* Sheldon was requested from Miss G. O. Wineland of the U. S. Department of Agriculture, who has recently studied(45) the species exhaustively, and another from Dr. E. A. Bessey of the Michigan Agricultural College. The strain received from Dr. Bessey bears the number 299 and was originally received in 1924 from Dr. C. L. Shear of the U. S. Department of Agriculture under number 884. This culture was isolated from banana in Honduras and was sent to Dr. Shear by Dr. O. A. Reinking. Miss Wineland's culture bears the number 16 and was sent

as the most typical of the species from the strains studied by her. Diseased corn kernels were received from J. B. S. Norton of the Maryland Agricultural Experiment Station and from them was isolated a fungus producing catenulate *microconidia* in sporodochia. *Fusarium moniliforme* was also isolated from diseased ears of corn sent from Stockton, California, to Prof. W. T. Horne of the plant pathology laboratory of the University of California. All these strains were grown in parallel cultures with several strains of the fungus from figs and carefully compared on a great variety of media.

In some respects the fig fungus agrees fairly well with the descriptions given for *F. moniliforme* Sheld. by Sheldon(35), Wollenweber and Reinking(48), and Wineland(45). It differs, however, in the minimum measurements of all forms of spores, the fig fungus having shorter micro- and macroconidia. The fig fungus shows more aerial, more highly raised, and looser mycelium on some media. The aerial mycelium is more highly colored on some media and it has never been seen to be Isabella color or even any related color. The colors are white, pink, maroon, or reddish purple. Sporodochia are not usually produced on ordinary media, and when produced on steamed corn meal and elder stems they are formed in columns, or are filiform, suggesting possible catenulation. Macroconidia are not found in aerial mycelium. Sclerotia are not produced by the majority of the strains. Swollen cells in chains or singly are found in the mycelium; no pseudo or true pionnotes forms were ever observed. This form therefore is considered as a new variety, being named *Fusarium moniliforme* Sheldon var. *fici* n. var.

Cultural Characters.—As mentioned previously, many of the strains were grown on a variety of media in an effort to induce the production of macroconidia in culture. Strain 93 described above varied considerably on the several media employed and its characteristics on certain standard media will be described and measurements of the macroconidia given.

The media used were Coons's, Czapek's dextrose, Czapek's sucrose, and Sideris' synthetic agars; hard oat, soft oat, fig, lima bean, prune, potato, and corn-meal decoction agars; blackberry, tomato, *Melilotus*, fig, and elder stems; potato, fig, carrot, and coconut plugs; corn meal, rice, and oat meal in 150 c.c. Erlenmeyer flasks, and in tubes; crackers in deep Petri dishes and hard potato decoction agar with the addition of 5 per cent dextrose in Petri dishes to determine color production and rate of growth.

The most valuable of these media were: Coons's synthetic, hard oat, and fig agars in slants, blackberry and elder (*Sambucus nigra*) stems, potato plugs, rice in test tubes, oat and corn meal in 150 c.c. Erlenmeyer flasks, and hard potato plus 5 per cent dextrose agar in Petri dishes. These media were made according to directions given by Sherbakoff(36), Coons(7), and Sideris(37). Fig agar was made by boiling 100 gms. of dried figs for 30 minutes, straining, filtering, adding 2 per cent agar and sterilizing at 15 lbs. pressure for 20 minutes. Cornmeal and oatmeal flasks were prepared by adding one part of the meal by volume to three parts of water and steam sterilizing for one hour on three successive days.

Coons's agar: The aerial growth was scanty, powdery, white, dotted with salmon-colored sporodochia. The color on the substratum was Bishop's purple, non-diffusing. The mycelium and the spores were very granular and the septation in the macroconidia indistinct. 1-septate and 0-septate macroconidia were found; 0-septate macroconidia measured $8.2 \times 3.1\mu$; 1-septate, 16.4μ; 3-septate $28.7 \times 4.1\mu$. Swellings occurred in the hyphae.

Hard-oat agar: The aerial growth was hydrangea-pink, downy, pinkish vinaceous, abundant, with sporodochia in groups. The medium was dark mineral red. Swellings occurred in the hyphae.

Fig. agar: The abundant powdery growth ranged from pinkish vinaceous to dark Corinthian purple. The medium was Hay's maroon. No sporodochia were found.

Blackberry stem: The fine white growth was pale vinaceous fawn with dull Indian purple spots and the large scattered sporodochia were salmon buff. Macroconidia in sporodochia were mostly 3-septate, $28.2–37.6 \times 3.4–4.7\mu$ (average $33.0 \times 4.2\mu$); microconidia measured 7.0 to 8.8μ long. Sporodochia are not produced when there is much water in the tube. Certain strains produced sporodochia containing microconidia exclusively.

Elder stems (*Sambucus nigra*): Abundant aerial white growth developed. Many sporodochia were found, in color between light and pale ochraceous buff, gregarious and solitary, effuse. Two hundred macroconidia were studied regarding septation, using the method suggested by McWhorter(20). The percentages were as follows: 0-septate, .5 per cent; 1-septate, 10 per cent; 2-septate, 5 per cent; 3-septate, 74 per cent; 4-septate, 10.5 per cent; no 5- or 6-septate spores found. Microconidia measured from 4.7 to 10.6μ in length (average 7.8μ); 3-septate macroconidia measured 28.2 to 33.7μ in length (average 28.2μ).

Potato plug: Abundant matted white growth developed with the substratum blue in spots. Macroconidia were produced occasionally, but these were seldom in columns. Swellings occurred in the hyphae.

Steamed rice: After 30 days, there was abundant aerial white growth. Interstices were pinkish vinaceous, kernels light Corinthian red. In sixty days the interstices turned Corinthian pink, the kernels Corinthian red and Indian red. When a smaller amount of water (1:1) was added to the rice the colors were purple drab to pallid vinaceous.

Cornmeal flask: This medium was found very valuable for the production of normal macroconidia. The aerial growth upon it was abundant and white, slightly tinged with salmon. Sporodochia, salmon-colored and in tendrils or columns, developed in great abundance after some time (50 days). The macroconidia measured as follows:

1-septate, 12.9–16.4 × 2.3μ (average 14.2μ).
3-septate, 20–40 × 2.4–4.1μ (average 30.5 × 3.4μ).
4-septate, 40–50 × 3.5–4.1μ (average 46.5 × 4.0μ).
5-septate, 43.4–51.7 × 3.5–4.7μ (average 46.8 × 3.7μ).

Oatmeal flask: Abundant growth was made, shell pink, slightly vinaceous in color. An abundance of sporodochia developed as in cornmeal, salmon or greenish-blue in color. Pseudochlamydospores occurred in abundance on the mycelium.

5 per cent dextrose hard potato agar in Petri dishes: In the dark, aerial mycelium was very woolly and raised, abundant, white to pale grayish vinaceous; on the medium dark Perilla purple to maroon. Growth from center of Petri dish to the edge (5 cm.) was made in six days. Spores occurred in chains but mostly in loose balls on the end of the much-branched conidiophores.

The organism was grown on these media repeatedly with identical results.

It is mentioned by Wineland (45) and several other investigators that strains of *Fusarium moniliforme* when grown in culture for a long time or when only mycelium or microconidia are used in the transfers, lose their power of producing macroconidia as well as their chromogenic properties. The strain just described of the organism from the fig has been under culture for almost three years and transfers of it have been allowed to dry for over a year at high temperatures. When transferred to suitable media they did not seem to differ in the least from the original descriptions. However, the same cannot be said of other strains of this fungus.

In the course of this work it was suspected that light and the moisture content of the medium exercised an effect on the color formation and the production of sporodochia. To test this point 8 gm. portions of cornmeal were put in each of seven 150 c.c. Erlenmeyer flasks and water was added in increments of 4 c.c., from 8 c.c. of water up to 32 c.c. The series was prepared in duplicate. The flasks were autoclaved at 15 pounds pressure for 20 minutes, cooled in the refrigerator and immediately inoculated with .5 c.c. of a heavy suspension of both macro- and microconidia. One series was kept constantly in the dark at room temperature while the other was exposed to a rather weak diffused daylight near a window of northern exposure shaded by large eucalyptus trees. After 20 days the growth was profuse on all the members of the series, but sporodochia developed in great abundance only on the members of the series exposed to the light and in inverse proportion to the amount of water initially added. A suspicion of sporodochia only showed in the members of the series not exposed to light that contained 8, 12, and 16 c.c. of water respectively. On the other hand, the color developed on the medium was very much more intense and bright in the series kept in the dark and especially so in the members of the series that contained 24, 28, and 32 c.c. of water, respectively. These results are summarized in table 2.

TABLE 2

EFFECT OF LIGHT AND WATER CONTENT ON COLOR AND SPORODOCHIA PRODUCTION OF F. MONILIFORME VAR. FICI GROWN ON CORNMEAL

Water (cc.)	Diffused light		Dark	
	Sporodochia	Color of substratum	Sporodochia	Color of substratum
8	++++	Flesh pink	−	Shell pink
12	++++	Flesh pink	±	Light Corinthian red
16	+++	Light Corinthian red	±	Buff pink
20	++	Russet vinaceous	−	Buff pink
24	++	Onion skin pink	±	Deep Corinthian red
28	+	Onion skin pink	−	Deep Corinthian red
32	+	Onion skin pink	−	Deep Corinthian red

This is in accordance with the findings of Sherbakoff(36) regarding the intensity of color produced by *Fusarium* grown in darkness. Coons(7) has found that diffused light is necessary for the pycnidia formation of *Plenodomus fuscomaculans.*

The effect of the hydrogen-ion concentration on the development of pigment in *Fusarium* has been recently discussed by Sideris(38) with a review of the literature on the subject. He found that *Fusarium moniliforme* Sheld. produced a range of diffusible colors from flesh to colorless when grown in liquid media with adjusted H-ion concentration ranging in pH from 3 to 7.5. This reaction was maintained constant by additions of acid or alkali throughout the growth period. When the reaction was not kept constant the colors ranged from hydrangea pink to lilac, indicating that in the alkaline members of the series where no color was produced when the reaction was maintained constant, pigment developed through the shifting of the pH by the metabolic products of the organisms.

Fusarium moniliforme var. *fici* (strain 93) was grown in Petri dishes on a series of solid media ranging in pH from 4 to 8. The media were prepared according to the directions of Sideris(38). Ten c.c. portions were poured and the dishes were kept in the dark. The rate of growth and the pigment development was frequently noted. No difference in the rate of growth was observed. The organism grew equally well and fast on plates of pH 4, 5, 6, 7, and 8. The pigments, however, differed on the different members of the series and also from those given by Sideris(38). Table 3 presents the colors diffused through the agar.

TABLE 3

EFFECT OF HYDROGEN-ION CONCENTRATION ON PIGMENT FORMATION IN FUSARIUM MONILIFORME VAR. FICI

pH Initial	Color after 6 days diameter of disc 30 mm.	Color after 14 days diameter of disc 45 mm.	Color after 33 days diameter of disc 50 mm.
4	Maroon to Van Dyke red fading to hydrangea pink	Victoria lake to Van Dyke red	Burnt lake
5	Dark Indian red to vinaceous rufous to salmon	As in 4 but brighter	Burnt lake
6	As in 4 for one-half of disc; as in 5 for remainder	Perilla purple to colorless	Burnt lake
7	Black to purplish-lilac to colorless	Neutral red to colorless	Dark Corinthian purple
8	White except on center which is dark blue slate	Deep livid purple to colorless	Indian purple

Strain 16 received from Miss Wineland was also grown in a similar series. The colors corresponded to those given by Sideris, viz., hydrangea pink to salmon. In the fig fungus the pigment is of the brighter maroon on the acid side, grading at first to dark reddish purple on the alkaline side; the colors of the alkaline side approach those of the acid side when the reaction is increased in acidity because of the action of the fungus.

