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FRUIT-ROTTING SCLEROTINIAS

II. The American Brown-Rot Fungi*

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SUMMARY

Comparative studies of a large number of single-spore strains of Sclerotinia have furnished information as to the identity of forms present in this country, as well as confirming Wormald's conclusion that the common American brown-rot organism is distinct from *Sclerotinia cinerea,* which he found only in Europe, and establishing additional differential characteristics. On the basis of these differences *Sclerotinia cinerea* (Bon.) Schr. forma *americana* Wormald has been raised to specific rank as *Sclerotinia americana* (Wormald) Norton and Ezekiel.

Sclerotinia americana can be distinguished from *S. cinerea* macroscopically by differences in the habit of growth on potato dextrose agar, the rate of oxidase production in liquid culture media, and the character of growth on inoculated fruits. The habit of growth in drop culture, under standardized conditions, furnishes conclusive identification, the cells of hyphae of *americana* averaging almost twice the length of those of *cinerea* forma *pruni* as well as showing marked differences in the mode of branching and general mycelial habits of growth.

Sclerotinia cinerea is reported in this country for the first time. The Oregon spur-blight Monilia, mentioned in previous literature, and three strains isolated by the writer from fruit from Mountain View, California, were identified as *S. cinerea* forma *pruni.* A single collection from Washington, D. C., coincided with a culture of "*Monilia cinerea* forma *mali*" obtained from Wormald. *S. fructi-*

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gena was not isolated in this investigation from this country nor has it been authentically reported here in the literature.

Anastomosis occurred between adjacent colonies of similar and dissimilar strains grown simultaneously in Petri dishes, but no further activity could be detected. Development of apothecia from fruits inoculated with single-spore strains showed *S. americana* to be homothallic.

The minimum, optimum, and maximum temperatures for growth in culture of *S. fructigena, cinerea,* and *americana* fell near 3°, 25° and 33° C., respectively.

Strains of *S. americana* show wide variation in cultural characteristics, effect on rotting fruits, size of conidia, and shape of apothecia. Except for the last, these variations have been proved genetic constants of the respective strains.

A classification of *S. americana* into six varieties, of which var. I is considered the type variety for the species, is presented on the basis of the cultural characteristics.

INTRODUCTION

The brown-rot diseases of stone and pome fruits, caused by species of Sclerotinia, constitute a most cosmopolitan and destructive group of plant diseases. In the United States injury occurs through a range nearly co-existent with the distribution of the host plants but is generally limited to fruit rot and blossom blight of stone fruits. It has usually been assumed that the same organism was responsible for brown-rot throughout the country and that it was identical with the earlier known *Sclerotinia cinerea* of Europe. The recent work of Wormald[1] (38), who concluded from extensive investigations that "*Sclerotinia cinerea* forma *americana*" was different from the European forms, showed the need for further investigation along this line.

Information has also been lacking as to the extent, persistence and significance of variation within the American species. The fact that such variations existed was early reported by Reade (29) who, however, stated that these variations disappeared when the fungi were cultivated on the same medium under uniform conditions.

The purpose of the investigation reported herewith has accordingly been to establish the relationship of the organisms causing brown-rot in this country to each other and to the European forms.

The work was initiated in 1920. During the first year material assistance was rendered by Mr. W. J. Sando, who was at that time a student in the department. The writer wishes also to express his appreciation to Dr. J. B. S. Norton, in whose laboratory this work was done, for his constant assistance and advice. Many others have assisted by furnishing specimens and cultures.

[1]Reference is made by number to "Literature cited," p. 141.

HISTORICAL

The European Brown-rot Fungi. In Europe the brown-rot fungi have been known for many years. Persoon (24) discovered *Sclerotinia fructigena* in 1796, naming it at first *Torula fructigena* and later (25) *Monilia fructigena.* The considerable economic importance of the fungus was not recognized until near the end of the nineteenth century (12, 33). Schröter (32) transferred the fungus to Sclerotinia before the apothecial stage had been seen; and Woronin (42) also considered it to be a Sclerotinia without having found any apothecia. The apothecial stage of *Sclerotinia fructigena* was grown and described by Aderhold and Ruhland (1).

Sclerotinia cinera has not been known for such a long time and has been frequently confused, in Europe with *S. fructigena,* and in America with *S. americana.* It was first described by Bonorden (3) in 1851. Not until Woronin (42) showed that the conidia of *cinerea* are smaller and of a more grayish color than those of *fructigena* were these species generally differentiated.

A specialized form on apricots was described by Ehrenberg (8), and Aderhold and Ruhland (1) found an apothecial stage arising from apricots which they named *"Sclerotinia laxa."* Another form has been described by Miss Westerdijk (35) from apothecia on cherries. The only forms generally differentiated, however, until recently have been *S. cinerea* and *fructigena,* on the basis chiefly of Woronin's investigations. Wormald has in extensive investigations in England (38, 39) found that he could also distinguish two forms of *Monilia cinerea* which caused different diseases on fruit trees and could be distinguished by their cultural characteristics from *Sclerotinia cinerea* forma *americana.* He has since found (40) the apothecial stage of his *S. cinerea* forma *pruni.* Killian (17) made an elaborate study of two types of *Monilia cinerea* isolated from sour cherries and sweet cherries and apparently corresponding to Wormald's forma *mali* and *pruni.*

Brown-rot Fungi in America. In this country Peck (23) described brown-rot in 1880 as one of the commoner diseases of fruits. Apparently the first mention of the occurrence of the perfect stage of a brown-rot fungus is a description by Winter (37) of *Ciboria fructicola* from Pennsylvania in 1883. This name, however, cannot be assigned to any of the species now known, since the description does not differentiate between them. The name generally applied to the pathogen in earlier publications was *Monilia fructigena,* and after Norton (19) discovered the apothecial stage in 1902 the fungus was designated as *Sclerotinia fructigena.* Later investigations by Aderhold and Ruhland (1), Pollock (26), Matheny (18), Conel (6), Valleau (34), and others, have led to the general belief that the common American brown-rot fungus corresponded "more closely to *S. cinerea* than to *S. fructigena.*"

Reade (29) was the first to note the presence of cultural strains

in the fungus. Ames (2) noted that the temperature relations of a strain of Monilia isolated from peaches were not the same as in a plum strain. Jackson (15) described in Oregon a disease of pears due to a Sclerotinia which was studied by Posey (27), who considered it different from the fungus commonly attacking stone fruits in this country. Cook (7) has recently found variations in the rate of growth and the size of conidia in cultures isolated from rotted apples.

In preliminary reports (21) of the results submitted in the present paper it has already been stated that, as found by Wormald, the common American brown-rot fungus is distinct not only from *S. fructigena,* but also from *S. cinerea;* and Wormald's name *S. cinerea* forma *americana* raised to specific rank as *S. americana.* The presence of distinct genetic varieties of differing cultural characteristics within *S. americana* (10) was reported at the same time.

METHODS

Points of Attack. Previous classification of the brown-rot fungi in this country has been based chiefly on the morphological characteristics displayed under natural conditions. Since no method of producing the apothecial stage at will with this type of Sclerotinia has been published, studies of apothecial characteristics were necessarily with material of this sort. In the present investigation more exact methods as used by Wormald and Killian in recent detailed studies of the European forms have been utilized. Instead of basing classification on material collected under natural conditions, and hence to be obtained in the same condition again only under fortuitous circumstances, an attempt has been made to use only material from artificial culture, grown under reproducible conditions and accordingly representing the responses of the organisms concerned to a definite environment rather than to an unknown complex of conditions.

The particular methods used are discussed in connection with experiments in which they were involved. The more significant studies have been on the characteristics of the strains in artificial culture; on various fruits inoculated in the laboratory under controlled conditions; the comparative oxidase production in liquid culture media; and the type of growth produced by germinating conidia in drop cultures.

Strains. The word "strain" is used throughout this paper to designate a pure line culture and with no further taxonomic significance. Strains from the same or different sources may be identical in their characteristics and yet be referred to by separate strain numbers.

Strains were collected from five different regions of Maryland, a number were secured from other parts of the United States, and some from England and Holland for comparison. A list of the strains studied is given in Table I.

TABLE I.

List of strains of Sclerotinia studied. [*Those derived from the same individual fruit or apothecium are indicated by a "θ" between the strain numbers. With strains not isolated by the writer the date of receipt of the culture is given, in parentheses, instead of the date of collection.*]

Strain	Origin		Date collected
	Geographical	Host, etc.	
S 1 θ S 2	Maryland	Peach, isolated from old mummy	Dec. 19, 1920
S 3	Maryland	Plum, "Red June"	June 14, 1921
S 4 θ S 22	Maryland	Peach, apothecium	March 26, 1921
S 5 θ S 21	Maryland	Peach, apothecium	March 26, 1921
S 6	Maryland	Apple, "Bonum"	Oct. 6, 1920
S 8	Maryland	Apple, "Smokehouse"	Oct. 6, 1920
S 10 θ S 23	Washington, D. C.	Apple, "King David," collected in home storage cellar by Mr. W. J. Sando	Oct. 31, 1920
S 11	New York	Peach canker, isolated by Dr. R. A. Jehle	(Jan. 10, 1921)
S 13	Washington State	Apple, isolated by Dr. Jehle	(Jan. 10, 1921)
S 14 θ S 19	Washington State	Apple, isolated by Dr. Jehle	(Jan. 10, 1921)
S 20	Maryland	Peach, apothecium	March 26, 1921
S 21 θ S 9			
S 22 θ S 4			
S 23 θ S 10			
S 24	Maryland	Peach	June 20, 1921
S 25	Maryland	Peach	June 20, 1921
S 26	Washington, D. C., market, probably from Georgia	Peach	June 15, 1921
S 27	Maryland	Plum, "Red June"	June 14, 1921
S 28	Maryland	Plum	June 20, 1921
S 29	Maryland	Plum	June 20, 1921
S 30	Maryland	Plum	June 20, 1921
S 31	Maryland	Plum	June 20, 1921
S 33	Maryland	Peach	Aug. 2, 1921
S 34	Maryland	Peach	Aug. 2, 1921
S 35	Maryland	Peach	Aug. 2, 1921
S 36	Maryland	Peach	Aug. 2, 1921
S 37	Washington, D. C., market, probably from Maryland	Peach	Aug. 4, 1921
S 40 θ S 41	New Jersey	Peach twig canker, isolated by Dr. Mel. T. Cook	(Dec. 17, 1921)
S 42	England	Apple, *Sclerotinia fructigena* isolated by Dr. H. Wormald	(April 3, 1922)
S 43	England	*S. cinerea* forma *mali* isolated by Dr. Wormald	(April 3, 1922)
S 44	England	*S. cinerea* forma *pruni* isolated from ascospore by Dr. Wormald	(Nov. 3, 1922)

TABLE I.—*Continued.*

Strain	Origin		Date collected
	Geographical	Host, etc.	
S 45	California	Apricot, "Blenheim," sent by Mr. B. A. Rudolph	Aug. 1, 1923
S 46 & S 47	California	Peach, sent by Mr. Rudolph	Aug. 1, 1923
S 49	California	Cherry, "Napoleon," sent by Mr. Rudolph	June 25, 1923
S 50	Maryland	Apple, "Winesap"	Nov. 11, 1923
S 51	Maryland	Apple, "Winesap"	Nov. 11, 1923
S 52	Holland	*Sclerotinia fructigena* isolated from *Pyrus malus* by Dr. Johanna Westerdijk	(Nov. 24, 1923)
S 53	Holland	*S. cinerea* isolated from "Bismarck" apple by Dr. Westerdijk	(Nov. 24, 1923)
S 54	Holland	*S. cinerea* isolated from sour cherry by Dr. Chr. Berkhout	(Nov. 24, 1923)
S 55	Holland	*S. cinerea* isolated from *Prunus pseudo-cerasus* by Miss Kruseman	(Nov. 24, 1923)
S 56	Oregon	Pear. "Pacific Coast spur-blight Monilia" isolated by Prof. H. P. Barss	(Aug. 24, 1923)
S 57	Maryland	Peach, apothecium	March 14, 1924
S 58	Maryland	Peach, apothecium	March 14, 1924
S 59	Maryland	Peach, apothecium	March 28, 1924

Except with S 42, 43, 44, 53, and 54, single-spore cultures were prepared by the Keitt (16) method, which gave excellent results. Spores were allowed to germinate for 18 hours in potato dextrose agar, the location of isolated sporelings then marked on the reverse of the Petri dish using the 16 mm. objective, and the spore transferred to a new dish. Here observations with an 8 mm. objective established the presence of growth from only a single spore.

Stock cultures of the strains were generally carried in tubes of potato dextrose agar.

Culture Media

Potato decoction. 200 gm. of pared potatoes, cut into small pieces, placed at once, before oxidase activity browned the edges, in 1000 cc. of cold distilled water. Allowed to simmer for 1½ hours, strained through cheesecloth, filtered through absorbent cotton using suction, and made up to 1000 cc. Unless it was to be used in preparation of another medium, as below, it was then tubed and autoclaved at fifteen pounds pressure for twenty minutes. After autoclaving, the pH was usually between 5.4 and 6.0.

Potato dextrose decoction. 10 gm. of dextrose ("Difco") dissolved in 1000 cc. of potato decoction prepared as above, filtered rapidly through absorbent cotton, tubed and autoclaved. The hydrogen-ion concentration of fourteen lots of media prepared in this manner was determined electrometrically and also in some cases by the Gillespie drop-ratio method (11), with similar results by either method. The pH for all the autoclaved material, ready for use, fell between 5.0 and 5.8; mostly between 5.3 and 5.6. Before autoclaving the pH was around 6.1. New and old potatoes have been used in preparing this medium without affecting the reaction.

Potato dextrose agar. 10 gm. of dextrose and 20 gm. of granulated agar ("Difco") dissolved in 1000 cc. of potato decoction. Filtered through absorbent cotton, tubed and autoclaved. It is assumed that the addition of agar does not change the pH of media much (28), so that this medium would have the same average acidity as the potato dextrose decoction, or pH 5.35. This was checked in a few cases by colorimetric determinations, adding the indicator to the melted agar and making comparisons after it had solidified, as described by Hopkins (14).

It is of interest to note the variation in pH between this medium and the potato dextrose agar described by Hopkins. The materials were the same except that Hopkins used tap water and dialyzed his agar, and also followed a slightly different method of preparation, obtaining a medium with a reaction of pH 6.9 to 7.8.

Prune agar A. 120 gm. of prunes (stoned before weighing) simmered in 1000 cc. distilled water in double boiler for 1½ hours. Cooled. Filtered through double filter. Made up to 1000 cc., adjusted with N/10 NaOH to pH 5.5. 20 gm. of agar dissolved in solution in autoclave; strained through absorbent cotton, tubed, autoclaved.

Potato plugs. Plugs cut from potatoes with a cork borer were used without treatment other than the addition of a small quantity of distilled water before autoclaving.

PRESENTATION OF RESULTS

I. CULTURAL CHARACTERISTICS

A number of culture media were employed in studying the cultural characteristics of strains of Sclerotinia. However, special attention was paid to the growth of the fungi on potato dextrose media, which proved particularly valuable not only in separating strains of *S. americana,* but in differentiating it from *S. fructigena* and *S. cinerea.*

Method of Presentation. The macroscopic cultural characteristics will be shown in summarized tabular form. The column "hyphae" refers to the relative extent of mycelial growth. In strains producing few or no conidia the symbol is relative to the

portion of the surface of the substratum covered and the height of this hyphal covering. Thus "+++" and "++++" are used only for strains in which the hyphae not only covered the surface of the slant, but grew up into the air. On the other hand, in strains producing an abundance of conidia which obscured the mycelium, hyphal growth was recorded "tr." when the mycelium was entirely covered by conidia and "+" when traces of the white hyphae were visible through the conidial pustules.