Associated Organisms.—Two bacterial organisms, a red chromogenic and a colorless form, were found as constant associates of *Fusarium moniliforme* var. *fici* in diseased tissues of the fig and also alone in caprified figs, both ripe and green. The red chromogen was isolated the greater number of times from figs in the San Joaquin Valley. The colorless organism was more often found in figs from the Sacramento Valley, but both organisms were many times obtained from the same fig throughout California.

Morphologically the two organisms appear identical. They are both short rods $1 \times .8\mu$, arranged singly, asporogenous, motile, staining easily with ordinary stains, Gram negative. Culturally they are also identical except for chromogenesis. On agar slant at 30° C. they make an abundant, filiform, raised, glistening, opaque, viscid growth and have a decided odor which is similar in both and rather putrid. They liquefy gelatin rapidly. There is no surface growth on nutrient broth; the clouding is slight, the odor decided and the sediment scant. The liquid is colored red by the chromogenic form. They both possess a slight diastatic action, and produce an alkaline reaction on milk in two days with a rennet curd and peptonization. There is no reduction of litmus. They ferment dextrose and sucrose with the production of gas and acid (pH 4.5) but do not ferment lactose. Indol is not formed and nitrates are strongly reduced to nitrites.

The white strain answers the description of *Achromobacter nitrificans* (Burri-Stützer) Bergey(1). The chromogenic strain could not be assigned to any one of the species of Serratia described by Bergey(1). It differs in a number of ways from the type species *Serratia marcescens* Bizio (*Bacillus prodigiosus* Flugge); namely, in the alkaline reaction with rennet curd and peptonization in litmus milk, in the thick, raised, undulated margin of the colonies on agar, and in the fact that it has never been observed to form filaments or chains.

Both the red and the white organisms were found rather polymorphic on agar plates, especially when developing from fig tissue.

Plate 10 illustrates some of the differences. In dilution plates the red organism produces round, raised, glistening colonies with smooth margins. Such colonies are seen at the lower left of Petri dish 1109 in plate 10. It soon, however, begins spreading in fan shape, producing a colorless edge. This is more characteristic when the organism spreads from fig tissue placed in the center of the Petri dish. It then spreads in slender, faintly pink streams which unite, producing an undulate, dark red edge (Petri dish 1084, plate 10).

The appearance in nature and in the same habitat, of chromogenic and colorless forms, otherwise physiologically and morphologically identical, appeared to the writer to be of interest in the light of modifications and variations experimentally produced by selection or otherwise in *Serratia marcescens* Bizio (*B. prodigiosus* Flugge). Scheurlen(34) obtained colorless strains of *B. prodigiosus* by growing the organism on potato and selecting always from the least colored portion of the growth for subsequent transplanting. Wolff(46) also obtained long white forms of *B. prodigiosus* by careful selection and repeated transfers. Rettger and Sherrick(30) developed through selection white and red strains of *B. prodigiosus* from a culture which was weak in chromogenic properties. The chromogen from the fig has never shown weakness in chromogenesis, although it has been grown on acid media and has been kept without transferring for a long time; similarly, pigmentation has never been observed in the white strain.

Porter(27) found that *Fusarium lini* was inhibited in its growth by a bacterium. The fig fungus does not seem to be affected by the proximity of growth of either the white or the red bacterium described above, as can be seen from plate 6.

Pathogenicity.—Experiments to determine the pathogenicity of the organisms isolated have met with many difficulties. Inoculations could not be made on Calimyrna figs, since they may have already become infected in the process of pollination (caprification) which is essential to the development of the fruit of this variety. Fortunately it was observed that when Adriatics contract the disease through chance caprification they exhibit the same symptoms as Calimyrnas. Normally, and unlike the Calimyrnas in this respect, they develop fruit without caprification and on repeated examination were found to be sterile up to the time of ripening. Through the kindness of Mr. Taylor of the Pasa Rica ranch near Fresno, a row of large Adriatic trees was put at the disposal of the writer for inoculation purposes.

Keeping inoculated figs in moist chambers was found to be impractical because as soon as the ripe fig is put into a humid atmosphere it is invaded by a variety of saprophytes. The method of removing the glass lids and covering with cheesecloth was tried. This was found unsatisfactory because the figs dried too fast to show maximum development of the rot. It was finally found that by inoculating the figs at stages 4 or 5 of development, while still attached to the twig, and then bagging the entire twig with a three-pound-size manila grocery bag, satisfactory results could be obtained. The figs were thus inoculated while still sterile, since the eye is scarcely opened at stage 4, and were subsequently protected by the paper bag from wind and insect-borne infections as well as from excessive and rapid drying. No inoculated figs could be lost, since when they ripened and dropped they remained in the bags.

The entire collection of *Fusarium,* including approximately 250 isolations and the collection of other miscellaneous organisms including a variety of green fungi (*Cladosporium* sp., *Alternaria* sp., *Helminthosporium* sp., *Hormodendrum* sp.), and other moulds which appear occasionally on the plates, as well as the red and white bacteria described above, were inoculated, in duplicate, into figs. The inoculations were made by introducing mycelium and spores through the eye of the fig by means of a sterile platinum needle. The eye of the fig had been previously sterilized by swabbing it with a piece of cotton wetted in an alcoholic solution of $HgCl_2$.

The results have been very striking and conclusive. The typical symptoms of endosepsis, including the disintegrated pulp, and water-soaked purple or pink skin spots, the eye and stem-end rot, the "slip-skin" condition and the yellow gum at the eye were produced by the inoculations of strains of the *Fusarium moniliforme fici.* The green fungi and miscellaneous organisms were not found to be parasitic on the fig. Their growth when inoculated did not progress beyond the point of inoculation. They occasionally fill the cavity with a mass of superficial mycelium, utilizing the free sugar in the juice of the ripe fig; *F. moniliforme fici,* on the other hand, penetrates and disintegrates the pulp and the meat and permeates the affected tissues in every direction. Plates were poured from all the figs inoculated and in every case the organisms were regained in pure culture.

Examination of these plates showed that no changes had been effected in the organisms by passing them through the host; i.e., strains weak in chromogenesis or fruiting did not regain such lost properties or acquire others. A greater amount of aerial mycelium

was found in inoculated figs than is usually found in natural infections, but this is probably due to the large amount of inoculum introduced into the cavity.

Twenty sterile Adriatic figs were inoculated with the red and the white bacterial organisms, both in moist chambers and on the tree. The organisms were readily regained from the inoculated figs twenty-five days after inoculation but no change was observed in the fig. This is in accordance with what was observed in isolation experiments. Normal-appearing figs showing absolutely no deterioration yielded either the red or the white organism when plated. Finally, numerous inoculations were made by using one of the strains of the fungus together with either the red or the white bacterium. The results have not been very conclusive but suggest that the bacteria help in the disintegration of the pulp and contribute to the odor but cannot initiate the rot.

Transmission

The Flora of Caprifigs.—The difference in the flora of caprified and non-caprified edible figs suggested a study of the flora of caprifigs. The methods used in this work were the standard pathological ones. Caprifigs in all stages of development, before and after caprification (pl. 11) were collected from many parts of California. Usually not less than five figs were taken from one tree and not less than twenty-five from one orchard at one time. The methods used were those previously described. Duplicate plates from the same fig, using the same or a different medium, were often poured, but finally, as the flora was identical in both plates, and examination of a greater number of figs was desirable, a single plate was poured from each fig. However, care was taken to sample the pulp in such a way as to obtain a correct picture of its flora. A blank plate was poured as a check for every twenty-five plates.

The flora of caprified male figs was surprisingly uniform. At times a variety of saprophytes, usually *Alternaria* and other green fungi, was found, but not in such a way as to indicate a definite connection with the disease occurring in the edible figs. A variety of bacteria was also found in the course of the work, but two organisms, the red and the cream-colored one, were almost constantly obtained from the pulp of caprifigs. These two organisms were the same as those constantly isolated from Calimyrna figs. The fungus associated with the internal rot of the Calimyrna was also frequently isolated from caprifigs. Table 4 presents in a tabular form the flora of these caprifigs, as it was obtained from the different sections of California.

TABLE 4

THE FLORA OF CAPRIFIED MALE FIGS FROM COMMERCIAL ORCHARDS IN CALIFORNIA DURING THE YEARS 1922, 1923, 1924, AND 1925

	Number of figs examined	Per cent *F. moniliforme* var. *fici*	Per cent red bacterium	Per cent white bacterium	Per cent green fungi	Per cent Torula yeast	Per cent sterile figs
Mamme 1922 and 1923:							
Sacramento Section	52	30.7	13.4	61.5	.....	11.5	19.2
Modesto	45	35.5	40.0	35.6	2.22	2.22	20.0
Merced	22	54.6	63.6	59.0	4.55	4.55	9.08
Fresno	29	31.0	44.8	34.5	3.45	3.45	17.2
Reedley	49	32.6	61.2	28.6	18.4	18.18	2.04
Tulare	24	41.7	70.8	62.4	4.17	.....	16.6
	221						
Profichi 1923:							
Modesto	63	46.0	68.3	44.4	12.7	.....	4.76
Merced	45	51.1	82.1	40.0	6.66	.....	4.45
Fresno	115	40.9	65.2	18.3	10.4	.....	13.9
Reedley	46	52.2	78.2	21.7	4.35	.....	.....
Tulare	54	46.3	92.5	38.9	3.70	.....	7.41
	323						
Profichi 1924:							
Modesto	16	50.0	43.8	68.7	25.0	12.5	6.25
Fresno	43	69.8	67.4	67.4	.....	.....	.....
Reedley	47	51.1	80.8	51.0	4.25	8.50	....
Tulare	44	25.0	43.2	51.0	.....	.....	6.81
Southern California	70	17.1	.....	.....	.....	11.40	10.00
Mammoni 1924:							
Sacramento	79	55.6	.....	.....	.....	.....	.....
Modesto	12	58.3	41.7	.....	.....	.....	.....
Fresno	5	100.0	.....	.....	.....	.....	.....
Reedley	7	28.6	.....	100.0	.....	.....	.....
Southern California	5	60.0	.....	.....	.....	.....	.....
Mamme 1924:							
Sacramento	500	59.4	.....	.....	.....	.....	.....
Modesto	64	45.3	20.3	23.4	.....	.....	.....
Total Mamme	785						
Total Profichi	543						
Total Mammoni	108						
	1,436						

The State is divided for convenience into seven sections: the Sacramento Valley section; the Modesto section, comprising the Modesto, Ceres, and Turlock fig orchards; the Merced section, comprising the Merced, Planada, and Le Grand fig orchards; the Fresno section, comprising the Fresno, Figarden, and Clovis fig orchards; the Reedley-Dinuba section; the Tulare section, comprising the Orosi, Farmersville, Lindsay, and Strathmore orchards; and the southern California section, comprising the orchards south of Tulare and including the Coachella and Imperial valleys. During the last two years, after the relation of the fungus to endosepsis had been definitely established, media favoring the growth of fungi were used rather than neutral media permitting a great bacterial development. For this reason the percentages relating to the presence of the bacteria usually found in caprifigs are low or lacking. The red organism does not develop color on acid media; it forms pin-point colonies which grow very slowly, and their differentiation and identification is difficult.

This table shows that the forms of *Fusarium moniliforme* var. *fici* (*Oospora verticillioides*) which are always present in cases of endosepsis, together with the two bacterial forms, constitute what might be termed the normal flora of the male fig in its entire succession of crops, except in non-insectiferous caprifigs. The percentages of occurrence vary considerably but this is to be attributed to the small number of figs examined from one place at one time. The total number of figs examined is large enough to preclude chances of error. The fungus and the bacteria have been obtained from caprifigs and edible caprified figs at all stages of development, but only after the entrance of the blastophaga.