With conidia the symbols refer directly to the portion of the surface of the colony occupied by conidia, "++" indicating that macroscopic observation showed approximately a quarter to a half was covered, etc.

The production of microconidia was estimated from the abundance of the milky or yellowish droplets on the surface of the cultures. This generally parallels conidia production, but not always as microconidia are also produced directly on the hyphae.

"White hyphal mass" is used as the name for a cultural characteristic of Sclerotinia that, so far as the author knows, has not been previously described. In many of the strains of *S. americana* more or less densely felted masses of hyphae, often pure white in color, rise perceptibly above the general level of the colony. These masses may be almost points, not so long as they are tall—pencils of felted hyphae protruding into the air—or raised ridges, with definite margins, running entirely across the colony. Quite typically the mass is plateau-like, often including a half to two-thirds of the surface of the slant, elevated perhaps 2 mm. above the level of the rest of the colony which may be quite flat and covered with conidia and microconidia. Such shelf-like masses have flat upper surfaces parallel to the surface of the colony, and are of various shapes; circular, square, oblong, or sometimes dichotomous. They appear early in the history of the culture as, for instance, on potato dextrose agar at 20-25° C., in 5-7 days; and their development does not interfere with the normal development of the black sclerotioid crust along the surface of the substratum, so that the presence of such masses does not show from the back of an agar slant.

The hyphal masses develop more characteristically and occupy a greater portion of the colony surface in cultures in narrow tubes than in those of larger diameters as 16 or 18 mm.

While conidia are frequently borne in small numbers on the hyphal masses, these bodies are not primarily sporiferous. More commonly they appear almost white, while the remaining lower part of the colony may be covered with grayish-buff conidial pustules.

Microscopically, the mass is composed of the regular aerial hyphae of the fungus, of varying sizes. This is in contradistinction to the hyphae of the "hyaline mound," which are all of nearly the same diameter.

In the case of the "hyaline hyphal mound" more definite structures are observed. Macroscopically, these are recognized as hemispherical bodies of a translucent, water-soaked appearance. The mounds appear to emerge from the white aerial hyphae, which surround the base of larger ones and cover over the smaller ones with a sparse coating, presenting a characteristic moundlike swelling in the colony. From the rear it can be seen that the black sclerotial crust is absent at the center of the mound, which extends down into an agar substratum. When these bodies are examined with a needle, the "hyaline mound" is found to be of an exceedingly tough, coriaceous though elastic texture, while the "raised hyphal mass" is readily torn to pieces.

Microscopically, the mound in cross-section is made up (fig. 1) of homogenous, intricately entwined hyaline hyphae. Mixed with these are innumerable microconidia, which are formed in masses around the descending slopes of the mound and also in spherical, pycnidia-like bodies, 90-140 μ in diameter, imbedded in the sclerotial

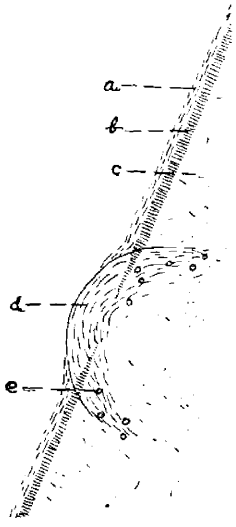


Fig. 1.—Diagrammatic sketch of x-section of a "hyaline hyphal mound," from a potato dextrose agar slant of S 23. (*a*) superficial hyphae, (*b*) sclerotial crust, (*c*) agar substratum, (*d*) hyaline mound, (*e*) pycnidia-like masses of microconidia.

crust and in the agar below. As suspected from macroscopic observation, the sclerotial crust is absent under the center of mounds. Whether the latter arise only at points not occupied by the sclerotial plate, or the development of the mound results in the disappearance of the crust, has not been determined.

"Flatness" expresses the relative elevation of cultures, and is of particular interest in the case of those cultures that do not show the definite raised hyphal masses, but still have the hyphae arranged as raised masses, though of irregular height and outline, in part or all of the colony. Such variation in elevation appears to be a definite, genetic, cultural characteristic.

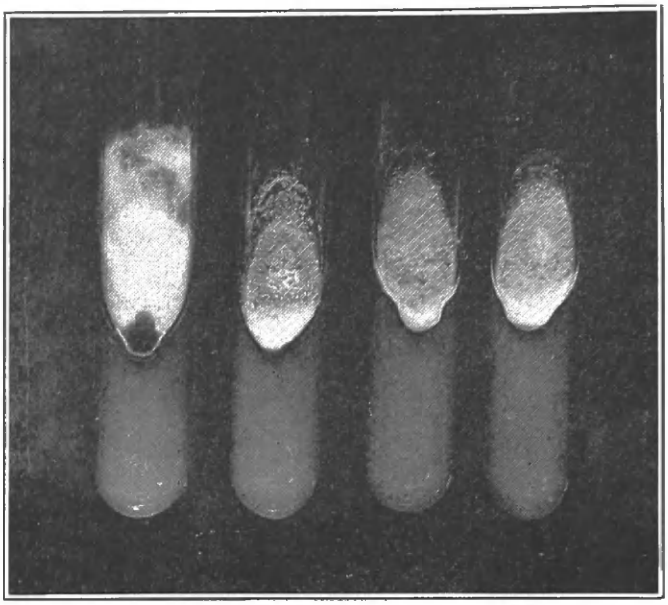


Fig. 2.—*S. americana* strains, left to right, S 27 (var VI) ; S 22 (var. I) and two tubes of S 13 (var. II). The two S 13 tubes show the maximum variation noted within a single spore strain. Potato dextrose agar cultures, 18 days growth at 27° C.

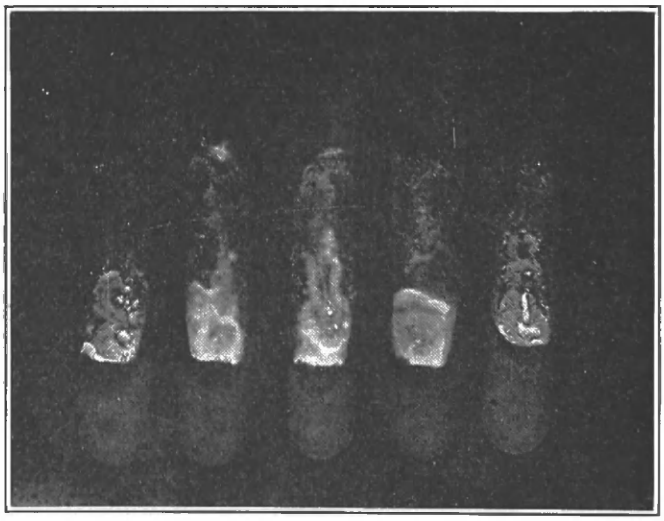


Fig. 3.—*S. americana* strains, left to right, S 4 (var. I) ; S 5 (var. III) ; S 20 (var. IV) ; S 21 (var. III) ; and S 22 (var. I). Note the preponderance of conidia and microconidia in S 4 and S 22, and the presence of raised hyphal masses in S 5, 20, and 21. Potato dextrose agar cultures, 12° C., two months.

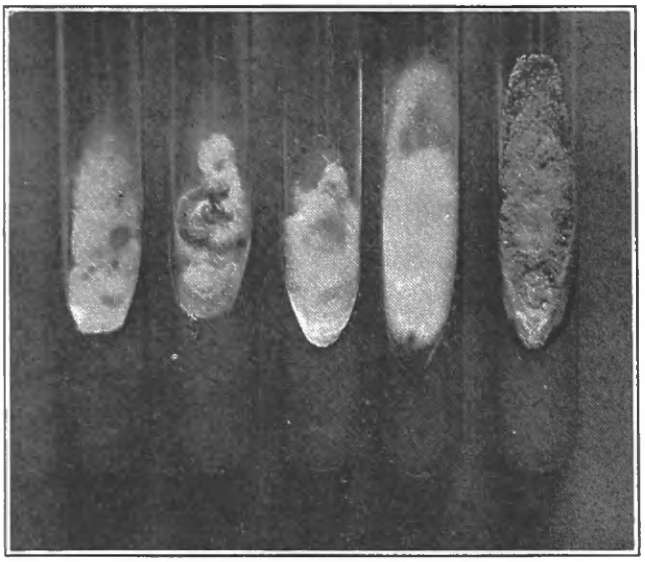


Fig. 4.—Front view.
Left to right, *S. cinerea* forma *pruni* (S 44) ; *Monilia cinerea* forma *mali* (S 43) ; *S. fructigena* (S 42) ; *S. americana* var. VI (S 27) and var. I (S 22). Potato dextrose agar cultures, 16 days growth at 22° C.

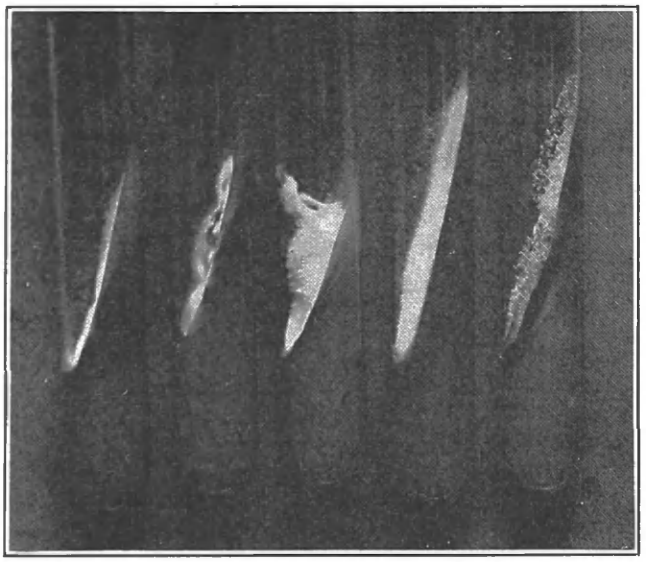


Fig. 5.—Turned sideways to show the fan-shaped hyphal masses protruding from the *S. fructigena* colony.

"Nigrescence" refers to the sclerotioid crust formed characteristically by all strains on the surface of the substratum. This is a black, carbonaceous, plate-like growth, usually .4 to .8 mm. in thickness, forming in strains marked "++++" or "+++" a layer under the entire surface of the colony. It appears to be analogous to the blackening of the skin in fruits attacked by brown-rot.

Characteristics on Potato Dextrose Agar Slants. This medium was used for carrying the cultures, as well as for many experiments. Descriptions of stock cultures were made periodically, and records accumulated for more than forty-five different series of potato dextrose agar cultures, though some of these included only a few strains. The tables presented summarize the results obtained at two temperatures.

From Table II it is at once evident that there is considerable variation between strains. Thus S 21 and S 22, strains derived from apothecia collected at the same place at the same time, are quite different in their habits of growth. By comparison of the actual cultures it is possible to differentiate even other strains so similar that they are described in the records by the same symbols. These differences, too slight to be described, are, however, of little significance, due to the variability of cultural characteristics with variations in temperature and other conditions (see below), and it is sufficient here to group the strains roughly by the characters recorded. This is done in Table III for those strains that under these conditions appear to fit the author's concept of *Sclerotinia americana*.

Of the 38 strains included in Table II there are 8 that will not fit into this grouping. S 42 is differentiated by the yellow [often Massicot yellow (30)] conidia and sheaf-like hyphal masses peculiar to *S. fructigena* (fig. 5). S 44, S 49, and S 56 exhibit in varying degree the characteristic smooth colony surface, made up of the segmented hyphae or "chlamydospores" (see fig. 6, *b*), characteristic of *S. cinerea* forma *pruni*. The color is also quite characteristically a dull brown [buffy brown to Saccardo's umber (30)].

S 54 and S 43 show low total growth, made up chiefly of a flat, hyaline, coriaceous mat of mycelium, covered in places by a scant and closely appressed web of white hyphae. In this respect they resemble S 10 and S 23, in which, however, the hyaline bodies are usually but not invariably moundlike and separated by the sclerotial crust instead of forming a continuous layer. These strains correspond to Wormald's *Monilia cinerea* forma *mali* or Killian's sour cherry Monilia.

TABLE II.

Cultural characteristics of Sclerotinia strains on potato dextrose agar slants, 14 days at 26-28° C.

Strain	Hyphae	Conidia	Micro-conidia	White hyphal masses	Hyaline hyphal mounds	Flatness	Nigrescence
1	++	++	tr.	—	—	+	++
2	+	+++	++	—	—	+++	++++
3	+++	tr.	+	—	—	—	++++
4	tr.	++	++	—[1]	—	++++	++++
5	++++	+	tr.	++++	—	—	++++
6	++++	tr.	+	++++	—	—	++++
8	+++	++	+	—	—	+	+++
10	+++	+	++	—	+++	—	++
11	++++	—	tr.	—	—[1]	—	++
13	++	++	—	—[1]	—[1]	++	++++
14	tr.	++	+++	—	—	++++	++++
19	+	++	++	—	—	+++	++++
20	++++	+	++	++++	—	—	+++
21	++++	++	tr.	++++	—	—	+++
22	tr.	+++	++++	—[1]	—	++++	++++
23	++	?	tr.	—	+++	—	++
24	+++	++	+	—[1]	—	tr.	+++
25	++++	++	+	++++	—[1]	—	++++
26	++++	+	++	+++	—[1]	+	+++
27	++++	—	tr.	—	—	—	+++
28	+	+++	+	—	—	++++	+++
29	++++	+++	+	—[1]	—	+	++++
30	+++	+++	+	+++	—	++	++++
31	+	+++	+	—	—	+	++++
33	+++	++	++	+	—	—	+++
34	++	+++	+	—	—	+	++++
35	++++	+	+	++	—	—	++++
36	+	+++	+++	—[1]	—	+++	++
37	+++	+++	+	++	—	—	++++
40	+++	++	+	—	—	—	+++
41	++++	++	tr.	—[1]	—	tr.	++++
42	++++	++	—	(+++)[2]	—	—	+
43	++	?	tr.	—	(+++)[3]	+++	—
44	++	(++)[4]	—	—	—	++++	++
45	+	++++	+	—	—	++	++++
49	+++	(+)[4]	tr.	—	—	++	—
54	++	—	+	—	(++)[3]	+++	—
56	+++	(++)[4]	+	—	—	++	+

[1]Recorded as present in some cases only.

[2]Sheaf-like or fan-shaped masses of hyphae, always larger at the apex than at the base, thus differing from the usual hyphal masses. Usually bear yellow conida on the apex. Characteristic of *S. fructigena.*

[3]Hyaline hyphal mat, rather than mound, forming the surface of the colony; sparsely covered with white aerial hyphae. Characteristic of *Monilia cinerea* forma *mali* Wormald.

[4]A smooth, flat, greenish-brown surface, made up of the segmented hyphae. No conidial pustules, but microscopically a few conida can generally be found. Characteristic of *S. cinerea* forma *pruni* Wormald.