In certain varieties of caprifigs, for example, Roeding No. 1, Roeding No. 3, and Milco, some of the fruit of the profichi crop remains on the tree and develops normally, producing pollen, although it does not contain insects. Such figs are commonly known as blanks and can easily be distinguished from the insectiferous figs by their smaller size and lighter color. Blank figs in different stages of development were used in pouring a large number of plates but they invariably proved to be either sterile or to possess a saprophytic flora distinct from the one occurring in caprified figs. Caprifigs as a rule do not soften or acquire sugar as do the edible figs, except in a few varieties like the Milco and certain seedlings and only in the mammoni crop. On account of this fact the symptoms of the disease are not clearly defined as in the edible fig. The profichi crop, which is the most abundant of the three, is usually picked before ripening and

hung in the edible fig trees in baskets to facilitate caprification. These profichi figs gradually become hard and dry. If such dried profichi are split open, they are found filled with the aerial mycelium of the fungus previously described which has developed in the cavity after the blastophagas issued and before the figs became bone dry. Occasionally profichi were found exhibiting a wet and mushy internal rot and in many cases no insects issued from such figs. The external symptoms of the disease, i.e., bright pink or purple spots around the eye and on the sides, have been observed on fleshy mammoni figs of the Milco and Maslin varieties.

The Blastophaga as Carrier.—The fact that the fungus was obtained from both Calimyrna and caprifigs and not from any of the non-caprified figs pointed to the only connecting link between the two, viz., the blastophaga. In order to study this point, caprifigs from an orchard showing a high percentage of endosepsis were taken to the laboratory just before the blastophagas issued. The eye and the surrounding skin of each were carefully sterilized in 1:1000 $HgCl_2$ in 50 per cent alcohol and a sterile homeopathic vial was attached to the eye of each fig by means of melted paraffine (pl. 9). The figs were placed in a test-tube rack in diffused light until the blastophagas issued. When about a dozen insects had collected in each vial, the vials were detached and the insects removed one by one by means of sterilized forceps and placed on a poured Czapek-dextrose-synthetic-agar plate. Care was taken to immerse the insect in the agar so that movement was stopped. The fig from which the insects had issued was also used in pouring a plate so that the presence of the fungus in the cavity of the fig could be ascertained. Media permitting the development of bacteria were also used and the experiment repeated a great number of times using caprifigs from different sources and crops. These experiments proved plainly that the blastophagas actually carry the organisms discussed from the caprifigs into the edible figs at caprification time. The flora of each fig as determined by pouring a plate was also obtained from the blastophaga, caught as previously described. All combinations of the three organisms were found to be carried by the insects. Plates 12, 13, and 14 show some of the many plates poured in these experiments. The lower Petri dish in plate 12 shows the development of a colony of fungus from each insect placed on the agar plate. The same results were obtained when individual gall flowers picked one by one from the cavity of the caprifig, were placed on another agar plate as shown in plate 12 (upper). The Petri dishes in plate 13 illustrate the development of

both the red and the white bacteria as well as of the white fungus when insects or gall flowers are placed on poured agar plates. However, not all the blastophagas, even from a fig that showed the white fungus, were found to carry that organism, but almost all of the blastophagas plated carried at least one of the two bacterial organisms.

Method of Transmission.—Insects have for a long time been known as carriers of plant diseases. Rand and Pierce(29) have given an extensive review of the whole subject of insect transmission in plant and animal diseases. They found that insects transmit pathogens in three ways: (1) mechanically, by picking up the spores on the exterior of their bodies and accidentally sowing them on the surface or inoculating them into punctures; (2) by making avenues of infection through wounds; (3) by transmitting them internally, either mechanically or biologically.

In our investigations it was found that the pathogen and the organisms associated with it are carried by *Blastophaga psenes* L. Extensive studies were made in order to ascertain the method of transmission, which might in this case be "external-mechanical," "internal-mechanical" or "internal-biological." The life of the adult blastophaga is very short. Grandi(15) has kept them alive in captivity for 4–5 days and Vallese(43) for 8 days. The female, after laying its eggs, usually dies in the cavity of the fig where the eggs were laid. The male usually does not come out of the fig where he lives. Neither the male nor the female adult insects have been observed feeding on any of the parts of the fig. Grandi(15) has found that they possess well-formed mouthparts which are used in opening their way out of the gall. The male opens his own way out of the gall; then he makes an opening into the gall containing the female and fertilizes her. The female later enlarges this same hole and comes out.

The body of the adult female is covered with spines and is black. On examining the wings of the blastophaga, which are also spiny but transparent, it was found that spores, as well as pollen grains, were sticking to them. Insects caught in the vials attached to the eye of caprifigs, as previously described, were found carrying such spores on their wings. Under the moist conditions inside the little vial, the result of transpiration by the fig, these spores were found germinating, and when the insects were left long enough in the vials they became covered with the mycelium of the fungus. A photomicrograph of a portion of the blastophaga wing carrying microconidia of the fungus is shown in plate 15, *a*. It appears reasonable that such

spores are present on other parts of the body of the insect but are not visible against the black background of the insect's body. It is concluded, therefore, that the transmission is "external-mechanical."

In order to determine whether the infection may be also carried by the third method of transmission, viz., internally (mechanically or biologically) the following plan of investigation was outlined.

1. Gall flowers were removed from a caprifig just before the insects issued and were placed on a poured agar plate; some unsterilized, some after sterilization by momentary dipping in 95 per cent alcohol, and others after dipping in mercuric chloride 1:5000 solution, for 1, 2, or 3 minutes, followed by washing in sterile water until the washings were free from chlorine as shown by adding a drop of silver nitrate. To facilitate the handling of such small objects through these steps, the material was kept in sterile Gooch crucibles through all the washings and picked out of the crucible by means of a sterile wire having one end flattened and bent at a right angle.

2. Insects were removed with the aid of sterile needles from their galls, which were sterilized as in (1). The insect and its gall in each case was plated separately.

3. Adults were collected as they were wandering in the cavity after they came out of the gall but before they left the receptacle, and plated, some unsterilized and some sterilized as in (1). After sterilization some insects were crushed and some were not.

Since the infection in the cavity of the fig cannot be determined by other than cultural means, the unsterilized gall flowers from the fig used in each experiment served as an index of infection and a check on the effectiveness of sterilization. Under these circumstances a large number of plates had to be poured from a considerable number of figs in order to secure sufficient data on which to base conclusions. The galls were removed from each receptacle by cutting through the stalk with a sterile knife. They were placed in a sterile Petri dish and mixed, after which they were divided into lots of ten and one lot was used in each experiment. The results showed that sterilization with $HgCl_2$ as described above, was effective. Flowers showing both fungus and bacterial infection when plated unsterilized, were found sterile after sterilization, both when crushed on the plate and when left uncrushed. The purpose of crushing was to facilitate the development of parasites if any were present. The results also showed that the insect is sterile inside the gall, since after external sterilization of infected galls, no more growth was obtained when the gall containing the insect was plated after crushing, or when the insect was removed

from the gall by means of sterilized needles and both insect and gall plated separately. It is possible that the $HgCl_2$ penetrated the tissue of the gall and thus disinfected the insect also, although this is rather doubtful, since a few of the insects were found alive upon extraction from the gall, and these were sterile.

The results from step three of the scheme of attack were not as conclusive as those of steps one and two. Insects picked from inside the cavity of the fig, from the vials (plate 9) and as they were coming out one by one through the eye of the fig were plated, both unsterilized and sterilized, as mentioned previously. When unsterilized insects were plated they usually developed the cryptogamic flora of the fig from which they were issuing. If the fig was sterile or contained the fungus or either of the two bacteria or any combination of the three organisms that constitute the cryptogamic flora of the caprified fig and caprifigs, the same was found to be true of the blastophaga in the majority of cases. Over four hundred insects were used in this phase of the studies. After the insects had become infected no method of sterilization proved completely effective. It has been mentioned before that the body and attachments of the adult insects are covered with spines. Spores lodged among these spines might easily escape killing even when alcohol is used to wet the surface of the body before applying the mercuric chloride solution. It was noticed that the growth on the plates was slow in appearing and not very abundant. In many cases sterilization was effective as many insects were found to be sterile.

From these results it may be safely concluded that the infection is carried externally and mechanically, and that the insect picks up the spores in the cavity of the fig after issuing from the gall and before coming out through the eye of the fig. It seems also probable that there is no internal mechanical or biological transmission. This can be also deduced from our knowledge of the life history of the insect. The egg is laid, according to Grandi(15), inside the ovule of the gall flower between the internal integument and the nucellus. The insect develops inside the walls of the gall, feeding on the endosperm, the female does not come out until the male opens a hole in the gall walls. Unless the infection were deposited along with or carried in the egg, the chances of infection of the insect while still in the gall would be very small.

Caprification Studies.—In caprifying the Calimyrna crop the insectiferous profichi are suspended in strings, baskets, or other containers in the trees and the blastophagas coming out of such profichi

and carrying pollen enter the edible figs and, incidentally, infect them with the endosepsis pathogen if they happen to come out of an infected caprifig. In order actually to prove the connection between infected caprifigs and diseased Calimyrnas the following caprification experiments were carried out at Planada in 1924 and at Davis in 1925. Through the kindness of Mr. W. Arnold of Planada, one hundred and twenty large paper hat bags were placed on Calimyrna twigs, two weeks before any blastophagas issued from caprifigs. In each bag a twig bearing from six to ten figs was enclosed. At caprification time one profichi fig was introduced into each bag from places that were known to be heavily infected. After caprification was over the bags were removed and the figs allowed to ripen. Similarly bags were placed on Adriatic, Kadota, and Black Mission trees in the Forkner Fig Gardens Experimental Plots, and on the Adriatics of the Pasa Rica ranch near Fresno. Caprifigs from the same source as for the Calimyrnas were used. The figs were picked as they ripened, taken into the laboratory and examined for rot. If the figs looked healthy, or were doubtful, plates were poured to determine the presence of infection. No plates were poured from figs showing plainly the symptoms of the rot which developed actively. The results are summarized in table 5.

TABLE 5

PERCENTAGE OF INFECTION OF CAPRIFIED CALIMYRNA, ADRIATIC, KADOTA, AND MISSION FIGS, CAPRIFIED WITH INFECTED CAPRIFIGS

Variety	Total number caprified	Number of infected	Per cent infected
Calimyrna	698	404	57.8
Adriatic	55	42	76.3
Kadota	55	20	36.3
Mission	26	6	18.7

In the experiments of 1925 at Davis, the exact flora of each caprifig was determined by plating. Two hundred and thirty-eight twigs were enclosed in bags. One profichi was introduced in each bag at caprification time. When caprification was over and the bags removed, each caprifig from which the blastophagas had issued was taken to the laboratory and examined. If the symptoms of the disease were evident no plate was poured. If the caprifig looked normal a plate was poured, using the method previously described.

As the edible figs thus caprified were ripening they were taken to the laboratory and carefully examined. The results were according

to expectations. Rather few caprifigs used in caprifying the edible figs in the bags were found to be free from infection, but those that proved to be so produced edible figs that were entirely normal. Specifically, in one instance, the caprifig used in caprifying 16 edible figs on a White San Pedro twig proved to be free from infection. All sixteen figs on this twig were normal. Other twigs on Calimyrna, Adriatic, Black Mission, Bourjassotte Panache, Verdal Longue, and seedling trees, which carried from one to six figs, were also free from infection when caprified with non-infected blastophagas. Not all the figs from twigs caprified with infected insects were diseased, indicating that not all the blastophagas, even from an infected receptacle, carry the infection.

Hand Pollination.—It has been mentioned before that certain varieties of the caprifig (Roeding No. 1, Roeding No. 3, Milco) develope pollen without the stimulus of caprification. Such pollen-iferous but non-insectiferous caprifigs are known as blanks and were found to be free from infection with the endosepsis organism. It was thought that if the pollen from such caprifigs was introduced artificially into Calimyrnas, figs should be developed free from infection. The pollen from blanks was shaken onto a sheet of sterile paper and introduced into sterile glass eye-droppers having one end pulled to a fine capillary. The other end was plugged with cotton and connected with a rubber bulb. The capillary end was introduced into the cavity of the fig and sufficient pollen blown into the cavity to secure pollination. The pollinated figs were protected from caprification before and after pollination by enclosing the twigs in manila paper bags. The pollen and the fig from which it was obtained were plated in order to determine their flora. Green fungi were occasionally found, but no *Fusarium* nor the bacteria described previously.

The results of these experiments were entirely convincing when proper precautions were taken to exclude the insects from both the blank and the pollinated fig. Twenty-five figs were pollinated by this method in 1925 and all proved to be free from disease. The pollen should be collected from the caprifig blanks before the eye of the fig opens because the larva of an unidentified species of thrips may later enter the cavity. Plates were poured repeatedly from blanks and edible figs in order to determine the presence of the thrips larva and its effect on the flora of the fig. Of 95 uncaprified figs examined, the larva was found only in two, and the flora of such figs was never found to parallel the flora of caprified figs. Most of the figs were sterile. Occasionally several types of green fungi that bear no relation to the disease were obtained.

Pathogenesis and Life History of the Pathogen

From the foregoing discussion it is seen that the spores of the fungus are introduced by the blastophaga into the edible fig at a very early stage of growth when the fig is small, green, hard, and ready to be caprified or pollinated. The symptoms of the disease do not appear in the tissues of the fig until it is ripe. In the meantime the fig develops normally, the cavity fills, and the seeds are formed. The question is raised as to what becomes of the spores of the fungus during this interim. Do they germinate and vegetate or remain dormant until the fig tissues can be invaded? Many figs at different stages of development were examined. Occasionally some white, aerial, fungous growth, produced by the endosepsis pathogen was observed in the cavity of unripe figs. This growth invaded very little tissue, but developed mostly on the dead body of the blastophaga which was lodged between the flowers. In many figs the stigmas of certain flowers were much darker than those of others, and this color was very prominent in the flowers surrounding a dead blastophaga. In figs from orchards highly infected, it was found that sometimes the entire cavity where the styles and stigmas come together in the center presented this scorched, dark brick-red pigmentation, although no fungous growth was evident to the naked eye. The styles were removed and examined under the microscope. To facilitate the examination the styles were fixed in absolute alcohol, stained in 2 per cent solution of Magdala red in 85 per cent alcohol, dehydrated in absolute alcohol, cleared in xylol and mounted in balsam. It was found that the withered stigmas were covered with slender hyphae. Plates were poured from figs showing the dark-red stigma, and also from unpigmented ones. The fig was split under aseptic conditions and the entire flower removed by cutting the stalk with a sterile knife. The flowers were placed on poured and cooled Czapek agar plates. It was found that *F. moniliforme* var. *fici* grew regularly from dark-red, orange, or brown-colored stigmas. White or slightly yellow-colored stigmas were sterile. The fungus could be very easily seen on the plates, forming colonies radiating from dark-red stigmas. In one case dark-red and colorless stigmas were selected from the same receptacle and while the former were found infected with the fungus, nothing grew from the latter. Plate 14 (2545 and 2571) illustrates this point. It was found that wherever insects were lodged, as mentioned previously, such brown discolorations could be seen on the

style, the stigma, the stalk, and even on the side of the seed. Fungus hyphae could be seen under the microscope on all of these discolored parts.

Adriatics caprified experimentally were also examined and compared with non-caprified figs. In non-caprified figs of the Adriatic variety the stigma is colorless almost to maturity, when it shrivels and turns yellowish-brown. In caprified figs, immediately after caprification the stigmas turn yellow or yellowish-brown. This discoloration of the stigma is uniform throughout the cavity and cannot be confused with the color of the stigmas showing fungous infection. The latter usually occur in spots, except in extreme cases, and are most abundant near the body of a dead blastophaga. The same is the case in the caprifigs. The fungus was found vegetating on the stigmas until the other tissues could be invaded (plate 15, *b* and *c*).

Rotting tissue of Calimyrnas was fixed in chromacetic solution, washed in water, dehydrated, cleared, infiltrated in paraffin, and sectioned. Various differential stains were tried, as well as Haidenhain's iron-haematoxylin, Delafield's haematoxylin, and Flemming's triple stain. In most sections the disintegration of the tissues was so far advanced that no picture could be obtained of the action of the fungus on the host. However, by making a large number of sections, especially of the meat around the eye, from the margin of watersoaked spots, it was found that the fungus follows at first the vascular system and then invades the cells, growing both intercellularly and intracellularly. Figure 2 and plate 16 illustrate these points.

The life history of the pathogen can now be recapitulated. Infection takes place at caprification time, from spores carried mechanically by the caprifying insect *Blastophaga psenes* L. The spores are usually deposited near or on the flowers about the eye of the fig. The spores germinate readily and the fungus grows slowly on the stigmas of the flowers and the body of the dead insect, until the pulp and the meat commence to ripen and are in turn invaded. The fungus does not attack any part of the tree except the fruit. It does not run into the twigs from the stalk of the fig. Figs are produced at the axils of the leaves of the current year's growth, therefore they could not be infected from old cankers even if such existed. No other source of infection has been found but that of caprification. Within edible figs, therefore, the life cycle of the disease is not completed as the blastophaga is unable to breed in them. The pathogen completes its life cycle in the caprifig paralleling exactly

that of its carrier. The fungus overwinters along with the blastophaga in the mamme figs. In April the adult females carry the spores of the fungus into the profichi where they germinate and grow on the stigma of the gall flowers and the body of the dead insect. In June the adult females come out of the profichi carrying spores of the fungus on their bodies. Some of these females enter Calimyrnas and infect them, others enter mammoni caprifigs, where they carry the infection and lay their eggs. The blastophaga coming out of the mammoni transmit the infection to the mamme in September and the cycle is completed.

Distribution

The disease discussed in this paper has not been reported definitely from other countries. In a series of monographs on the fig industry of the different districts of Italy by Guglielmi(17) on the district of Lecce, Portale(26) on the district of Mistretta, De Rosa(33) on the district of Castro, Siniscalchi(39) on the province of Salermo, and Ferrari(13) on the district of Cosenza, although the diseases of the fig are discussed, no mention is made of internal rot. A gummosis of the fruit of the Dottato (Kadota) is mentioned by Ferrari(13) from the district of Cosenza and discussed in relation to a bacteriosis of the tree. He states that those figs attacked by the disease when nearly ripe show a drop of liquid at the eye first yellow, then reddish, which increases in volume and if the infection is heavy, is exuded. This description corresponds rather to souring, especially since the Dottato is a parthenocarpic variety. No mention of internal rot is made by Trabut(42) or Guillochon(18) from North Africa (Algiers), or Esterlich(12) from Spain in their treatises on the fig. Vallese(44) describes a disease of the fruit in Italy that may possibly be the same as endosepsis. He states that the spoiled fruits ripen without presenting external signs of the disease. The pulp, however, turns pale brown and the sweet juices change to bitter and are extremely disgusting. The most susceptible variety is the red Dottato. Probably this rot is caused by a fermentation of the sugar of the fig started by a bacterium.

Efforts have been made to ascertain whether endosepsis is found in Asia Minor, Algeria, and Greece, where the practice of caprification is in general use. No reference in literature was found. Dried figs from Asia Minor secured by Mr. Condit were examined and plates poured, but without definite results. *Aspergillus* sp. and a few yeasts were the fungi obtained. It was thought that if caprifigs could be

secured from the above mentioned countries in the winter, the flora secured from plating these caprifigs would give an idea regarding the presence of the internal rot in these countries. Permission was obtained from the Federal Horticultural Board and letters were written to scientists and others in the Mediterranean countries. Several responded very kindly and I wish to express my obligation to Dr. Cass A. Reed, of the International College, Smyrna, Dr. L. Trabut, of Algiers; Mr. D. P. Caldis, of Mytilene, Greece; Mr. E. Kefalas, of Samos, Greece; and Mr. C. E. Dickerson, Assistant Trade Commissioner, American Embassy, Athens, Greece, for supplying me with caprifigs.

A number of fungi, mostly of the genus Fusarium, were secured from these caprifigs, but preliminary observations did not disclose these to be the same as *F. moniliforme fici,* the organism responsible for internal rot in California.

SUMMARY

A disease of the Calimyrna and other caprified figs is here described for the first time, and the synonymy and economic importance are discussed. The name endosepsis (internal rot) is proposed.

The disease is caused by *Fusarium moniliforme* var. *fici* n. var., which has been found permeating the diseased tissues, constantly isolated from them, proven to be pathogenic when inoculated and readily reisolated. Strain 93 is considered the type form of the new variety.

The above fungus, together with two bacteria, constitute a typical flora in caprified figs in California.

The organisms constituting this flora are described and their physiological and cultural reactions given. Their taxonomic relations are discussed.

These organisms were found to be transmitted mechanically on the body of the caprifying insect, *Blastophaga psenes* L.

The life history of the pathogen is outlined.

Figs were found to be sterile internally up to the time when they are entered by insects.