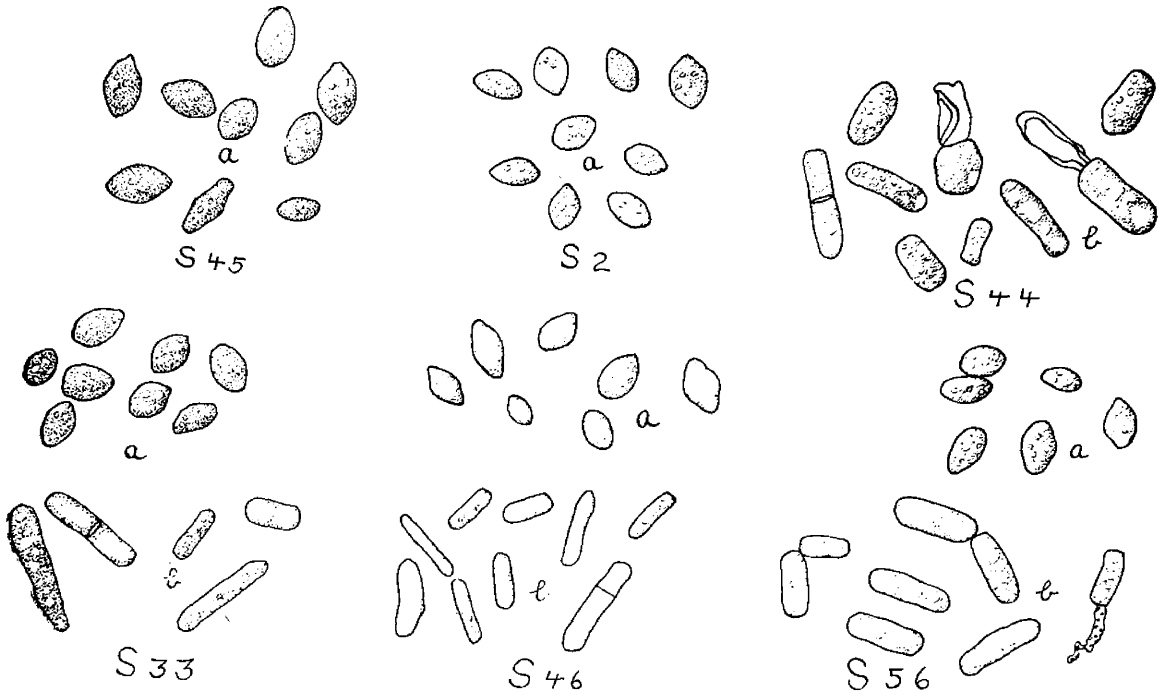


Fig. 6.—Conidia (*a*) and chlamydospore-like segments of old hyphae (*b*), from strains of *S. americana* and *S. cinerea* forma *pruni*. x 400.

TABLE III.

Strains grouped according to the cultural characteristics shown in Table II.

Group characteristics	Strains
1. Hyphae few, conidia abundant, hyphal masses none, surface usually flat.	S 2, 4, 14, 19, 22, 28, 31, 36, 45.
2. Hyphae and conidia abundant, hyphal masses none, surface usually not flat.	S 1, 8, 13, 24, 29, 34, 40, 41.
3. Hyphae and conidia abundant, hyphal masses present, surface not flat.	S 30, 33, 37.
4. Hyphae abundant, conidia few, hyphal masses abundant, surface not flat.	S 5, 6, 20, 21, 25, 26, 35.
5. Hyphae abundant, conidia few or none, hyphal masses none, surface not flat.	S 3, 11, 27.

TABLE IV.

Cultural characteristics of Sclerotinia strains on potato dextrose agar slants, 14 days at 13.5-15° C.

Strain	Hyphae	Conidia	Micro-conidia	White hyphal masses	Hyaline hyphal mounds	Flatness	Nigrescence
.....	+++	+++	+	—	—	+++	+++
2.....	++	+++	+	—	—	++++	++++
3.....	++++	tr.	—	—	—	—	++++
4.....	tr.	++++	+++	—	—	++++	++++
5.....	+++	++	+	++++	—	—	++++
6.....	++++	tr.	+	++++	—	—	++++
8.....	++++	++	+	+++	—	—	++++
10.....	+++	—	+	—	tr.	++	++
11.....	++++	—	+	—	—	++	++++
13.....	++	++	tr.	—	—	++	++
14.....	+	++++	+++	—	—	++++	++++
19.....	tr.	++++	++	—	—	++++	++
20.....	++++	++	++	++++	—	—	+++
21.....	++++	++	+	++++	—	—	++++
22.....	tr.	++++	tr.	—	—	++++	++++
23.....	+++	—	++	—	+++	+	tr.
24.....	+++	++	+	++	—	+	++++
25.....	++++	++	++	+++	—	—	++++
26.....	++	+++	++	—	—	++	++++
27.....	++++	—	+	—	—	—	++
28.....	tr.	++++	tr.	—	—	++++	+++
29.....	+++	+	+++	++	—	—	+++
30.....	++++	++	tr.	+++	—	—	+++
31.....	+++	++	+	+++	—	+	++
33.....	+++	++	+	—	—	++	++
34.....	+++	+++	++	—	—	++	++++
35.....	++++	++	+	++	—	—	++++
36.....	+++	++	++	++	—	++	+++
37.....	++++	++	+	—[1]	—	—	++++
40.....	+++	+++	+	—	—	++	++++
41.....	+++	tr.	+	+++	—	++	++++
42.....	++++	(++)[2]	++	(++++)[2]	—	—	+++
43.....	++	—	++	—	(++)[3]	++++	tr.
44.....	+++	(++)[4]	+	—	—	++++	+
45.....	++	++++	++	—	—	++	++++
46.....	++	(++)[4]	+	—	—	++++	+
47.....	++	(++)[4]	+	—	—	++++	++
49.....	+++	(+)[4]	+	—	—	++	+
50.....	tr.	++++	+++	—	—	+++	++++
51.....	+	++++	++++	—	—	+++	++++
52.....	++++	(++)[2]	++	(++++)[2]	—	—	++
53.....	++++	—	+	—	—	—	+
54.....	++	—	++	—	(+)[3]	++++	tr.
55.....	+++	(++)[4]	+	+	—	++++	++
56.....	+++	(++)[4]	+	—	—	+++	++
57.....	++	++++	++	—	—	++++	+++
58.....	++	++++	++	—	—	++++	++++
59.....	+++	+++	+	++	—	+	++

[1], [2], [3] [4] same as in Table II.

The cultural characteristics of strains on potato dextrose agar at a lower temperature are given in Table IV. Here, as in Table II, distinct variations show between the different strains of *S. americana*. The strains generally show almost the same characteristics under these conditions and the grouping given in Table III will apply almost without modification.

S. fructigena is again differentiated by its large yellow spore pustules. Most of the strains of *S. cinerea* forma *pruni* show again the characteristic colony surface, smooth and with deep brownish colored areas, composed of the chlamydospore-like hyphal segments. S 10, 23, 43 and 54 are as before characterized by their featureless type of growth, consisting mainly of hyaline mycelium.

Characteristics on Prune Agar Slants. (Table V). On prune agar an interesting phenomenon is the absence of the "raised hyphal masses" in all except one strain of *S. americana*. Increased conidial production is also general. Accordingly, strains which in Table III fell in groups 3 and 4 would now frequently fit in group 2.

Characteristics on Potato Plugs. Cultures incubated at 25° gave results rather like those of the potato dextrose agar slants so far as concerned grouping of the *S. americana* strains. These showed for the various strains a ratio of conidia and hyphae production much as that described above. S 22, for instance, produced an abundance of conidia in a flat colony in which very few hyphae were visible; while S 6 showed an abundance of white hyphae forming the typical hyphal masses and bearing conidia only sparsely.

As Wormald (38, 39) found, when *S. cinerea* f. *pruni* strains are grown on this medium pustules of conidia appear, though in some of the strains studied here, as S 53, only very few developed. However the smooth brownish surface composed of mycelial segments, characteristic of this form, also showed in parts of some cultures, so that they could be distinguished from those of *S. americana*. With strains of the *cinerea* f. *mali* type the growth of aerial hyphae was unusually abundant, but not sufficiently so to hide the hyaline "substrate mycelium" which constitutes the greater part of each colony and by which these strains are distinguished. The sparse aerial mycelium covering this was often of a definitely alveolate appearance.

When cultures were grown at 14° C. the usual differentiating characteristics of *S. cinerea* were absent, hyphal growth being still more pronounced. A new characteristic, however, was observed. While in strains of *S. americana* the aerial mycelium was more or less matted together into an even though not necessarily dense mass, S 44, 47, 49, and 55 showed over the surface of the colony numerous separate spicules of white hyphae, projecting well beyond the general surface of the colony.

TABLE V.

Cultural characteristics of Sclerotinia strains on prune agar slants, 11 days at 25° C.

Strain	Hyphae	Conidia	Microconidia	White hyphal masses	Hyaline hyphal mounds	Flatness	Nigrescence
S 1	+++	+	—	—	—	tr.	++
S 2	+	++	—	—	—	++	+++
S 3	++++	+	—	—	—	—	+++
S 4	+	+	—	—	—	++++	++
S 5	++	++	++	—	—	++	+++
S 6	++	++	+	+	—	tr.	+++
S 8	++	++	—	—	—	+	++
S 11	++++	++	tr.	—	—	—	++
S 13	++++	+	—	—	—	—	tr.
S 14	+	+++	—	—	—	+++	+++
S 19	++	+++	—	—	—	+++	++
S 20	++	++	—	—	—	++	++
S 21	++	+	?	—	—	++	++
S 22	+	+++	—	—	—	+++	++
S 23	+++	+	—	—	+++	—	++
S 24	++	+	—	—	—	++	++
S 25	++	++	—	—	—	++	+
S 26	+++	++	+	—	—	++	++
S 27	++++	+	tr.	—	—	—	++
S 28	++	+++	—	—	—	+	++
S 29	+++	+	+	—	—	+	++
S 30	++	+	tr.	—	—	++++	+++
S 31	++	+	—	—	—	+++	+
S 33	++	+	—	—	—	+++	+
S 34	+++	++	—	—	—	++	+
S 35	++	++	—	—	—	+++	++
S 36	+++	++	—	—	—	++	++
S 37	++	+++	—	—	—	++	+++
S 40	++	+++	—	—	—	+++	++
S 41	+	+++	—	—	—	+++	+++
S 42	++++	(++)[1]	+	(++++)[1]	—	—	—
S 43	++	—	+	—	—	++++	—

[1]Sheaf-like or fan-shaped masses of hyphae, always larger at the apex than at the base, thus differing from the usual hyphal masses. Usually with the yellow conidia. Characteristic of *S. fructigena.*

Characteristics in Potato Dextrose Decoction. Cultures of *S. americana* grown on this medium at 20° C. show strain differentiation as described previously on other media, except that the white hyphal masses develop less abundantly. S 6 and S 20 still show these bodies, but strains such as S 21 no longer develop them.

Strains of *cinerea* forma *pruni* cannot always be distinguished from *americana;* however, most of them still differ by showing the typical smooth, brownish surface over a portion of the colony.

Characteristics in Potato Decoction. With *S. americana* this medium proved of even less value than the preceding for strain differentiation. However, though the distinctions to be seen were not so pronounced as on other media studied, comparison of those displayed gives essentially the same groupings as in previous cases. Thus S 22 and S 40, while they produced more hyphae than usually, showed still a predominance of conidial production and absence of the white hyphal masses. S 6 and S 20 again showed predominance of hyphae with the development of the hyphal masses and only a trace of conidia, while S 11 and S 27 formed an abundance of hyphae, but no hyphal masses.

Most strains of *S. cinerea* grew on this medium merely as masses of white mycelium and could not be differentiated with certainty from mycelium-forming types of *S. americana.*

Characteristics on other Culture Media. A few *S. americana* strains of different types were grown on pieces of peach tissue sterilized in culture tubes, on decoctions prepared from peaches and apples, on boiled rice, etc. Growth occurred in all cases in the usual manner, strains that normally showed an abundance of conidial over hyphal growth doing so here, etc. None of these media gave results as satisfactory as potato dextrose agar, though the rice afforded better comparison in the case of the relative nigrescence of the substratum.

Petri Dish Cultures.

In Petri dish cultures of potato dextrose agar, strains of *S. americana* have their strain differential characteristics suppressed so that they resemble each other more closely than under many other conditions; but they are all clearly distinguishable from strains of *S. cinerea* forma *pruni* and forma *mali,* and, as always, from *S. fructigena* (Table VI).

Wormald (39) has already described the growth typical for these species on plates of prune agar. He was able to distinguish cultures of *S. cinerea* by their zoned and lobed growth (see fig. 8). *S. fructigena* and American strains, on the other hand, grew out in plate culture without forming definite zones and with an edge almost entire rather than lobed. Killian (17) in his elaborate study

of two types of *cinerea*, isolated from cherries, found that he could distinguish them in culture by the zoned and lobed growth of the sweet cherry form, while the sour cherry Monilia, which grew more slowly, formed instead a featureless mat of flat, hyaline "substrate mycelium."

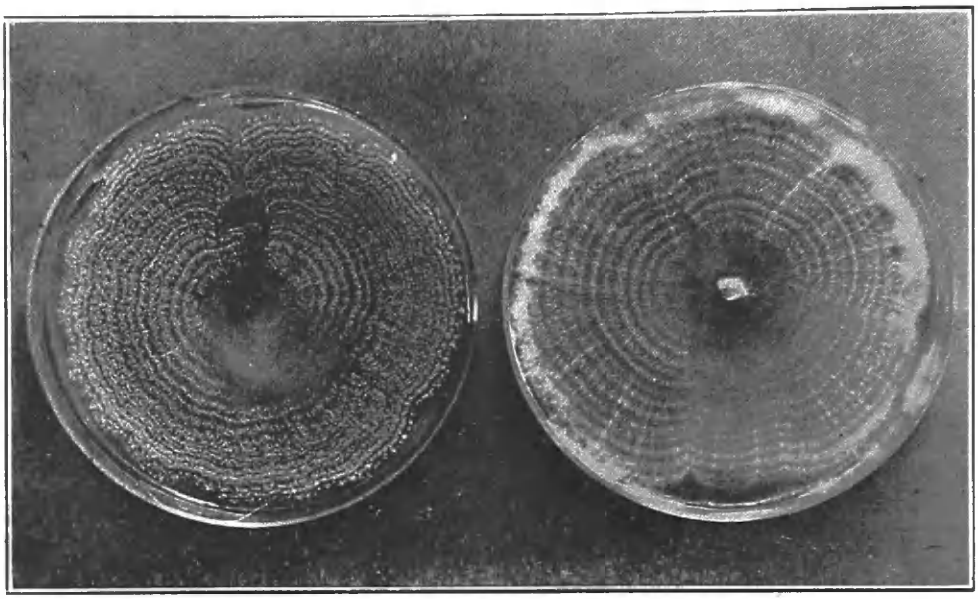
In Table VII the salient characteristics are given of the growth of some strains of *S. americana* when inoculated in the centers of plates of set potato dextrose agar. All of these strains show in plate culture the characteristics of *S. americana:* rapid growth,

TABLE VI.

Strains of Sclerotinia grouped in species by their characteristics on potato dextrose agar Petri dish cultures. Incubated at room temperature, 14-20° C.

Characteristics	Strains
Sclerotinia americana. Growth rapid, whole plate covered with colony homogeneous except for numerous concentric circles of conidia, or in some strains, of mycelium. Edge almost entire.	S 1, 2, 4, 5, 6, 11, 13, 14, 20, 21, 22, 26, 27, 34, 36, 37, 40, 41, 45, 50, 51.
S. cinerea forma *pruni.* Growth only half so rapid as in *americana.* Much lobed and zoned. In this series, 2 to 7 zones per plate culture of 90 mm. diameter.	S 44, 46, 47, 49, 55.
S. cinerea forma *mali.* Growth still slower. Colony of flat, hyaline "substrate mycelium," frequently with closely packed concentric circles barely visible in the mycelial mat.	S 10, 23, 43.
S. fructigena. Yellow conidia produced on large fan or sheaf-like masses of hyphae.	S 42, 52.

filling the whole plate without zone formation, and a definite concentric ringing around the point inoculated (fig. 7). These circles are generally composed of pustules of conidia but in a few cases they are produced instead by hyphae. The typical Petri dish culture of *S. americana* under these conditions consists macroscopically of numerous concentric circles of conidia, the hyphae being completely obscured by conidia. As will be noted in Table VII growth in plate culture causes a shift from the production of hyphae to conidia formation. S 6 which in slants of potato dextrose agar produces a predominance of hyphae, in plate culture grew almost the same as S 22, which shows a predominance of conidia under all conditions. However, even in plate culture S 27, while it made a colony very similar in general characteristics



Petri dish cultures on potato dextrose agar.
Fig. 7.—*S. americana.* Left, S 6 (var. IV), right, S 11 (var. V). 26 days at 13.5° C.