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(47) WOLLENWEBER, H. E., C. D. SHERBAKOFF, O. A. REINKING, H. JOHANN, and A. A. BAILEY
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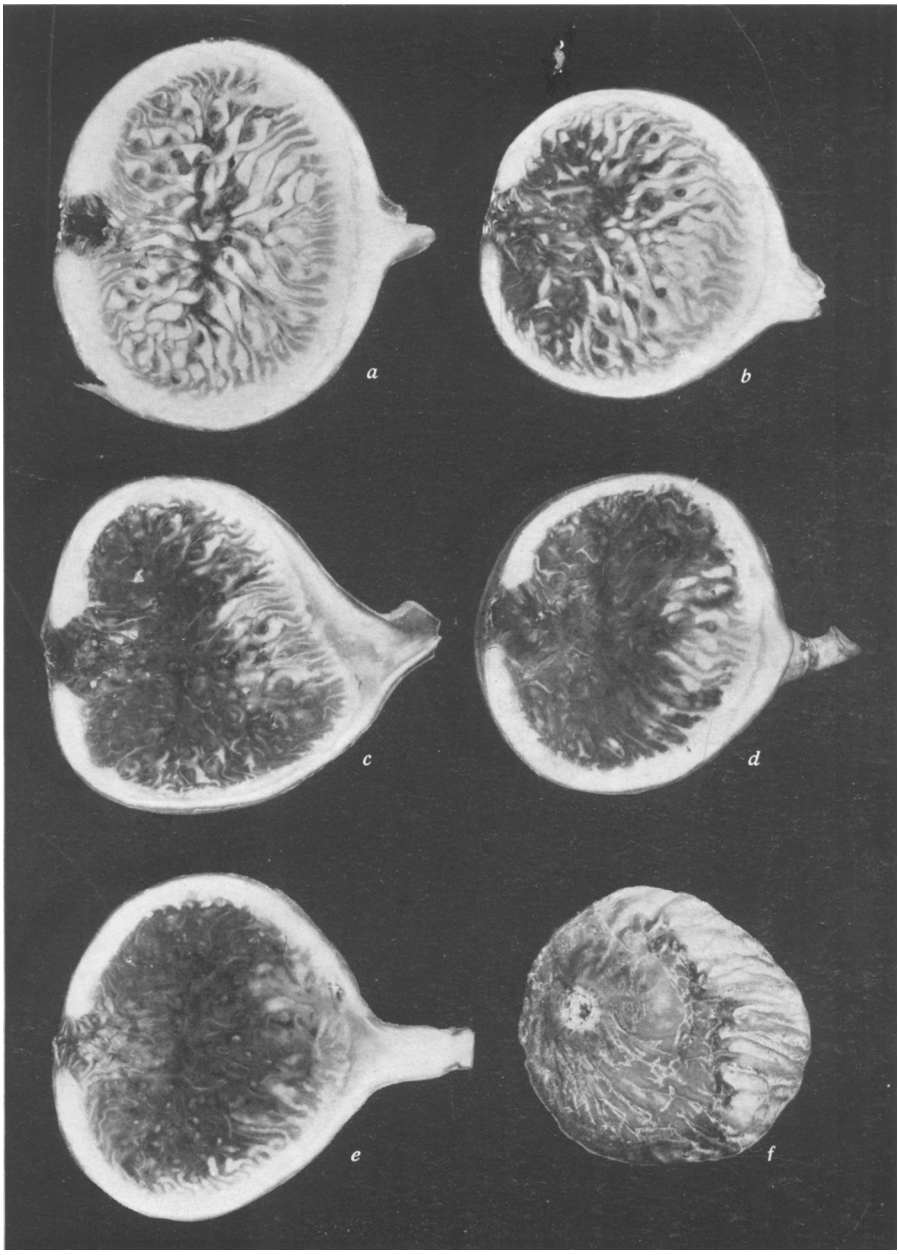
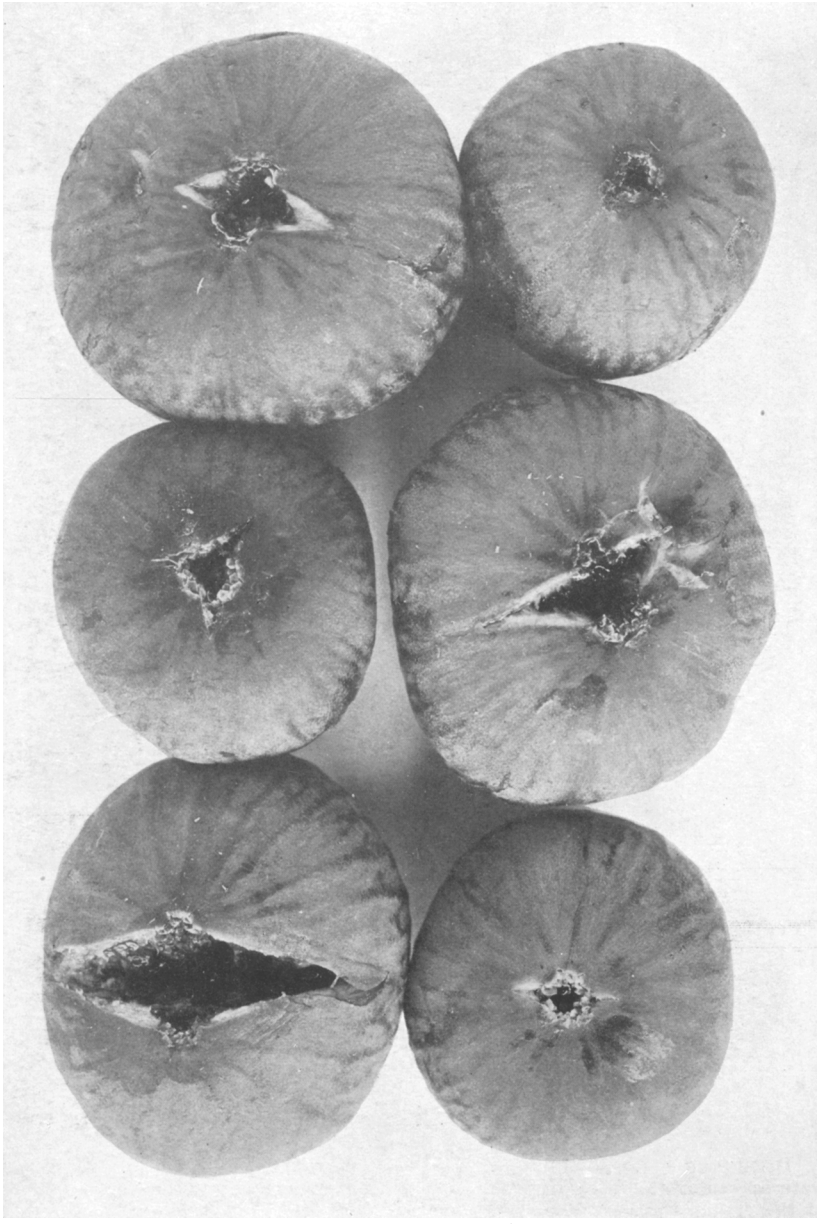
PLATES 1-16

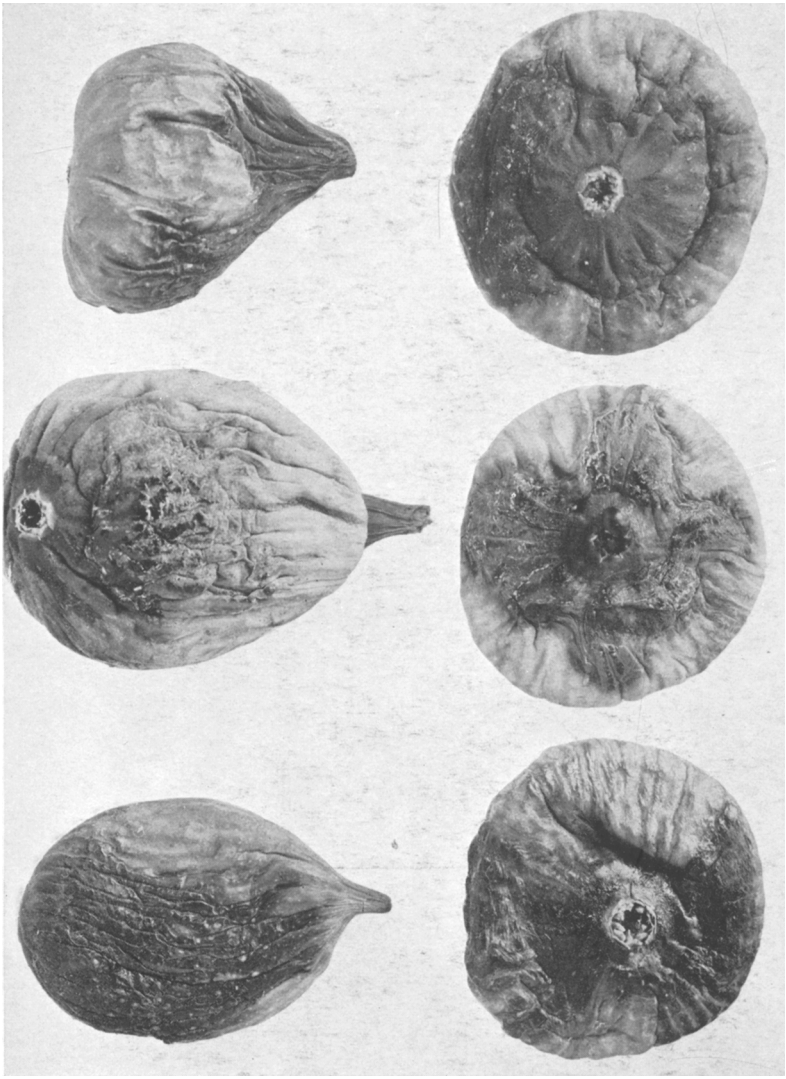
Transmitted September, 1926.



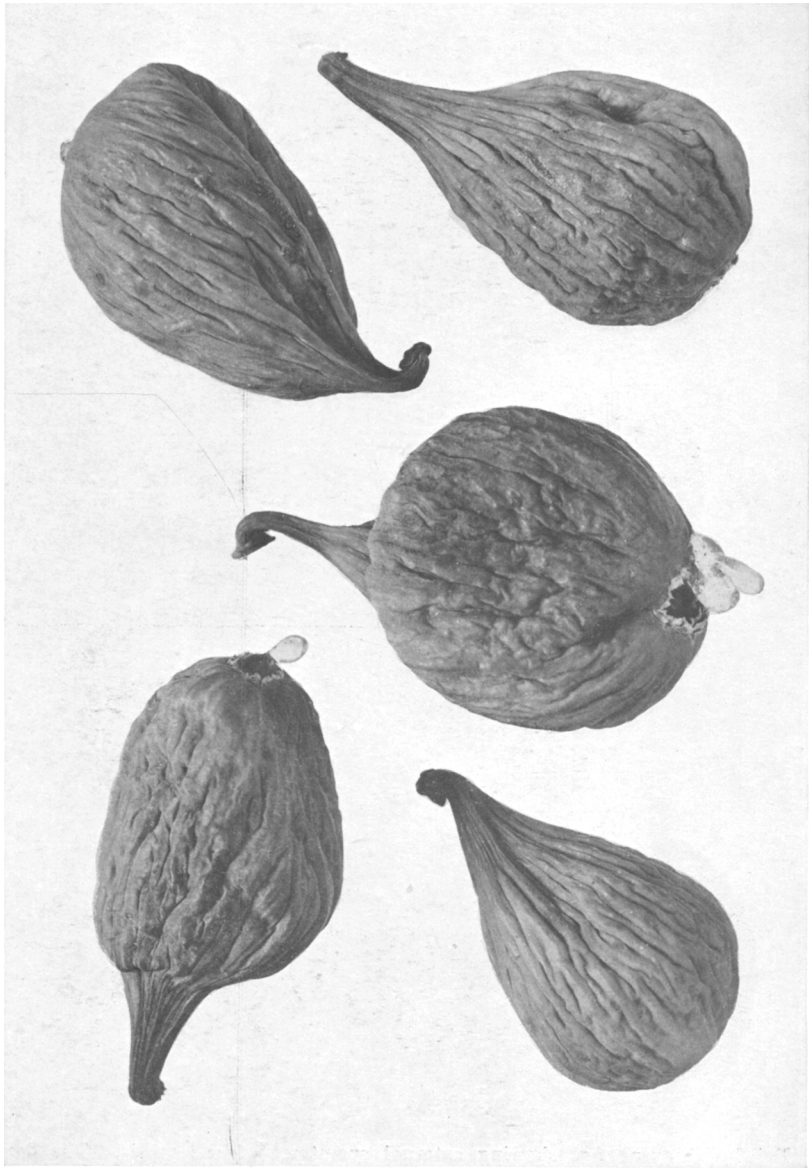
Calimyrna figs at stage of maturity ready for picking for fresh shipment or canning, showing progressive internal symptoms of endosepsis. External appearance normal in the first five. *a*, Normal fig. *b*, Flowers around the eye diseased. *c*, Three-fourths of the pulp diseased. *d*, Seven-eighths of the pulp diseased. *e*, Entire pulp diseased. *f*, External symptoms. Meat invaded.



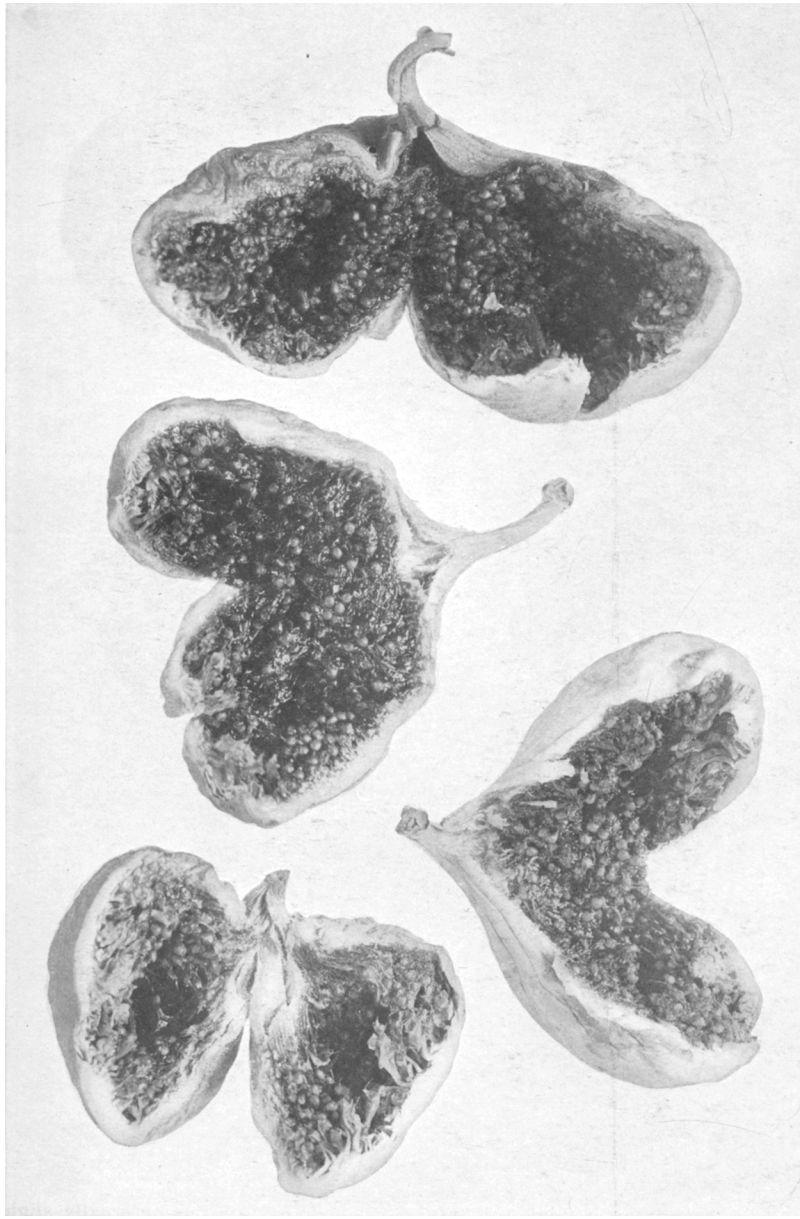
Calimyrna figs showing external symptoms of endosepsis.



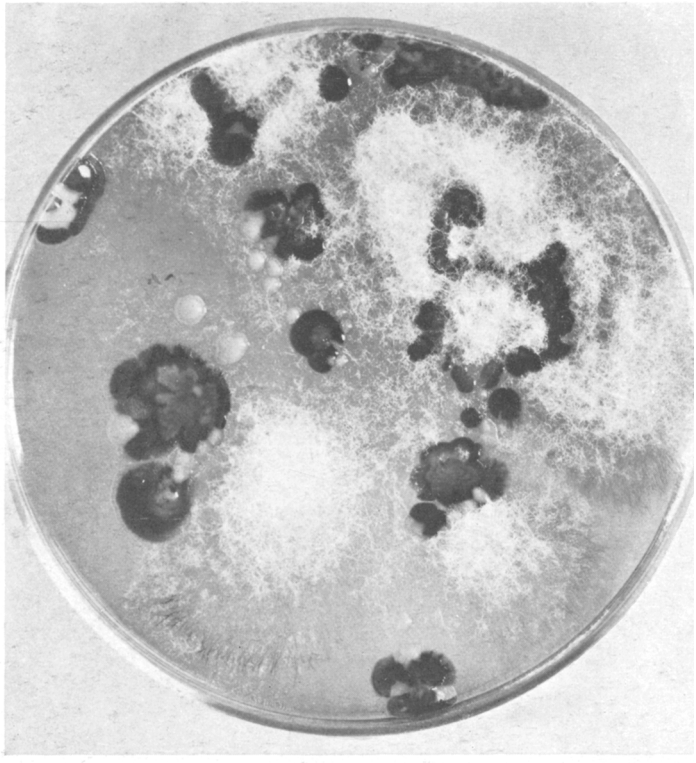
Dried Calimyrna figs showing external symptoms of endosepsis. The dark watersoaked spots are pink, red, or purple in color. Notice the small drop of gum at the eye of one fig.



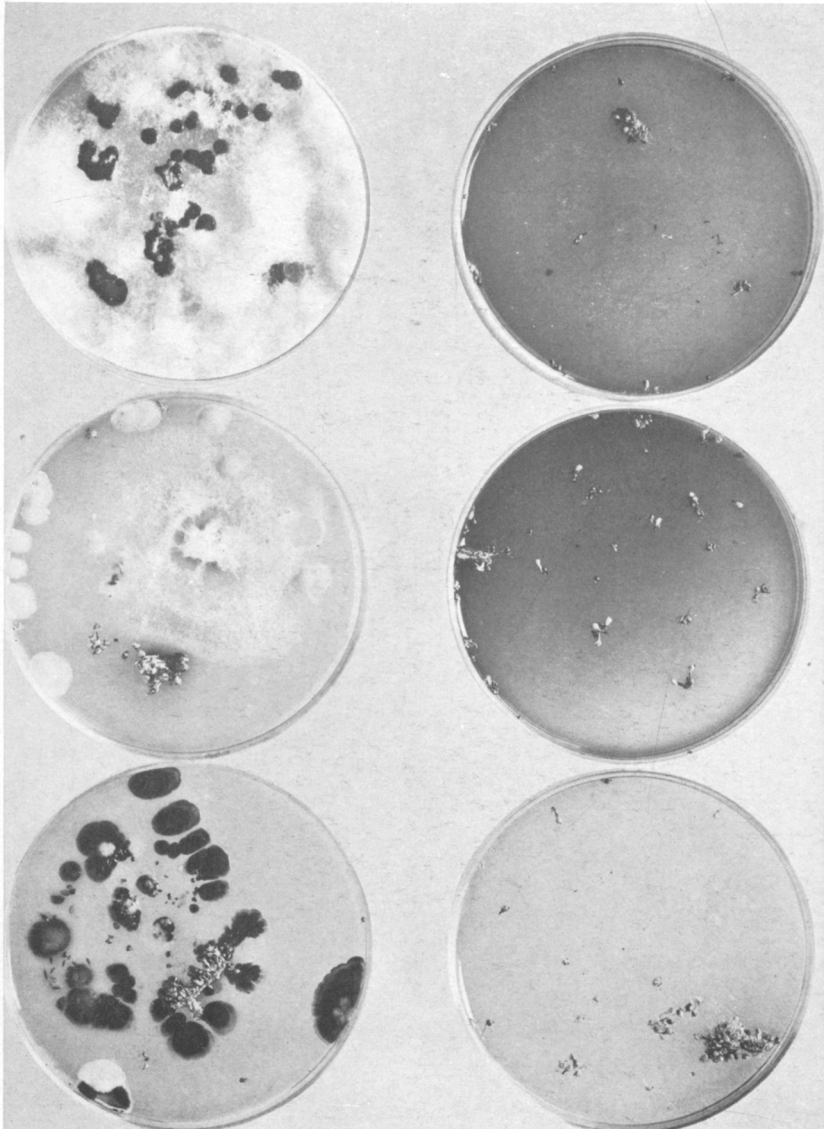
Dried Calimyrna figs of practically normal appearance but internally slightly affected with endosepsis.



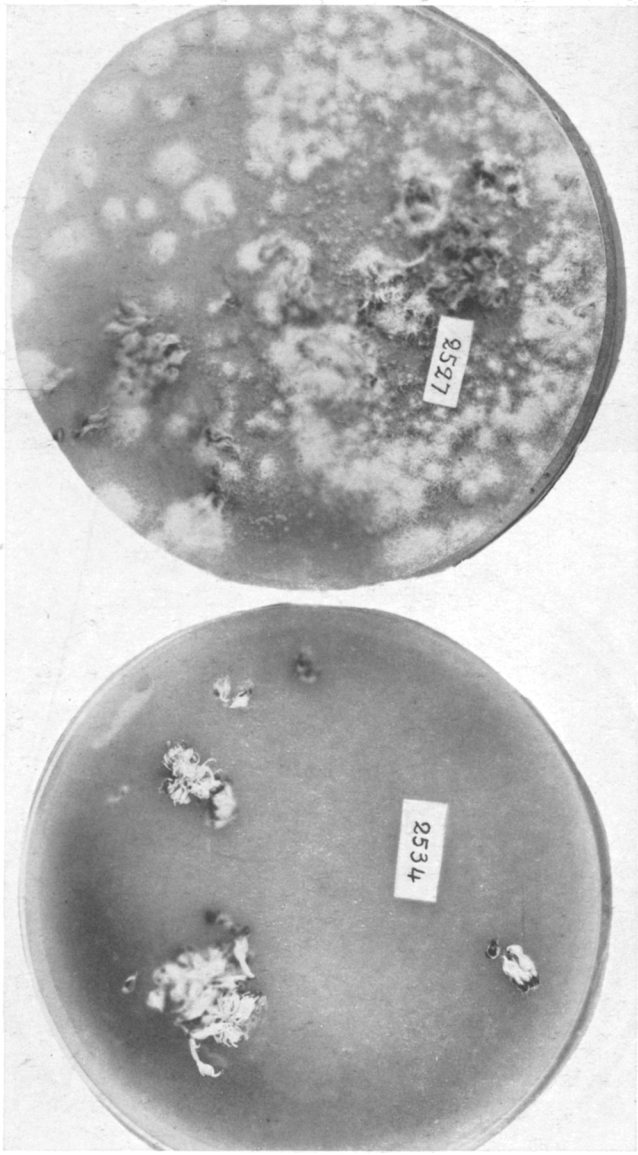
Interior of dried figs shown in plate 4, slightly affected with endosepsis. The pulp is dry and "seedy" and has a peculiar flavor.



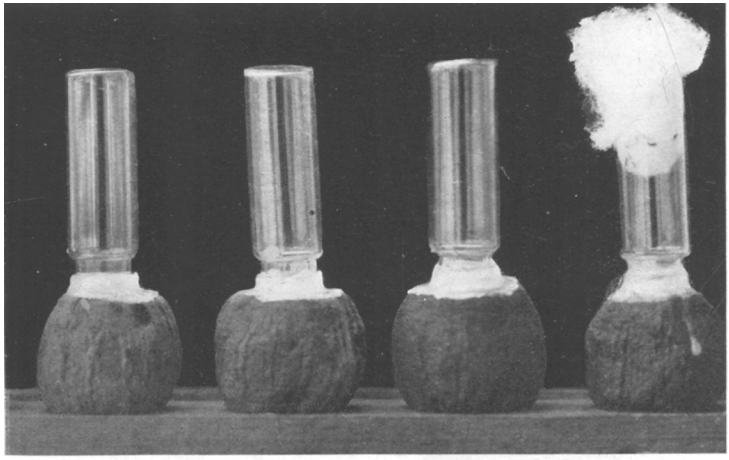
Typical flora of a caprified fig, showing the three characteristic organisms.

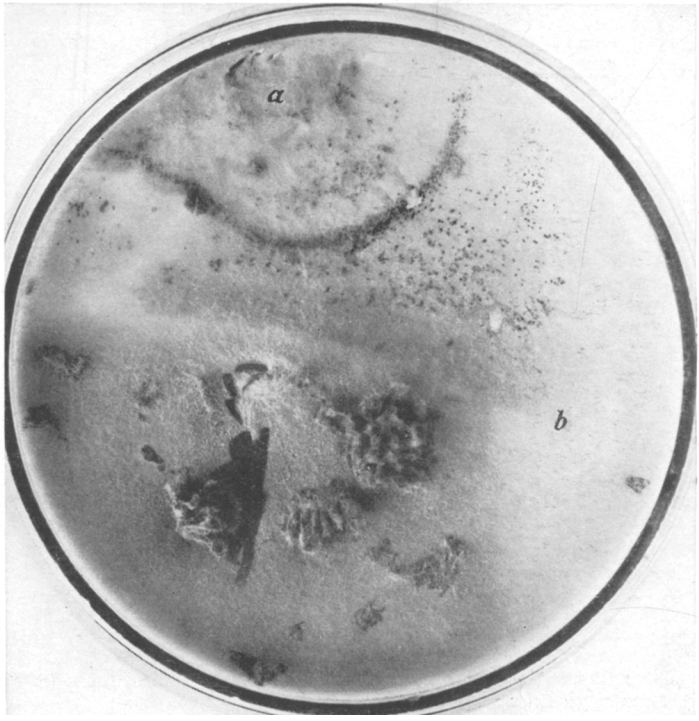


Left, typical plates from unripe, caprified, Calimyrna figs. Right, plates poured from uncaprified, unripe, Mission, Kadota, and Adriatic figs; sterile.