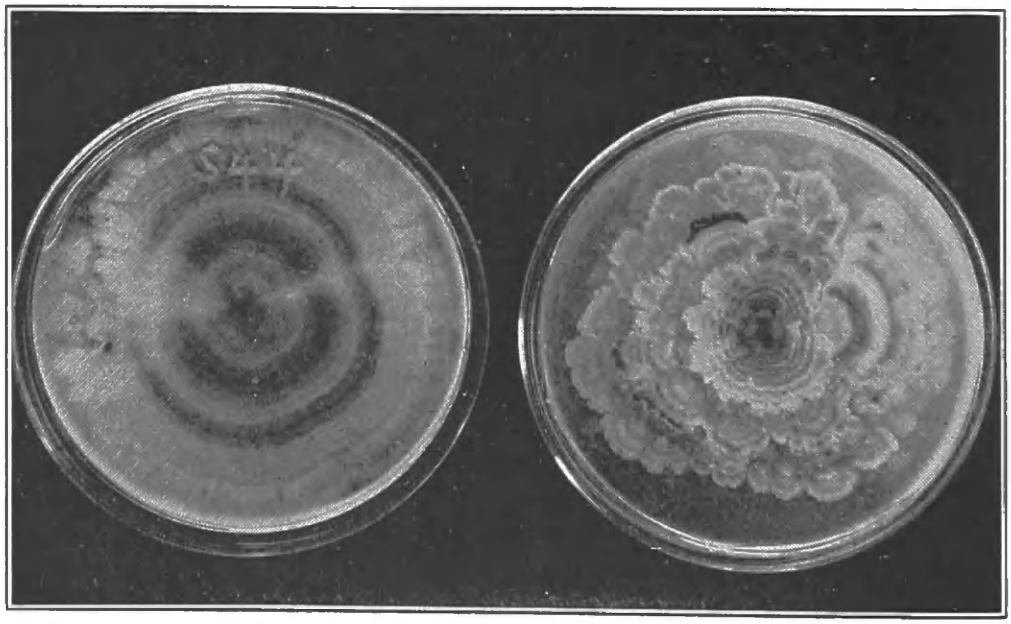


Fig. 8.—*S. cinerea* forma *pruni.* Left, S 44, showing the characteristic zoning and lobing only at the edge of the colony. Right, S 47, a California strain, appearance more characteristic for the species. One month, at room temperature of about 15° C.

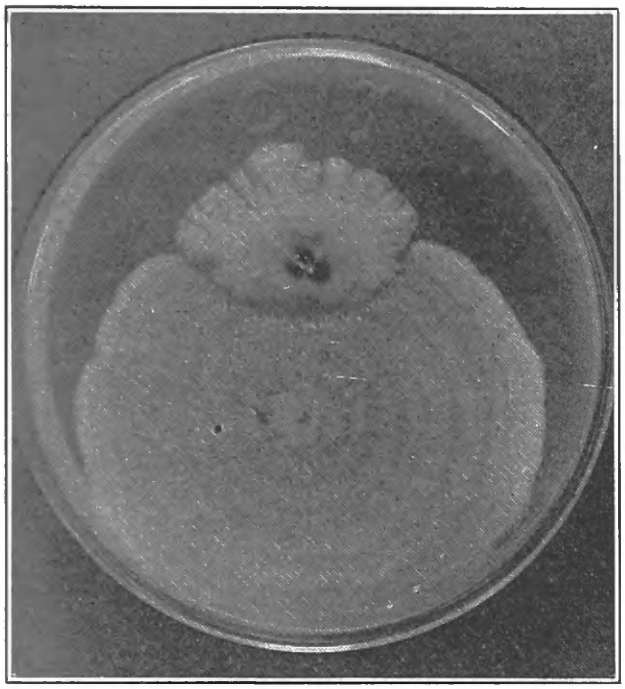


Fig. 9.—*S. cinerea* forma *pruni* (S 44) upper, and *S. americana* var. I (S 22) lower colony, inoculated at the same time at the same distance from the center of a plate of set potato dextrose agar. Note difference in character and rate of growth. Photographed after 11 days growth at 13.5° C.

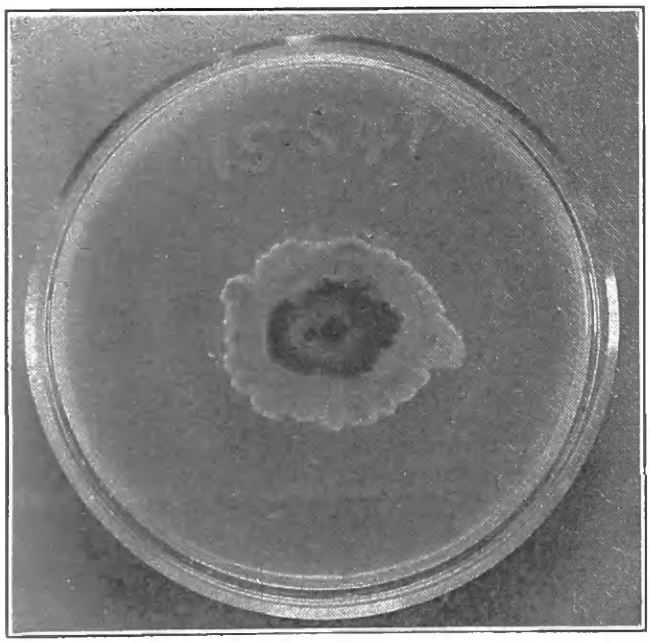


Fig. 10.—A young *S. cinerea* plate culture showing two well developed zones and a third just starting. S 47. Potato dextrose agar, 15° C., 21 days.

to the S 22 colony, formed only hyphae which grew in the concentric rings shown in fig. 12.

TABLE VII.

Cultural characteristics of some strains of Sclerotinia americana on potato dextose agar plates at room temperature 13.5° C. Notes after 17 days.

Strain	Hyphae	Conidia	Flatness	Average diam. of colony, mm.
S 6	+	++++	+++	82
S 11	++	++	++	85
S 20	+	++++	+++	88
S 21	tr.	++++	+++	85
S 27	++++	?	+++	84
S 45	tr.	++++	+++	90

Strains of *S. fructigena* are differentiated in plate cultures by the production of the typical yellow conidia on fan-shaped hyphal masses. Their rate of growth is nearly the same as that of *S. americana* though the concentric ringing effect is absent.

Strains of "*Monilia cinerea* forma *mali*," S 10, 23, and 43, showed resemblance to each other just as had been noted in the earlier cultures. Their growth was the same as that described by Killian for his sour cherry Monilia; an extremely low rate of growth, the colony consisting of a flat, almost featureless, mass of hyaline mycelium forming a tough layer on which only traces of aerial hyphae and no conidia were observed.

The following measurements give the diameter of typical plate cultures, on potato dextrose agar, incubated for 10 days at 16° C.:

Sclerotinia americana—S 22; 76 mm., S 27; 70 mm.
S. cinerea forma *pruni*—S 44; 29.5 mm.
S. cinerea forma *mali*—S 43; 22.5 mm.

Most of the strains included under *S. cinerea* forma *pruni* in Table VI had already been assigned to this species on the basis of their resemblance in tube culture to a type strain (S 44) from Wormald. However, while study of the strain differences in tube cultures serves best to differentiate groups of strains within the species, the use of Petri dish cultures results in more decisive species differentiation. As shown above, individual strain variations are largely suppressed, the differences in the rate of growth of the different species are emphasized, and the characteristic zonation and lobing of *S. cinerea* forma *pruni* appears.

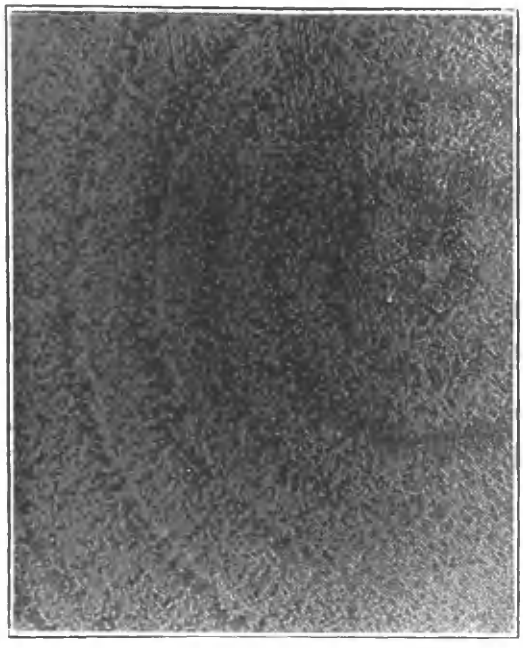


Fig. 11.

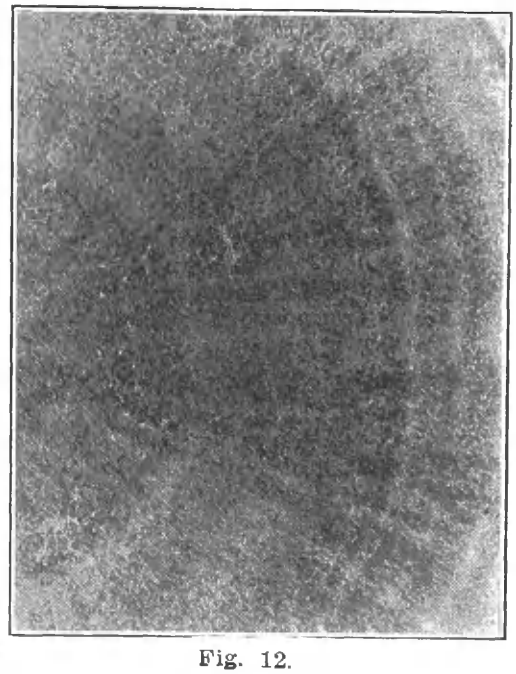


Fig. 12.

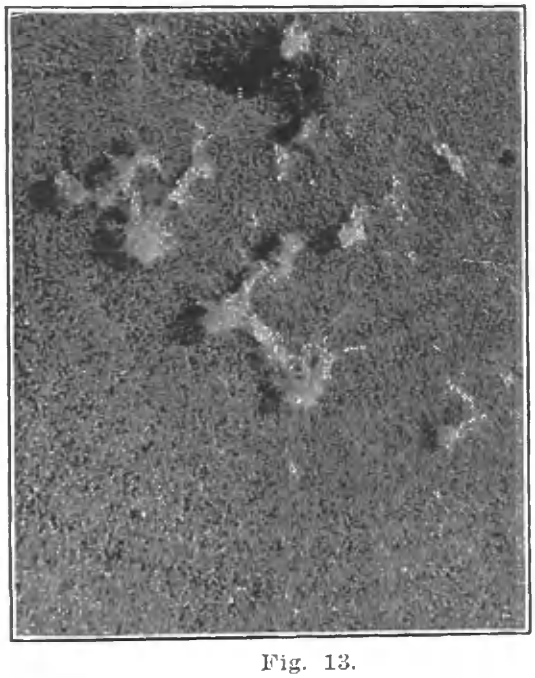


Fig. 13.

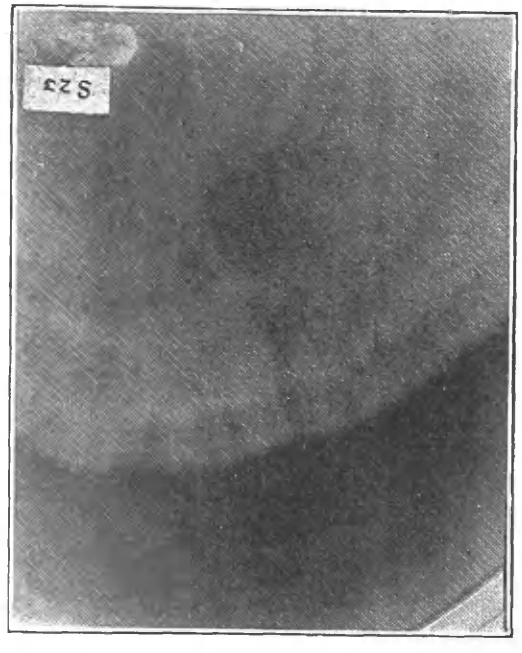


Fig. 14.

Figs. 11-14.—Detailed view of growth in Petri dish cultures, on potato dextrose agar. x 1½.

Fig. 11.—*S. americana* var. I (S 22).

Fig. 12.—*S. americana* var. VI (S 27). Note absence of conidia.

Fig. 13.—*S. americana* var. III (S 21). Shows presence of raised hyphal masses

Fig. 14.—*S. cinerea* forma *mali* (S 23). Shows the characteristic hyaline, featureless growth.

Double Inoculations in Petri Dishes.

At the suggestion of Prof. H. H. Whetzel, a number of plate cultures were simultaneously inoculated at different points with two strains (fig. 9), to see if any heterothallic action would be displayed at the junctions of the colonies. Potato dextrose agar was the medium used and the plates were grown at a varying room temperature.

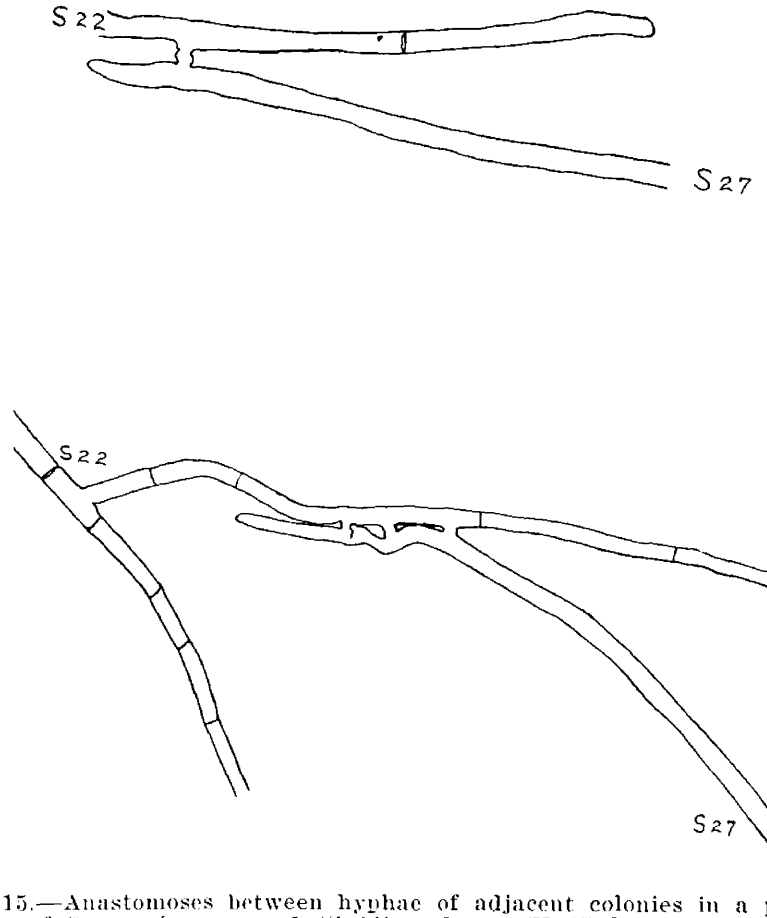


Fig. 15.—Anastomoses between hyphae of adjacent colonies in a potato dextrose agar plate, of *S. americana* var. I (S 22) and var. VI (S 27). x 400.

In one series, S 13, 22, and 27 were used in all possible combinations. The two colonies of each plate grew evenly, no inhibiting effect being observed before the colonies actually came in contact. When this occurred extension of the colony ceased but no macroscopic evidence of any reaction between the strains appeared. Microscopically, anastomoses were observed between all the different strains, but no further growth then occurred. Some anastomoses observed between S 22 and S 27 are shown in fig. 15.