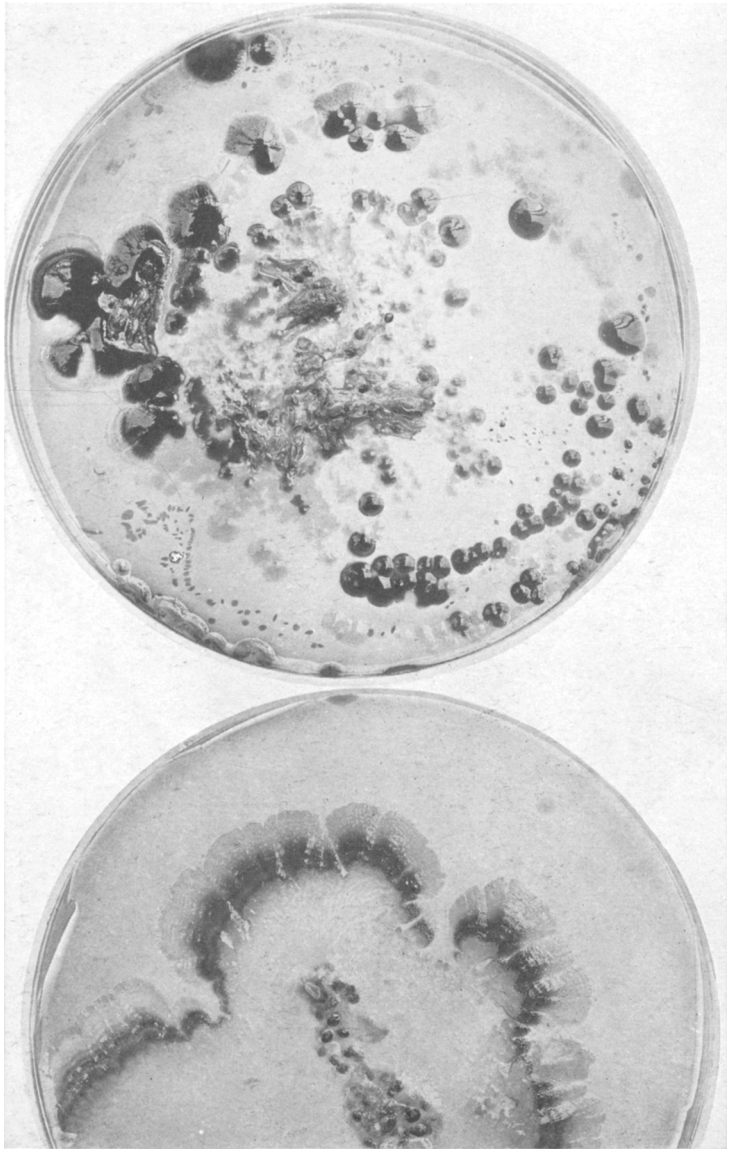


2527, flora of a caprified, unripe Adriatic. 2534, flora of a non-caprified Adriatic; sterile.

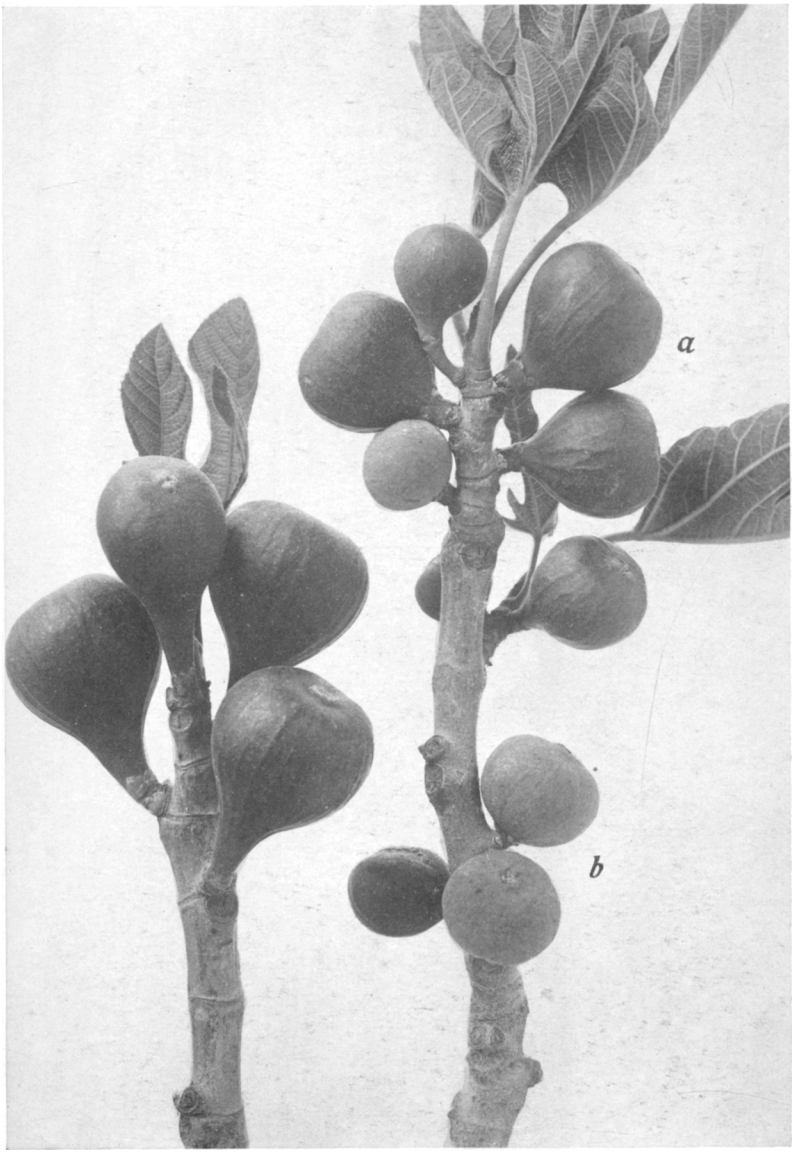




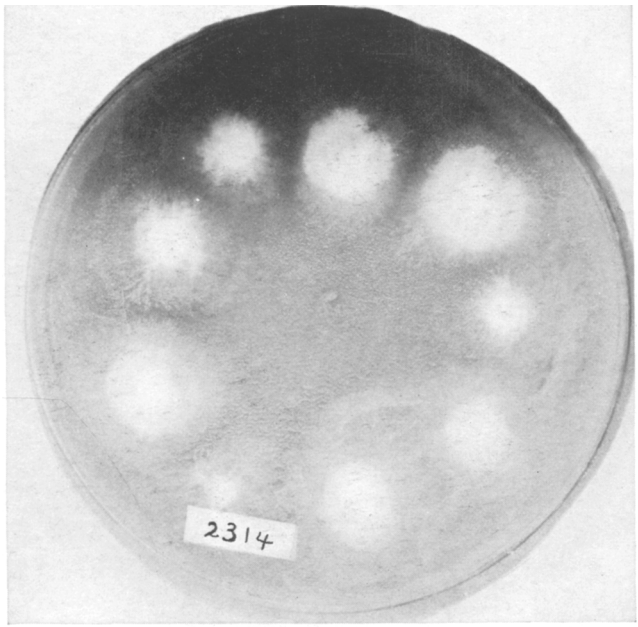
Above, method of catching blastophaga as they issue from caprifigs. Below, formation of perithecia in *F. moniliforme fici.*

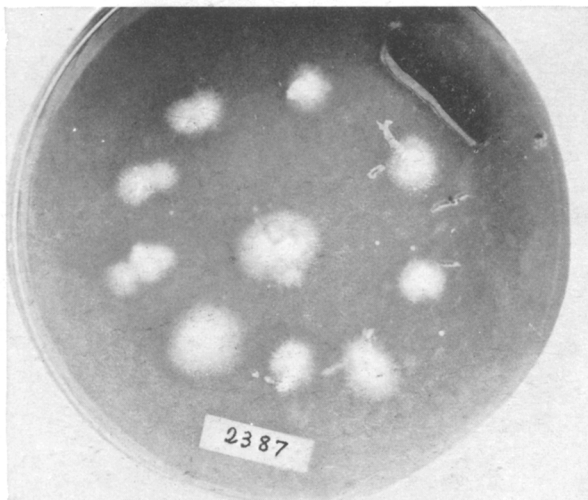


Two plates illustrating the growth of the red chromogenic bacterium obtained regularly from caprified figs.



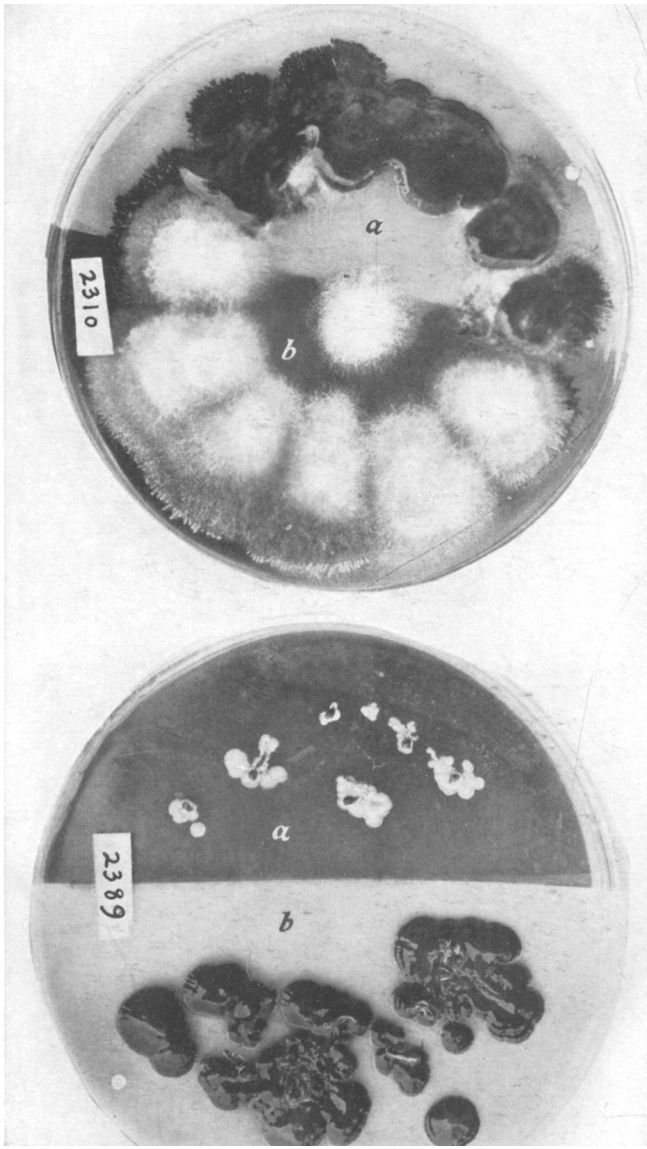
Caprifigs at the time of infection. *a*, Profichi. *b*, Mamme.