In a second series all combinations were tried with S 22, 27, 42, 43, and 44. The results as to general growth were as before, but no

anastomosis was observed except between S 22 and S 27, and in a single case between S 22 and S 43.

The results above, and the subsequent development of apothecia from fruit inoculated with single spore strains, indicate that the organisms concerned are of a homothallic nature.

II. GENETIC NATURE OF STRAIN VARIATION IN SCLEROTINIA AMERICANA.

In other sections the more striking variations noted between strains of *Sclerotinia americana* are described. The following experiment was planned particularly to demonstrate that these variations are actually genetic characteristics of the respective strains, and not as Reade (29) stated responses to previous environmental differences which disappear when the strains are grown under uniform conditions.

Five strains, S 4, 5, 20, 21 and 22 were used. These were all isolated at the same time from apothecia of the same origin and cultivated side by side until the experiment was started five months later. S 4, and S 22, both from the same apothecium, form in culture an abundance of conidia and microconidia, few hyphae, and no hyphal masses. S 5 and S 21, again derived from separate ascospores from a single apothecium, form fewer conidia but more hyphae, and produce characteristically well-developed white hyphal masses, while S 20 develops an abundance of hyphae and even fewer conidia. While these differences are not great as compared to the distinctions between *S. cinerea* and *americana,* they are sufficient to make these strains readily distinguishable.

The general plan followed was to run the five strains through successive series of inoculations on apples, then on artificial culture media, to apples again, etc., as shown diagrammatically in fig. 16. Each series of cultures listed included three cultures in standard potato dextrose agar and two in potato dextrose decoction for each strain.

In the apple inoculations three were used for each strain of each series. Those in the "d" series were of the Mother and Bloomfield varieties, and in the "e" and "f" series York Imperial. The fruits were carefully selected of even size and free of imperfections, and surface sterilized as described under fruit inoculation methods. After inoculation through wells cut with a sterile scalpel the three apples for each strain were incubated together under a large glass dish.

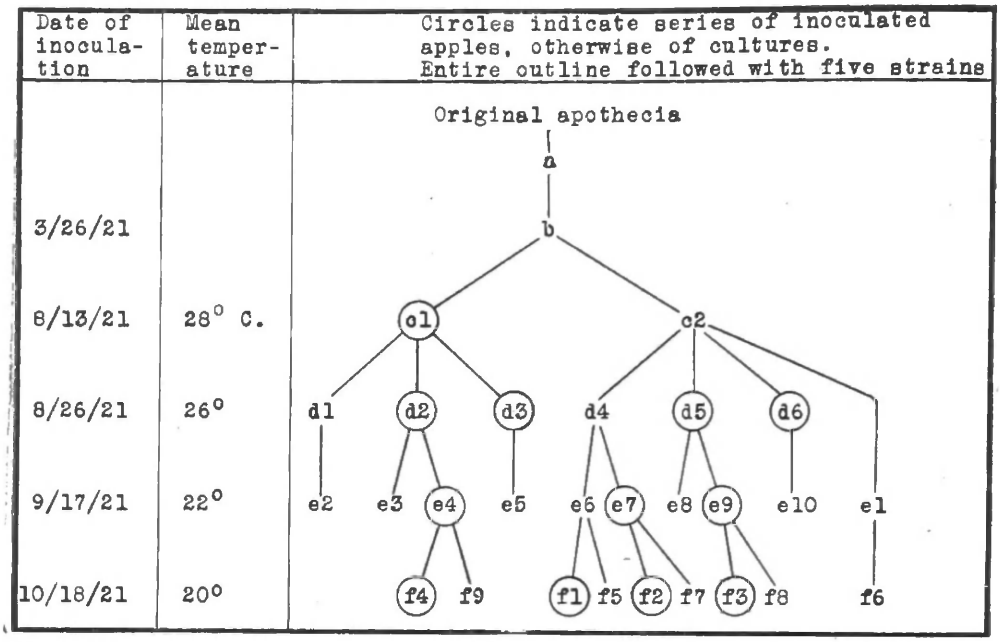


Fig. 16.—Scheme of inoculations in section II.

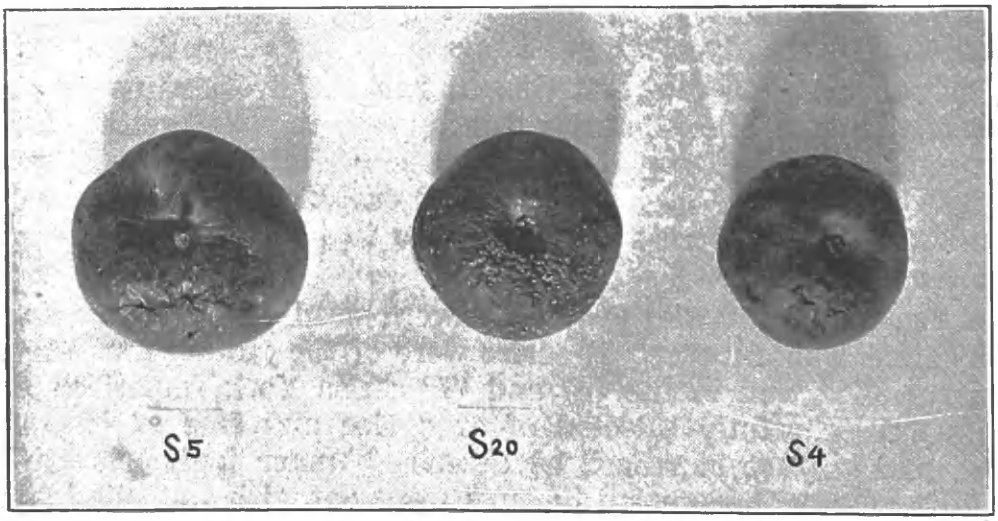


Fig. 17.—Apples inoculated with *S. americana* strains, in series "c1" of fig. 16. Note the development of conidia on S 20 (var. IV) while none show on S 4 (var. I) or S 5 (var. III). Photographed 13 days after inoculated.

It will be noted from fig. 16 that, by the method followed, it was possible to compare the cultural characteristics of cultures which had just been passed one, two, or three times through rotting fruits with those carried directly from the apothecia in culture. Similarly, comparison could be made between apples inoculated direct from culture and those inoculated with the fungus after passing it through the rotting fruit.

The strains studied attacked inoculated apples at approximately the same rate. However, differentiating characteristics appeared in the first series of apples, c 1, in the relative production of conidial pustules and in the nigrescence of the skin of the rotted apples. In this series S 4 and S 22 produced conidia only at the points of inoculation and most of the surface of the skin was blackened. With S 5 and S 21, a few conidial pustules appeared away from the point of inoculation, but nigrescence was observed only around the point of inoculation and involved only about a tenth of the surface of the apple. Finally, in S 20 there was abundant development of conidial pustules over inoculated fruits, and also considerable nigrescence, though not so much as in S 4 and S 22. The differences in the production of conidia are shown in fig. 17.

In the next series, d, the fruit generally did not produce any conidia away from the point of inoculation, except in d 5. Here, with Mother apples, conidia were abundant on S 4, 20, and 22, while none appeared on S 5 or S 21. That this was a varietal influence of the host was indicated by the failure of the d 6 series, Bloomfield apples inoculated from the same source, to produce conidia.

Further description of the results is unnecessary since in every remaining series the typical strain characteristics appeared. In the e and f series of apples the description above for the c series applies exactly to most cases. With the cultures, the description already given would fit any series throughout the experiment, no varietal host differences entering in here. Thus the cultures of f 5 were the same as the original cultures; and cultures f 7, f 8, and f 9, passed respectively once, twice, and three times through apples, were so closely like those of f 5 that cultures of the same strains in these different series were indistinguishable.

In another experiment an effort was made to develop new strains by "selection" of cultures of atypical appearance.[1] S 13 was used here, this strain showing more variation in the conidia-hyphae ratio in cultures of the same series than any other *americana* strain. From stock cultures, two tubes were selected that showed the maximum variation in this respect (which was not wide enough to describe in the condensed tabular analyses). From each of

[1]This experiment was suggested by Mr. G. B. Posey.

these, five transfers to potato dextrose agar slants were grown at 27° C. From these transfers, selection was made among those inoculated from the culture tending toward hyphal predominance of the transfer most advanced in this respect, and in the other set a transfer was similarly selected for conidial growth, and transfers made as before. Even after repeating this process a third time no intensification of the characteristics selected for could be observed, both series still showing merely the usual minute variations between individual cultures, with no grouping of variations into the sets by the history of the cultures. Fig. 2 shows the maximum variation, almost imperceptible, observed in this whole series between any of these S 13 cultures.

Considering these results, and also the fact that stock cultures of many strains have been carried in the laboratory for more than three years without changing from their original characteristics, it seems safe to conclude that the characteristics of *Sclerotinia americana* strains are invariant, under given conditions, and that cultivation under similar conditions does not remove the distinctions between strains. Cultivation under diverse conditions, in the cases tried above, did not result in the introduction of permanent modifications of strain characteristics.

III. OXIDASE TESTS.

Wormald (38) found that his forms of Sclerotinia under certain conditions exhibited marked differences in the activity of the oxidase system produced, as measured by the reactions with guaiacum emulsion or pyrogallic acid. In infected apples and in infected flowering shoots, as well as in liquid culture media, his forma *mali* gave a much stronger oxidase test than the forma *pruni;* while American cultures gave a more vigorous reaction than either of the others.

The tests below were performed with guaiacum emulsion as the reagent. The method used by Wormald was employed, except that the time necessary to produce a definite color reaction was determined rather than recording the color produced in a definite time.

The reagent was prepared as follows: 10 grams of gum guaiac were soaked in 100 cc. of 96% alcohol for three days, and the solution then filtered. The same tincture was used in all the tests listed. The emulsion used in the tests was prepared by shaking 5 cc. of the tincture with 95 cc. of distilled water.

Cultures were grown in the standard potato dextrose decoction already described. 1 cc. portions of the liquid medium were pipetted into large culture tubes, and 5 cc. of the guaiacum emulsion added to each tube. All tests were run in duplicate and the duplicates invariably gave similar results. Tests were performed at a

room temperature of about 24° C., varying not more than a degree during the series. The time was recorded (1) to the first change in color from that of a blank guaiac emulsion held next to those being tested; and (2) to "pale blue" (Ridgway, 30). This latter color was not reached in all strains, but it was necessary to record it because strains with strong oxidase activity effect the first change so rapidly that little comparison can be made between them on this basis.

TABLE VIII.

Guaiacum emulsion tests of the oxidase activity of culture media in which Sclerotinia strains had been cultivated. Series 1.

Strain	Weight of mycelium, in grams	Time before standard pale blue (30) color developed
S 11	.3299	1 min.
S 3	.1688	1 min. 30 sec.
S 36	.2323	1 min. 35 sec.
S 40	.2873	1 min. 43 sec.
S 6	.1958	2 min.
S 45	.2898	2 min. 30 sec.
S 22	.2503	2 min. 30 sec.
S 41	.3388	3 min.
S 14	.2093	3 min. 15 sec.
S 13	.2153	4 min. 15 sec.
S 25	.2273	4 min. 30 sec.
S 29	.1633	6 min.
S 2	.2613	10 min.
S 26	.2483	11 min. 30 sec.
S 42	.3073	12 min.
S 44	.3023	12 min. 30 sec.
S 47	.2103	15 min. 45 sec.
S 49	.1981	19 min. 15 sec.
S 27	.3320	22 min. 45 sec.
S 43	.2745	2 hr. 7 min.
S 23	.2643	.....[1]

[1]No reaction in 48 hours.

Series 1. Cultures were grown at a room temperature of 16° C. for 15 days. The weight of the hyphal mat produced, after being dried on filter paper for one minute, was then determined just before running oxidase tests. In Table VIII the strains are arranged in the order of their oxidase reactions, starting with the most vigorous.

When the weight of mycelium produced is compared to the oxidase activity, it is at once evident that there is no correlation in this respect. Little variation exists between strains included

as to growth produced and almost the entire range of variability is shown by the two strains showing the most oxidase activity.

In oxidase activity, the *S. americana* strains generally show more than three times the rapidity of change of the *cinerea* strains. However, S 27 falls in with the *S. cinerea* group, showing low oxidase activity. S 23 again shows its resemblance to the "*Monilia*

TABLE IX.

Guaiacum emulsion tests of the oxidase activity of culture media in which Sclerotinia strains had been cultivated. Series 2

Strain	Time before standard pale blue (30) color developed
S 11	15 sec.
S 41	50 sec.
S 20	2 min. 10 sec.
S 21	2 min. 15 sec.
S 45	2 min. 30 sec.
S 53	2 min. 45 sec.
S 40	3 min. 5 sec.
S 13	5 min. 20 sec.
S 22	5 min. 35 sec.
S 6	7 min. 30 sec.
S 42	7 min. 45 sec.
S 46	10 min. 30 sec.
S 55	17 min.
S 27	41 min. 30 sec.
S 10	52 min.
S 56	53 min.
S 47	60 min.
S 33	61 min.
S 3	137 min.
S 43	[1]
S 44	[1]
S 23	[1]
S 49	[1]
S 54	[1]

[1] S 23, 43, 44, 49 and 54 did not turn pale blue in 24 hours. These strains are arranged in the table in the order of their rapidity in changing from the initial color.

cinerea f. *mali*" group, represented here by S 43. The latter showed only very slow action and the former none in 48 hours.

Series 2. These cultures were grown at a mean room temperature of 19.3° C. for 8 days. Table IX shows again a general segregation by this test of strains belonging to the various species, but not a distinct grouping as afforded by other methods. However, it will be noted that all the *cinerea* strains in this table, with the exception of S 53, took more than 10 minutes to produce the

"pale blue" color; while most of the *americana* strains were able to produce that color in less than 8 minutes.

The *Sclerotinia fructigena* strain included in these tests, S 42, falls between the *americana* and *cinerea* groups in both tables.

To sum up these oxidase tests, it seems that under the conditions used they provide a rough but not a definite separation of the *cinerea* and *americana* species. Almost all strains fell into the proper species groupings when arranged by oxidase activity. Considerable variability is shown between individual *americana* strains, but the oxidase test does not give specific group differentiations corresponding to the results of other methods.

IV. FRUIT INOCULATIONS.

A number of strains were studied on apples, peaches, plums, and cherries. In all series the fruits were kept in improvised moist chambers. The chambers used were low cylindrical glass dishes, sterilized with 1 to 1000 $HgCl_2$ solution and inverted on mats of blotting paper or newspaper that had also been saturated with the same solution. When small fruits such as plums or cherries were used ordinary glass tumblers served equally well as covering dishes. Tests showed that with the same strains, inoculations on fruits produced similar characteristic effects in chambers of 300 cc. capacity as in larger ones of 2000 and 3500 cc. capacity.

Fruits for each series of inoculations were always of the same variety and often from the same tree, free of imperfections and of the same degree of ripeness and approximately the same size. They were washed well in tap water and sterilized in 1 to 1000 $HgCl_2$ solution. Apples and peaches were treated for 3 to 5 minutes and plums and cherries for 2 to 3 minutes, then rinsed twice with sterile water and immediately placed under the moist chambers previously prepared. The treatment was found to be severe enough, if the washing was omitted, to cause surface injury, as light brown sunken spots, especially to plums and peaches.

Before any inoculations were made the surface-sterilized fruits were left for 3 days to a week, in different series, to allow any undeveloped infection to appear. This preliminary incubation period was sufficient to detect infected fruits, as was shown by the absence of any brown-rot in check fruits during the experiments.