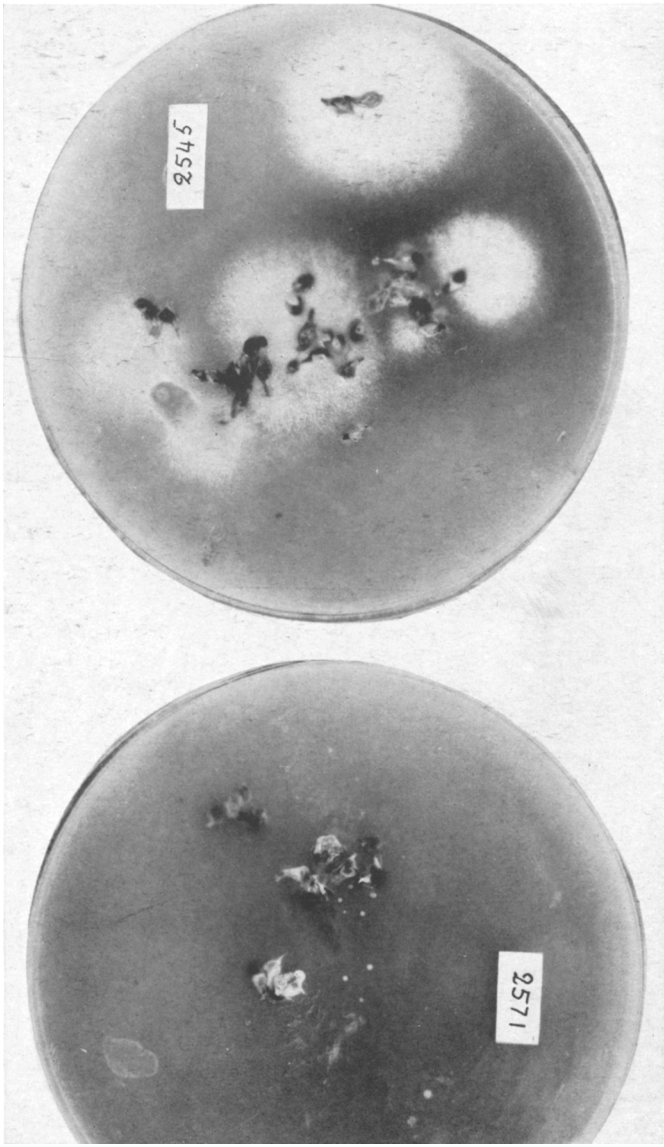
2387, galls picked from the cavity of an infected profichi. Notice the typical growth of *F. moniliforme fici.*

2314, blastophaga caught in the traps shown in plate 9 were placed on this poured agar plate. Notice the growth from each. The central puncture represents the control.



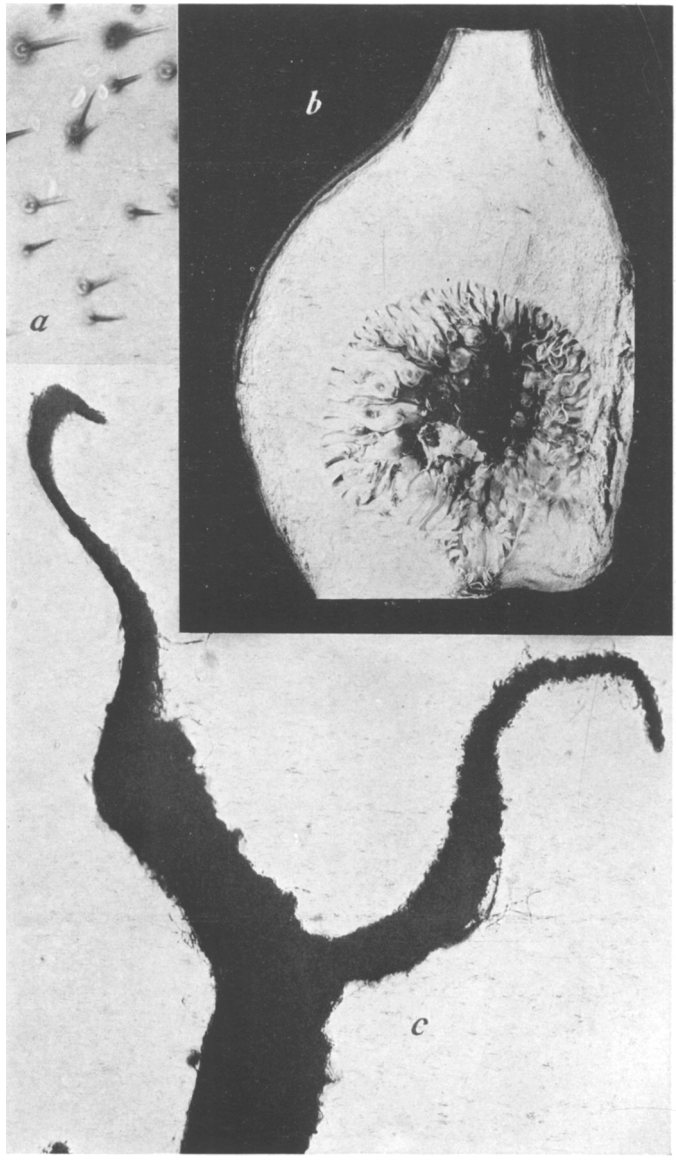
2310. *a*, Growth from blastophaga taken from a fig infected with red bacterium and white fungus. *b*, Growth from blastophaga taken from a fig infected only with white fungus.

2389. *a*, Growth from galls from a fig infected with white bacteria. *b*, Growth from galls taken from a fig infected with red bacteria.

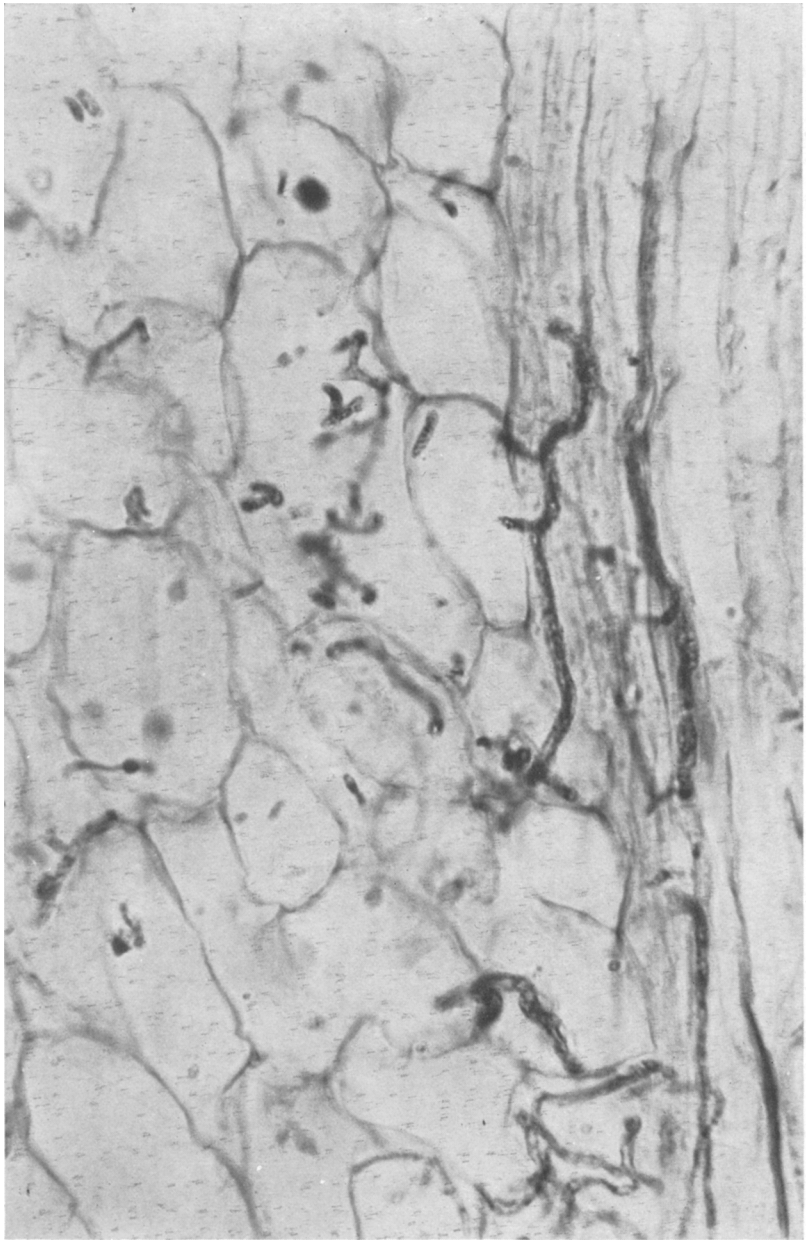


2545, plate poured from flowers showing a brick-red colored stigma. Notice the fungous colonies.

2571, plate poured from flowers showing a light yellow colored stigma. Notice the absence of fungus.



a, Part of the wing of a blastophaga, showing the spores of the fungus. *b*, Profichi fig, showing growth of the fungus in the cavity. *c*, Stigma of infected fig flower, showing fungus.



Photomicrograph of a section of ripe Calimyrna meat tissue, showing intercellular and intracellular penetration of the fungous hyphae.

The titles of the Technical Papers of the California Agricultural Experiment Station, Nos. 1 to 20, which HILGARDIA replaces, and copies of which may be had on application to the Publication Secretary, Agricultural Experiment Station, Berkeley, are as follows:

1. The Removal of Sodium Carbonate from Soils, by Walter P. Kelley and Edward E. Thomas. January, 1923.
3. The Formation of Sodium Carbonate in Soils, by Arthur B. Cummins and Walter P. Kelley. March, 1923.
4. Effect of Sodium Chlorid and Calcium Chlorid upon the Growth and Composition of Young Orange Trees, by H. S. Reed and A. R. C. Haas. April, 1923.
5. Citrus Blast and Black Pit, by H. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.
6. A Study of Deciduous Fruit Tree Rootstocks with Special Reference to Their Identification, by Myer J. Heppner. June, 1923.
7. A Study of the Darkening of Apple Tissue, by E. L. Overholser and W. V. Cruess. June, 1923.
8. Effect of Salts on the Intake of Inorganic Elements and on the Buffer System of the Plant, by D. R. Hoagland and J. C. Martin. July, 1923.
9. Experiments on the Reclamation of Alkali Soils by Leaching with Water and Gypsum, by P. L. Hibbard. August, 1923.
10. The Seasonal Variation of the Soil Moisture in a Walnut Grove in Relation to Hygroscopic Coefficient, by L. D. Batchelor and H. S. Reed. September, 1923.
11. Studies on the Effects of Sodium, Potassium, and Calcium on Young Orange Trees, by H. S. Reed and A. R. C. Haas. October, 1923.
12. The Effect of the Plant on the Reaction of the Culture Solution, by D. R. Hoagland. November, 1923.
13. Some Mutual Effects on Soil and Plant Induced by Added Solutes, by John S. Burd and J. C. Martin. December, 1923.
14. The Respiration of Potato Tubers in Relation to the Occurrence of Blackheart, by J. P. Bennett and E. T. Bartholomew. January, 1924.
15. Replaceable Bases in Soils, by Walter P. Kelley and S. Melvin Brown. February, 1924.
16. The Moisture Equivalent as Influenced by the Amount of Soil Used in its Determination, by F. J. Veihmeyer, O. W. Israelsen and J. P. Conrad. September, 1924.
17. Nutrient and Toxic Effects of Certain Ions on Citrus and Walnut Trees with Especial Reference to the Concentration and Ph of the Medium, by H. S. Reed and A. R. C. Haas. October, 1924.
18. Factors Influencing the Rate of Germination of Seed of Asparagus officinalis, by H. A. Borthwick. March, 1925.
19. The Relation of the Subcutaneous Administration of Living Bacterium abortum to the Immunity and Carrier Problem of Bovine Infectious Abortion, by George H. Hart and Jacob Traum. April, 1925.
20. A Study of the Conductive Tissues in Shoots of the Bartlett Pear and the Relationship of Food Movement to Dominance of the Apical Buds, by Frank E. Gardner. April, 1925.