Inoculations were made through pyramidal "wells" cut with a sterile scalpel in the side of the fruit. With apples and peaches three such places were cut on each fruit, with plums two and cherries one, and a liberal amount of inoculum placed in the bottom of each. Even with green fruits this gave almost perfect inoculation.

The method outlined was not intended to yield any information as to the relative infective powers of the various strains, but merely to show the course of the diseases caused by them after infection had been produced, under these strictly comparable conditions.

Results.—All the strains studied were found capable under these conditions of causing brown-rot of the familiar kind on all the fruits on which they were tried. The following appeared to be differentiating characteristics:

(1) Rate of growth. Strains of *Sclerotinia americana* and *S. fructigena* rotted fruits distinctly faster than those of *S. cinerea.*

(2) Production of conidia on the surface of the fruit (away from the point of inoculation). This occurred with *S. fructigena*

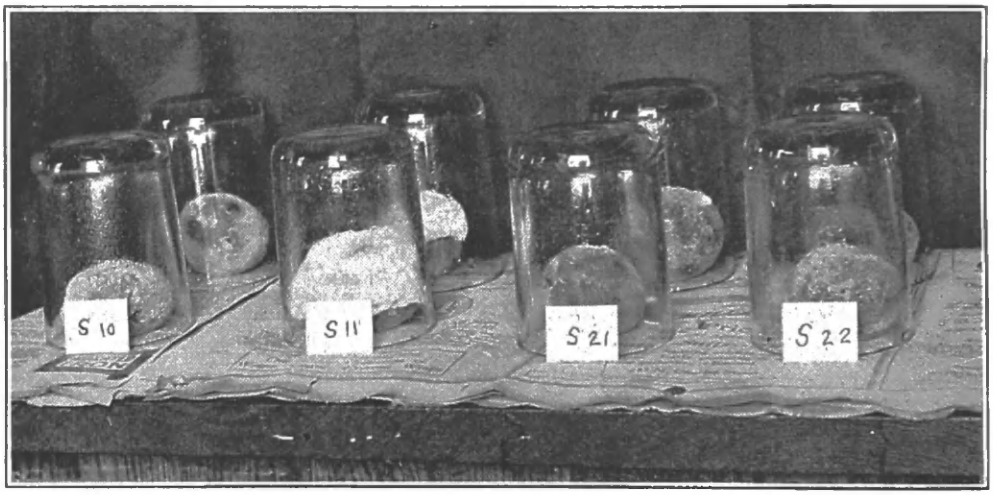


Fig. 18.—Greensboro peaches inoculated with some Sclerotinia strains, photographed after 5 days incubation at 28° C. Note the profuse growth of white mycelium over the fruits inoculated with S 11 (var. V).

in large pustules, often 2 to 5 mm. in diameter and bearing yellow conidia. With *S. americana* the size of individual pustules seldom exceeded 0.5 mm, and the spores were of the familiar buff color. Conidial pustules of *S. cinerea* were still smaller.

(3) Growth of hyphae on the exterior of rotting fruits. Under suitable conditions certain strains will develop on the outside of the fruit, and even envelope it in a mass of mycelium. (See fig. 18.)

(4) Nigrescence of the skin of the fruit. Though this is undoubtedly influenced by environmental conditions, as Heald (13) found, under definite environmental conditions different strains of Sclerotinia vary in a fixed way in regard to whether they blacken the skin of a rotting fruit, and what portion of it is blackened.

The results of two series of inoculations will be given in detail because they bring out well the difference shown between the

three species of Sclerotinia included as well as illustrating some of the differences between different types of *S. americana.*

Table X summarizes the result of inoculation on some sour cherries picked slightly unripe. Five of these were placed under a single tumbler for each strain.

In this series, conidia appeared abundantly in two days on the cherries inoculated with *americana* and *fructigena.* No conidia were produced even after a month's incubation on the cherries inoculated with the two *cinerea* strains. These were also distinctly slower in extending the rotted area than either of the other species involved. While with the first two species three quarters of each inoculated cherry was rotted in two days, the infection in the case of S 43 and S 44 included only about a third of each fruit in the same length of time.

TABLE X.

Characteristics of rot produced on sour cherries inoculated with Sclerotinia strains and incubated at 26.7° C.

Strain	Days to produce complete rotting	Production of conidia, at end of 9 days
S 4	4	++++
S 5	4	+++
S 20	4	++++
S 21	4	+++
S 22	4	++++
S 41	4	+++
S 42	4	++++
S 43	7	—
S 44	9	—

Table XI gives a summary of inoculations on some small Shockley apples inoculated in the laboratory in January, 1924. Three apples, each under a separate tumbler, were used for each strain, and in addition S 22, 43, 44, and 47 were inoculated into four apples per strain for incubation under large glass dishes. The appearance of the infection on the different apples of each strain tallied exactly as to nigrescence, spore production, etc.

The results were outstanding with regard to species differentiation. Of many strains of *S. americana* included, many of which produce conidia in abundance under other conditions, not one developed any pustules of conidia except at the point of inoculation. S 42, a *S. fructigena* strain, in 9 days produced the large spore pustules and yellow conidia characteristic of that species. Four of the *cinerea* f. *pruni* strains, S 44, 47, 49, and 55, developed

within 25 days after inoculation minute grayish pustules, scattered irregularly over the surface. This tardy development of conidia was in marked contradistinction to the rapid spore production by *S. fructigena* under these conditions, and to the rapid production customary with *S. americana* under conditions that result in spore production by strains of that species.

Of the other strains, S 23, 43, 53, and 54 were distinguished by their much slower rate of growth. The rest include all the *S.*

TABLE XI.

Characteristics of rot produced on Shockley apples inoculated with Sclerotinia strains, incubated at 18.5° C. (average). Notes after 27 days.

Strains	Conidia		Hyphae (at points of inoculation only)	Nigrescence	
	At point of inoculation	Away from point of inoculation		At point of inoculation	Away from point of inoculation
S 6	++	—	++	++	—
S 11	—	—	++	—	—
S 13	++	—	—	++	—
S 20	+++	—	—	+++	tr.
S 21	+++	—	—	++	+
S 22	++++	—	—	++++	+
S 23	—	—	tr.	—	—
S 25	+++	—	—	++	++
S 27	—	—	++	—	—
S 40	+++	—	—	+++	+
S 42	++++	++++	—	—	—
S 43	—	—	+	—	—
S 44	+	+	++	+	—
S 45	+++	—	—	++++	+++
S 47	++	++	tr.	+	—
S 49	++	+	—	+	—
S 51	++++	—	—	++++	++
S 53	—	—	+	—	—
S 54	—	—	++	+	—
S 55	+	++	—	++	—

americana strains of the series, which can then be grouped as in Table XII by the relative production of conidia or hyphae at the points of inoculation and the nigrescence of the skin of the apple.

Grouped in this way, it can be seen that strains which showed similarities under cultural conditions also produced rots of similar characteristics in this experiment.

From the many other series of inoculations which demonstrated differences between strains of *Sclerotinia americana,* some series

on plums are especially interesting. These rotted rapidly and no nigrescence could be noted, but as shown in Table XIII the few strains used could be grouped on the characteristics of conidial and hyphal production alone. It will be noted that the distribution of the strains in the groups here is roughly that of their distribu-

TABLE XII.

S. americana strains grouped according to characteristics of rot produced on inoculated Shockley apples, results shown in detail in Table XI.

Group characteristics	Strains
1. Conidia abundant, hyphae none, nigrescence pronounced and away from as well as at points of inoculation.	S 20, 21, 22, 25, 40, 45, 51.
2. Conidia medium, hyphae none, nigrescence around points of inoculation only.	S 13.
3. Conidia and hyphae medium, nigrescence at points of inoculation only.	S 6.
4. Conidia none, hyphae medium to abundant. Nigrescence none.	S 11, 27.

TABLE XIII.

S. americana strains grouped according to growth on inoculated plums after 7 days at 28° C.

Group characteristics	Variety and ripeness of fruit			
	"First," unripe	"First," fully ripe	"Waugh," quite green	"Waugh," almost ripe
1. Conidia abundant, hyphae few.	S 4, 14	S 14, 35	S 4, 5, 14, 20, 35	S 14, 35
2. Conidia and hyphae medium to abundant.	S 20, 35	S 4	S 36	S 4, 5
3. Conidia few, hyphae abundant.	S 5, 36	S 5, 20, 36	.....	S 20, 36

tion by cultural characteristics in earlier tables. S 4 and 14, for instance, also produce an abundance of conidia in culture, while S 5, 20, and 36, which here developed in some series so much mycelium as to completely envelope the plums, in cultures also form more mycelium than the other strains mentioned, and also develop the "raised hyphal masses".

Tables XIV and XV show groupings based on inoculations on apples and peaches. These rotted more slowly than the plums and showed more or less nigrescence, permitting the use of this characteristic in the grouping. While growth characteristics of the different strains varied according to the kind and variety of fruit, in every case there were clear-cut differences between different strains.

The inoculations performed demonstrated that the strains of *S. americana* which appear different in culture all produce "brown-rot" on fruits, but a rot of different characteristics for different cultural groups of strains. Thus strains such as S 22 which produce in culture a predominance of conidia tend to do the same on rotting fruits, while strains developing a predominance of hyphae in culture, as S 11, show few spore pustules and tend instead to a profuse display of superficial hyphae on inoculated fruits. Strains shown by other methods to belong in *S. cinerea* forma *pruni* or *S. cinerea* forma *mali* are again distinguished from *S. americana* by their slower rate of growth and differences in other characteristics.

V. TEMPERATURE RELATIONS

Series 1. Strains S 4, 5, 20, 21 and 22 were used in series of cultures, two Petri dish cultures and four slants of potato dextrose agar, at 3°, 13°, 20°, 25°, 30°, and 37.5° C.

No growth occurred at the highest temperature, and at the lowest temperature it was extremely slow and the same for the five strains. All grew well at intermediate temperatures. At 30° the production of conidia was inhibited in favor of hyphal growth. However, S 5, 20, and 21, which frequently show the "raised hyphal masses", did not show this characteristic at 30°, while at 13° they displayed it not only in tube cultures but also in some of the Petri dish cultures. Conidia also were more abundant, considering all strains, at this temperature than at any of the others.

No differences could be detected in the optimum temperatures which fell in all cases at about 25°.

Series 2. S 22 and S 27 represented *S. americana;* S 42, *S. fructigena;* S 43, *Monilia cinerea* forma *mali;* and S 44, *S. cinerea* forma *pruni.* Cultures were grown at 2°, 8°, 13°, 20°, 25°, 30°, and 35° C.[1]

Two tubes of potato dextrose decoction and two potato dextrose agar slants were incubated for each strain at each temperature. After two weeks the weight of the colony produced in the liquid medium was determined as follows: The hyphal mat was pulled out of the tube with a wire, allowed to drain for one minute on

[1]Dr. Erwin F. Smith kindly permitted the use of some of the incubators in his laboratory for the lower temperatures.

TABLE XIV.

Strains of Sclerotinia americana grouped according to growth on inoculated apples after 20 days at mean room temperature of 28° C.

Group characteristics	Variety and ripeness of fruit		
	"American Summer" unripe	"American Summer," ripe	"Red Astrachan," ripe
1. Conidia abundant, hyphae none, nigrescence pronounced.	S 22, 25, 35	S 8, 20	S 4, 35
2. Conidia medium, hyphae none, nigrescence medium.	S 36, 37	S 4, 6, 30	S 14
3. Conidia medium, hyphae few to none, nigrescence none.	S 21	S 5	S 5, 20
4. Conidia few to none, hyphae abundant, nigrescence none.	S 11	S 11	S 36

TABLE XV.

Strains of Sclerotinia americana grouped according to growth on inoculated peaches after 7 days at mean room temperature of 28° C.

Group characteristics	Variety and ripeness of fruit		
	"Late Crawford," green	"Elberta," nearly ripe	"Greensboro," fully ripe
1. Conidia abundant, hyphae none, nigrescence pronounced.	S 8, 20	S 4, 5, 8, 20, 24, 28, 29, 31, 37	S 22, 25, 30, 37
2. Conidia medium, hyphae few or none, nigrescence medium.	S 4, 5, 6, 30	S 6, 14, 25, 26, 27, 30	S 21, 26, 36
3. Conidia medium, hyphae none, nigrescence none.	S 13	S 13	
4. Conidia few or none, hyphae abundant, nigrescence none.	S 11	S 11	S 11

filter paper, and weighed at once. Averaging the results, the cultures of *Sclerotinia americana, S. fructigena,* and *S. cinerea* showed within the range of this experiment the same minimum, maximum, and optimum temperatures, except that neither S 43 nor S 44 grew at 30° C. Greatest growth was obtained in all cases at 25° and none at 2° or at 35°.

This indicated a lower maximum temperature for the two *cinerea* forms. Series of cultures grown at high temperatures to check this conclusion proved it erroneous, growth occurring equally at 30°, 31.5°, and 33° C. in the five strains considered.

With the methods used no consistent differences in the cardinal temperatures of the strains or species could be demonstrated, the minimum, optimum and maximum falling near 3°, 25° and 33° C., respectively, for all. These values agree well with those obtained by Ames (*2*) and Brooks and Cooley (*4, 5*) with strains isolated from various fruits. It should be noted that Miss Ames reported that the maximum temperature for germination with a Monilia strain isolated from a peach was 30° C., while with a plum strain it was 36° C.

VI. MEASUREMENTS OF CONIDIA.

The use of conidial measurements in differentiating *S. fructigena* from *S. cinerea* has been mentioned above, and is discussed by Wormald (*39*) with regard also to the variability in size of conidia of *S. cinerea* under different conditions. A recent note by Cook (7) mentions the isolation from apples of three Sclerotinia strains of which one developed spores ranging from 8 x 12 to 6 x 16 microns, while another developed spores measuring 20 x 20 to 24 x 36 microns.

In the present investigation this feature has not been developed extensively. In such measurements as were made the conidia were always allowed to stand at room temperature for at least ten minutes after mounting in distilled water, to allow for absorption of water. Spores were picked at random. Ranges when given apply only to the size of population measured.

Table XVI shows the mean values obtained by measuring conidia of the same five strains grown under three different conditions.

While the differences shown between the strains are slight, the strains maintain the same relative position as to size in all three cases. The conidia of S 21 are always larger, and those of S 4 smaller than those of any of the other strains. However, S 5, which is identical with S 21 as to source of origin and characteristics on cultures or rotting fruit, shows values resembling those for S 21, but closer to those for S 20 under most conditions.

Considering the range in size of the conidia the situation is again as above. Greater variability was found in conidia from

rotting apples. Spores from S 4 ranged in length from 8.3 to 20.3 microns, and those of S 21 from 11.3 to 27 microns; spores from the other strains fell between these extremes.

In Table XVII average measurements are given for 25 conidia of each of five strains of *S. americana* and one of *S. cinerea f. pruni*. Again, here S 21 shows a higher value among the *americana* strains than does S 20. However, S 45, which on its cultural and other characteristics is grouped with S 4, has larger spores than either of these other strains; while S 4 in the previous table is distinguished by its small conidia.

TABLE XVI.

Dimensions of conidia for certain strains of Sclerotinia americana.

[Mean values for 50 conidia each]

Strain	Source of conidia		
	Point of inoculation on rotted apples (lot f 1 of fig. 16), 20° C.		Potato dextrose agar slants, at 14° C. for 13 days
	22 days	28 days	
	microns	microns	microns
S 4	14.1 x 11.4	15.1 x 12.3	15.0 x 10.2
S 5	15.4 x 11.4	15.6 x 12.5	15.8 x 11.3
S 20	14.7 x 12.4	15.4 x 12.4	14.4 x 11.2
S 21	18.1 x 12.2	17.0 x 13.0	16.9 x 12.4
S 22	14.1 x 11.9	16.7 x 13.3	14.2 x 10.4

TABLE XVII.

Dimensions of conidia from potato dextrose agar Petri dish cultures, grown at 13.5° C.

[Measurements after 5 months. Mean values for 25 conidia each]

Strain	Average size of conidia
	microns
S 6	16.5 x 11.8
S 11	14.7 x 8.5
S 20	14.7 x 10.2
S 21	16.2 x 11.8
S 45	16.5 x 10.1
S 47	12.5 x 8.7

In Table XVIII some measurements are given for strains of the different species. As would be expected, the *Sclerotinia fructigena* strains show the highest values. The values for *Sclerotinia americana* and *cinerea* are not very different from each other and agree well with those given by Wormald.

TABLE XVIII.

Dimensions of conidia from cultures on potato plugs, grown 7 days at 25° C.

Species	Strain	Mean length	Mean width
Sclerotinia americana	S 22	14.5 ± 0.3[1]	10.8 ± 0.2
Sclerotinia americana	S 45	14.9 ± 0.1	10.6 ± 0.2
S. cinerea f. pruni	S 44	12.9 ± 0.4	9.0 ± 0.2
S. cinerea f. pruni	S 47	14.1 ± 0.2	11.0 ± 0.2
S. cinerea f. pruni	S 56	14.5 ± 0.2	10.6 ± 0.2
S. fructigena	S 42	18.0 ± 0.5	11.4 ± 0.3
S. fructigena	S 52	18.9 ± 0.1	11.7 ± 0.2

[1]Probable error computed by the formula P. E. $= .6745 \frac{\sigma}{\sqrt{n}}$

The size of conidia, from single-spore strains grown under different controlled conditions, has been shown above to be a constant for each strain. Significant differences are found not only between species but also between strains of the same species. Conidia of *Sclerotinia fructigena* are significantly larger than those of *S. americana,* and those of *S. americana* are frequently somewhat larger than those of *cinerea.*

Among the strains of *S. americana* variation in spore size does not seem to be correlated with the physiological cultural differences on which the varieties are described below, but appears to vary independently, perhaps to the maximum extent for the species, within any of these physiological varieties.

VII. DROP CULTURE STUDIES.

Wormald noted that different species of Sclerotinia show considerable variation in the germination of conidia in plates of prune agar. Conidia of *S. cinerea* f. *mali* and *pruni* characteristically germinated to form short germ tubes which very soon became branched. On the other hand, with the forma *americana* the germ tube was longer, straight, and usually remained unbranched until it had attained a length of at least 200 μ. Spores of *Sclerotinia fructigena* also germinated in the latter manner.

The following series of drop cultures was planned to check this distinction under conditions permitting of more detailed observa-

tion than in Petri dishes. A large number of strains were grown in hanging drop cultures, using shallow depression slides that provided an air cell of about 0.1 cc. capacity. The potato dextrose decoction already described was used as a nutrient in all cases. Clean cover glasses were kept in a small dish of alcohol. Just before use these were flamed off, a drop of the nutrient solution placed in the center with a wire loop, and the inoculum placed in the drop, distributing it well. It was found that considerable latitude in the density of inoculation of the drops would not interfere with the results; but observations are more readily made with drops not too heavily seeded. About 20-50 conidia in a drop carried by a 5 mm. loop make a convenient number. The cover glasses were sealed on the slides with vaseline in the customary way.

The type of germination was apparently not influenced by the size of the cell under the drop. Slides with slight depressions, with a capacity of only 0.1 cc., gave the same results as when glass rings of a capacity of 1.7 cc. were used. Brief tests showed also that with a given strain the same results were obtained irrespective of the type of inoculum used. Drop cultures inoculated with hyphae of S 40 developed the same characteristic *S. americana* growth as when conidia were used. Similarly, in S 46 typical *S. cinerea* development occurred if either conidia or the chlamydospore-like hyphal segments served as inoculum.

All series were incubated at 25° C.

Under the conditions specified, germination is rapid with all the strains of the three species studied. However, the rate and type of growth is so different that not only does microscopic observation yield the results illustrated and considered in detail below, but even macroscopic observation of the slides in two or three days shows that strains of *Sclerotinia americana* have made abundant growth, filling the drops with white hyphal growth, while growth with strains of *S. cinerea* is perceptibly less, individual colonies showing as minute woolly dots.

Microscopic study shows that these species differ not only in the rate of total growth but also in the dimensions of the individual cells, the types of the cells, and even in the type and frequency of branching.

Most of these characteristics can be observed within 16 to 24 hours (fig. 19). Just as Wormald found in his plate cultures, it was noted that the *americana* strains grew out to form long, straight germ tubes, which did not branch until they had attained considerable length and typically do not within 20 hours show any branching along the germ tubes close to the conidia. With *S. cinerea* strains on the other hand, the total length of the germ tube is decidedly less. Many branchings occur early in the growth of the germ tube so that some branches are always seen close to the conidium. Characteristically two branches of equal diameter

are found, so that in a culture of more than about 20 hours' growth it is usually difficult to identify the original germ tube. This frequent branching and the subsequent greater growth of one of the branches results in the typical geniculate or scorpioid form of growth.

Individual hyphal cells of *S. cinerea* are, also, characteristically of a slightly greater diameter, and the walls more deeply constricted at the septae, than with cells of corresponding age in *S. americana*. While the *americana* hyphal cells are generally straight, those of

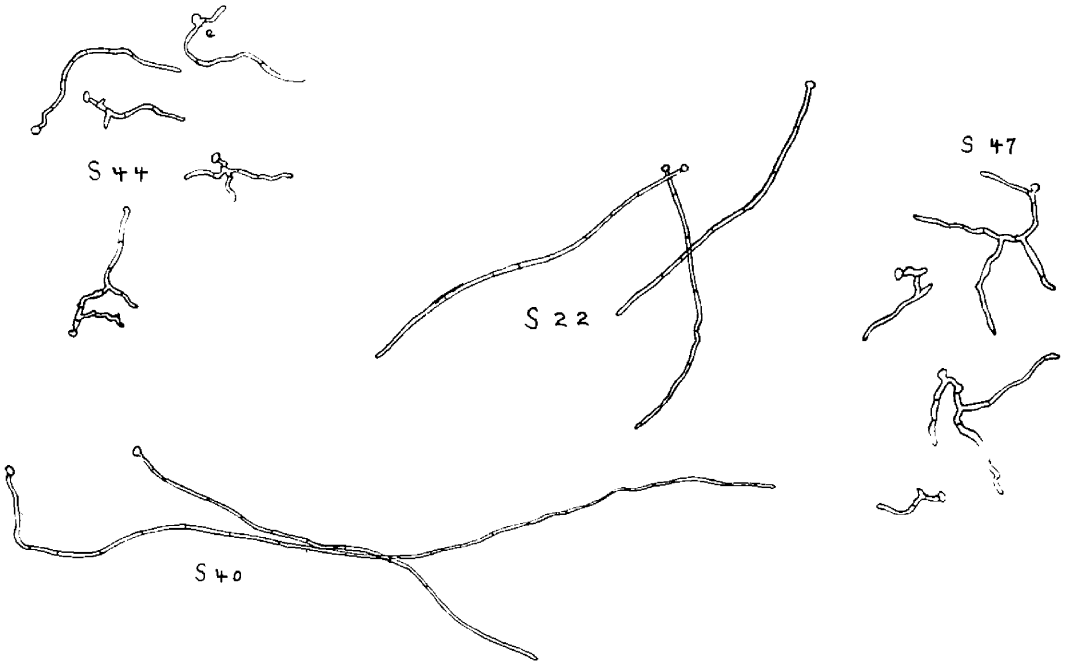


Fig. 19.—Germinating conidia of *S. americana* (S 22 and S 40) and *S. cinerea* forma *pruni* (S 44 and S 47). 18 hours growth in hanging drop of potato dextrose decoction, 25° C. x 80.

cinerea are frequenty contorted. Even as early as 20 hours it can generally be seen that the older cells of the *cinerea* hyphae are shorter than those of *americana* hyphae.

As an example some typical measurements recorded for a strain of *S. americana* and one of *cinerea* may be cited. These were averages of three typical sporelings; measurements were made after 18 hours of incubation.

	S 22 (*americana*)	S 44 (*cinerea*)
Average length of germ-tube	769.5 μ	140.6 μ
Number branches per conidium	1	$1\frac{1}{2}$
Total length of branches per conidium	30.4 μ	62.5 μ
Distance conidium to nearest branch	113.0 μ	13.8 μ

The general appearance of the culture is equally characteristic after three days growth (fig. 20), which is generally sufficient for vegetative development to be completed. With *S. americana* the hyphae are long, straight and branching is simple. In glancing over the slide it is easy to pick out at once the older hyphae from which the branches arise. On the other hand, the *cinerea* mycelium,

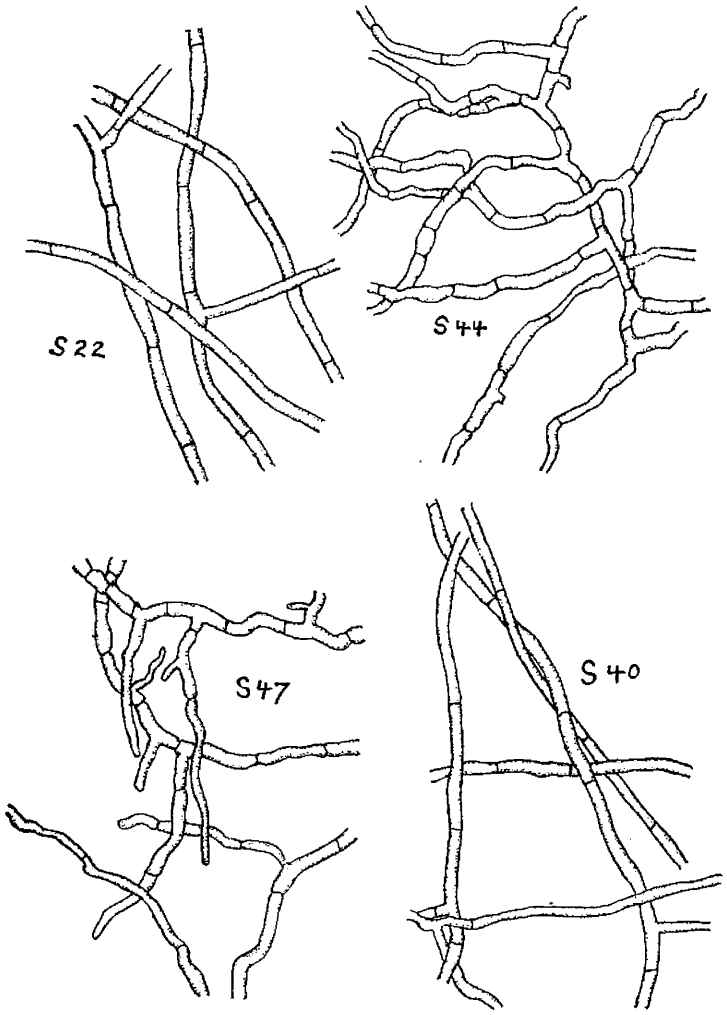


Fig. 20.—Hyphae of *S. americana* (S 22 and S 40) and of *S. cinerea* forma *pruni* (S 44 and S 47) in three-day-old standard drop culture. x 180.

while it occupies a much smaller total extent, is of a denser and more homogeneous nature. Almost every hypha arises from dichotomous branching, and all twist and bend so frequently that a network of contorted mycelium results and it is unusual for a hypha to be found that is straight for even the distance across a few fields of the microscope. This is markedly different from the *americana* hyphae, which can be traced straight, all the way across the drop.

Accurate observation of the length of hyphal cells is also possible in three day old cultures. Due to the enormous variability not only in length of individual cells, but in type of hyphal filament to be found in the same culture, no one figure can be obtained that will be representative of all hyphae of a given strain. It is however possible by selecting hyphae for measurement according to a definite plan to obtain measurements of a definite type of hypha, and such values can be reproduced quite accurately in subsequent series. In making the measurements given in Table XIX the following method of selecting hyphae was worked out.

The type to be measured is hyphae that have attained sufficient maturity so that septation is presumably complete. Hyphae were picked away from the edge of the colony. Only the larger hyphae from which good sized branches had arisen were used, and on these no measurements were made in the region that was inspected in selecting the hyphae. After examining a hypha to determine its apparent "maturity" the slide was then moved and measurements made of the first five consecutive cells along the filament selected, toward the center of growth, after passing out of the former field of the microscope. Thus the measurements were based on hyphae selected as typical of the larger cells of a culture but did not include the cells actually examined in making this selection.

For each measurement five hyphae were selected in this manner. The values shown are then averages of 25 cells for each slide examined and due to the variability mentioned above the actual averages obtained probably of more comparative than absolute value. The figures presented in Table XIX may then not represent absolute constants for the various strains but undoubtedly indicate the range of values likely to be obtained by the method described in differentiating *S. americana* and *S. cinerea* forma *pruni*.

A possible source of error that must be avoided is the selection of specialized hyphae, developing apparently as the first stage of apothecial development. These bodies have been described and illustrated by Woronin (42) from drop cultures of *S. cinerea*. They are readily recognized in cultures by their coiling form and trichotomous branching. The normal hyphal cells (fig. 21) are partially or completely vacuolate by the third day, while these ascogonial elements, arising late in the history of the culture, are generally growing vigorously at this time and their protoplasm is dense and homogeneous (fig. 22). In these studies such aggregates of hyphae have been noted in many strains of both *S. cinerea* and *S. americana*.

It should be emphasized that differentiation in drop culture is based on the characteristics of growth under the definite environmental conditions described and for observations made at the time indicated, in the case of measurements of cell lengths of hyphae, after three days growth. Additional incubation results frequently

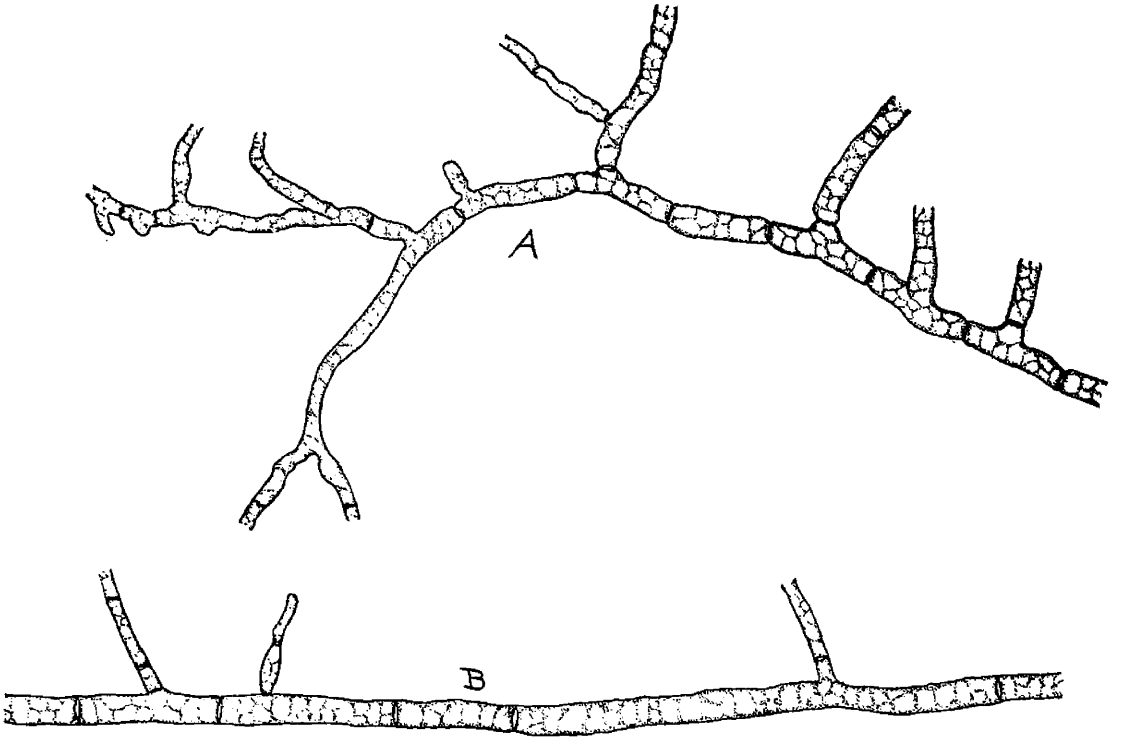


Fig. 21.—Type of three-day-old hyphal cells measured in drop cultures. A, *S. cinerea* forma *pruni* (S 44); B, *S. americana* var. I (S 22). x 400.

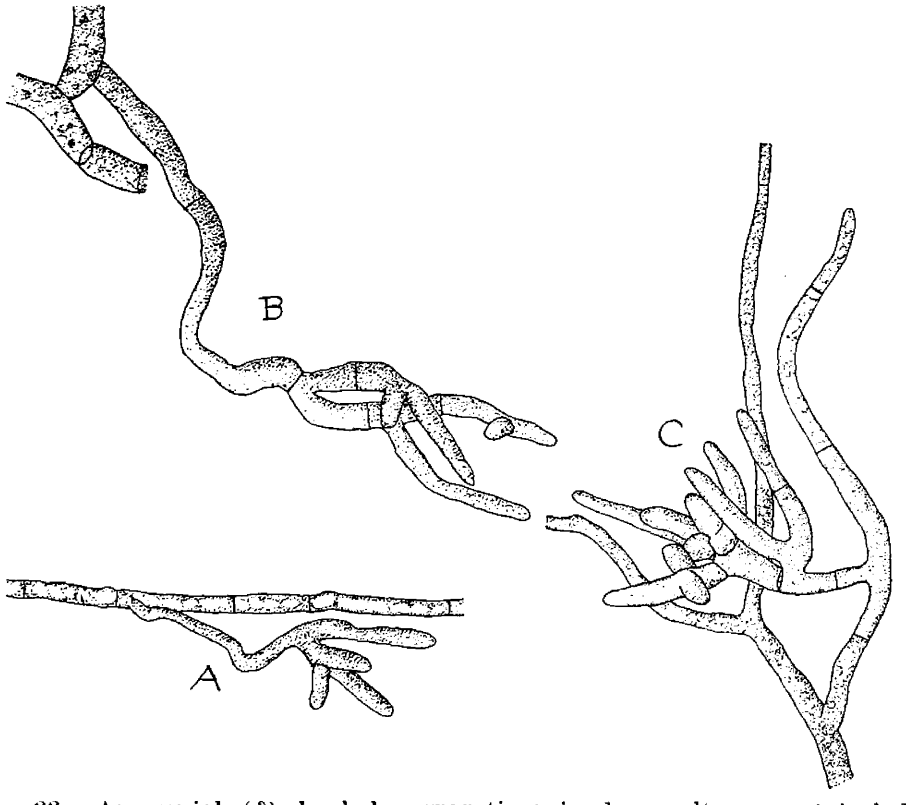


Fig. 22.—Ascogonial (?) hyphal aggregations in drop cultures, *not* included in measurements. A, *S. americana* var. III (S 5); B, *S. cinerea* forma *pruni* (S 46); C, *S. americana* var. II (S 34). x 400.

TABLE XIX.

Measurements of hyphal cells of strains grown in potato dextrose decoction drop cultures 3 days at 25° C.

Strains, **grouped by their germination and general growth characteristics (see text)**	Mean length (25 cells per test) in microns
S 2	69
S 6	65[1]
S 11	66[1]
S 13	68[1]
S 14	68
S 20	59[1]
S 21	59
S 22	62[1]
S 25	60
S 26	68
S 27	62
S 34	59
S 36	67
S 37	68
S 40	58
S 41	79
S 45	72[1]
S 50	75
S 51	78
Average for *S. americana* (above)	*66.2*
S 44	41[1]
S 46	25[1]
S 47	34
S 49	45
S 53	41
S 55	44
S 56	32[1]
Average for *S. cinerea f. pruni*	*36.0*
S 10	56
S 23	62[1]
S 43	64
S 54	74
Average for *Monilia cinerea f. mali*	*63.5*
S 42	40
S 52	68
Average for *S. fructigena*	*53.6*

[1]Average of two or more sets of measurements.

in so much growth of the ascogonial elements, which are at first easily distinguished from the normal vegetative hyphae, that some of these now vacuolate specialized hyphae may be selected for measurement instead of normal ones. In a few series in which late measurements of this sort were made, values were seldom obtained checking those obtained in other tests with the same strains or even in the same cultures when measured at the proper stage of growth.

In Table XIX the strains are grouped by species according to the general growth and branching characteristics of the hyphae, without considering the length of the cells. It will be noted that this classification coincides exactly with that given in Table VI based on the cultural characteristics of the strains. Furthermore, the length of typical hyphal cells in three day old cultures shows a grouping coinciding again with these previous ones.

Of the nineteen strains of *S. americana* included in these tests the lowest value obtained was 58 microns, while the average for the species was 66.2. With the seven strains of *S. cinerea* forma *pruni*, the highest value was 45 microns and the mean for the species 36.0. It is to be noted that the strains of *cinerea* found in this country agree by this test, as in general branching and growth habits, with the *S. cinerea* cultures from Europe.

Only two averages were computed for *S. fructigena* by this method. In general habit of growth in drop culture these strains resemble *S. americana* much more nearly than they do *S. cinerea.* A repetition of the test with S 42 would probably result in a value more nearly like that of S 52.

S 10, 23, 43, and 54 are listed separately. As has been previously mentioned, these strains resemble each other in their cultural characteristics as also in their rate of growth on inoculated fruits, etc. In the present series they were again the same in all showing mycelial growth habits and cell lengths in agreement with those of *S. americana* as described above. They are certainly distinctly different from any of the forms considered in this paper as *S. cinerea* forma *pruni.* S 43 was designated *Monilia cinerea* forma *mali* by Wormald, and the other strains of this group are apparently more closely related to it than to any other strains. It is then of considerable interest to note here the reversal in germination characteristics of this type of *M. cinerea,* if these strains are correctly placed as such, as compared to its germination in prune agar plates as described by Wormald, in which he found it the same as *S. cinerea* forma *pruni* and different from "forma *americana.*"

As a whole, the study in drop cultures under the standard conditions specified served to bring out distinct morphological differences between *S. americana* and *cinerea,* as described above, in not only the habit of germination but in branching and other mycelial characteristics visible at any stage of the life of the colonies. These distinctions held true throughout the entire population of *S. americana* and *cinerea* studied.

VIII. APOTHECIAL CHARACTERISTICS.

A detailed description of the apothecial stage of *Sclerotinia americana*, together with an account of some of its physiological relations, has already been published as the first paper of this series (22) and the literature along that line discussed there.

It will be noted from Table I that nine of the strains of Sclerotinia in the present investigation were derived from apothecia. Eight were of *americana* and a single strain, S 44, of *S. cinerea*. These agreed in all respects with corresponding strains from rotting fruits, etc.

As stated in the previous publication (22) distinct variations have been noted between apothecia of *Sclerotinia americana*. S 4, 5, 20, 21 and 22 were derived from such *S. americana* apothecia, which were used in experiments in which they were grown under identical conditions in the greenhouse (9) but which still showed differing characteristics. S 4 and S 22 came from an apothecium on a mummy that bore only cup shaped apothecia (becoming flat only when splitting in a stellate manner) in which the bottom of the cup was much wrinkled. S 5 and S 21 came from apothecia of the same form except with a flat bottomed cup; while S 20 was derived from a mummy showing apothecia perfectly flat and smooth across the top, with only a slight depression at the center of the disc and no wrinkling of the hymenial surface.

Whether such variations can be associated with the physiological ones on which the varieties of *S. americana* are described has not been determined. A series of peach mummies, inoculated with various single spore strains, started to develop apothecia only during the recent spring of 1924. Unusually high precipitation resulted in the "drowning" of those young apothecia located under what are usually the more favorable conditions, in the moister spots; while apothecia in more exposed places had already been killed in the customary way by drying.

Asci from apothecia of *Sclerotinia americana* measured at different times gave a mean size of 146.2x8.7 μ and ascospores of 12.0 x 6.5 μ. These values are for mature ascospores and monostichous asci. As Wormald mentioned (40) with *S. cinerea*, the asci swell just before the ascospores, arranged at that time in a distichous manner, are discharged.

These measurements are in close accord with those previously reported for *Sclerotinia americana* and also with those recently found by Wormald for *S. cinerea* (40).

DISCUSSION.

As well as differentiating a number of types among the strains studied, these studies have shown that the grouping developed from a given environment also holds true under another totally different. As has been shown above in detail, strains of European and American origin differentiated from the commoner American forms by the habit of growth of their mycelium in drop cultures (fig. 20) can be distinguished in the same drop cultures by the further morphological distinction that the average length of their hyphal cells is only half that of the *americana* forms. Furthermore, in Petri dish cultures on potato dextrose agar strains of the former groups, corresponding to *Sclerotinia cinerea* forma *pruni* Wormald, always have a typical zoned and lobed growth (fig. 8), while those of the latter kind consistently grow regularly out to the edge of the plate without zones or lobes showing, but typically with abundant concentric ringing of conidia or mycelium. Again, in their oxidase reaction the *americana* strains generally show greater activity than the *cinerea* ones. In tube, Petri dish, and drop cultures the rate of enlargement of *americana* colonies is greater than that of *cinerea* colonies. Inoculations on different fruits showed a corresponding difference in the rate at which the fruits were rotted, and also demonstrated that there was a difference in the conditions under which conidia would be produced from rotting fruits inoculated with these different forms.

With all these differentiating characteristics, especially the two morphological ones first mentioned above, it seemed advisable (21) to raise *Sclerotinia cinerea* forma *americana* Wormald to specific rank, becoming *Sclerotinia americana* (Wormald) Norton and Ezekiel. The differences are contrasted below:

Sclerotinia americana.	*S. cinerea (forma pruni)*
Growth in drop cultures of potato dextrose decoction at 25° C.	
Germ tube straight, 500-1000 μ long in 18 hours, frequently still unbranched.	Germ tube seldom straight, usually geniculate, 100-300 μ long and always branched in 18 hours.
Branching of hyphae typically simple, hyphae usually straight, mycelium loosely meshed.	Branching typically dichotomous, hyphae bending, often scorpioid; mycelial mat densely and intricately entwined.
Cells of hyphae, selected by standard method in 3 day drop culture, average 66.2 μ long.	Cells of hyphae, similarly selected, average 36.3 μ long.
Colony extending to limits of drop culture in 3 days, many hyphae pushing into the vaseline seal.	Rate of growth slower, total growth never so great, original drop rarely filled by colony.

Sclerotinia americana.	*S. cinerea (forma pruni)*
Petri dish cultures on potato dextrose agar at 15° C.	
Growth rapid and even, colony characterized by numerous concentric circles of conidia or mycelium, edge entire or sub-entire.	Growth less than half as rapid as *americana*. Characteristic colony with a number of zones, edge lobed.
Cultural characteristics on potato dextrose agar slants, 20-25° C.	
Growth rapid, conidia when present in definite, Tilleul buff, pustules.	Growth much slower. Conidia seldom produced, never in definite pustules. Surface of colony characteristically smooth, velvety, buffy brown to Saccardo's umber (30) in color.
Characteristics of rot on inoculated fruits.	
Rotting rapid, conidia if produced developing soon after inoculation.	Rotting slower, pustules of conidia developing if at all only after a considerable interval, and under conditions different from those favoring production of conidia of *S. americana*.
Oxidase production in culture media.	
With many strains, but not all, much stronger than with *cinerea*.	Always less than of most *americana* strains.

A partial synonymy of *Sclerotinia americana* is as follows:

Oidium fructigenum Knz. and Schm. Peck, N. Y. State Mus. Nat. Hist. Ann. Rpt. 34 (1880) : 34-36. 1881.

Monilia fructigena Persoon. Smith, J. Myc. 5: 123-134. 1889.

Sclerotinia fructigena (Pers.) Schroeter. Norton, Sci. 16: 34. 1902.

Sclerotinia cinerea (Bon.) Schroeter. Matheny, Bot. Gaz. 56: 418-432. 1913.

Monilia cinerea Bon. forma *americana* Wormald, Ann. Bot. 33: 361-404. 1919.

Sclerotinia cinerea forma *mali* Wormald was described by Wormald as differentiated from his *cinerea* forma *pruni* chiefly by a higher oxidase reaction and by causing a different kind of disease. In the present work a strain from Holland and two from Washington, D. C., have appeared similar to a strain of this type secured from Wormald. All of these are differentiated from *S. americana* and *S. cinerea* by a lower rate of growth, generally lower oxidase activity, and featureless colonies consisting mainly of "substrate mycelium." However, these strains showed in drop culture a close relation to *S. americana*; and it may also be of significance that the only anastomosis observed between forms not known to be of the same species, when grown together in Petri dishes, was between

S 43, a forma *mali* culture from Wormald, and *S. americana.* Thus forma *mali* should perhaps not be included under *S. cinerea;* however the number of cultures of it studied here were too few to justify any change at this time.

Sclerotinia fructigena is always to be distingushed from *americana* or *cinerea* by its larger pustules on fruit and in culture, bearing yellow conidia that are of a larger size than those of the other species.

In the present study, *Sclerotinia americana* has been found only in America. *S. cinerea* was sent in culture from England and Holland, and cultures of the Oregon twig-blight Monilia proved to be of *S. cinerea* forma *pruni;* as were also three strains from California. Two strains isolated from a single apple from a home storage cellar in Washington, D. C., in which so far as known only domestic fruit had been stored, were classified as *S. cinerea* forma *mali. S. fructigena* has not been found in this country in this investigation nor is there authentic record in the literature of its presence here.

Sclerotinia americana is the form predominating in this country, occurring abundantly on rotting fruits, peaches, plums, cherries, and apricots and less abundantly on apples and other fruits. Its different varieties have been isolated from these hosts as listed later. It is of interest to note that the only strain isolated from an apricot, S 45, proved to be a typical *americana.*

Varieties of Sclerotinia americana. It has been shown above that strains of *S. americana* vary widely in their characteristics in culture on rotting fruits, oxidase production, size of conidia, etc., and that such variations are genetic rather than modifications due to the environment. By considering a sufficient number of these physiological characteristics it is possible to separate any of the strains listed from any other. While it is hardly desirable to do this, it seems well at this time to establish in the species a few varieties, into which the strains can readily be grouped by their more prominent characteristics.

The varieties listed below are distinguished by the habit of growth on potato dextrose agar. This medium provides a good separation and involves the consideration of only a few characteristics. As has been shown above, the groupings of strains arrived at on this basis are significant also under other conditions, as on rotting fruits.

Diagnoses of varieties of S. americana. Descriptive terms are used here in the sense in which they have been described in Section I. Varieties are differentiated by their habit of growth on potato dextrose agar, at 25° C. in the case of tube cultures,* and at 15° C.

*At least some of the agar slants should be in narrow tubes (less than 15 mm. diameter). Observations may be made 7 to 14 days after inoculation.

with plate cultures, which are inoculations in the center of Petri dishes of set agar.

Var. I. Tube culture: conidia and microconidia abundant, hyphae only trace, no hyphal masses. Cultures flat.
Plate culture: conidia very abundant in concentric circles, hyphae inconspicuous, no hyphal masses.
Type, S 22.

Var. II. Tube culture: both hyphae and conidia medium to abundant, hyphal masses rarely if ever present.
Plate culture: conidia abundant in concentric circles. Hyphae few but visible macroscopically.
Type, S 13.

Var. III. Tube culture: hyphae and conidia medium to abundant, hyphal masses present.
Plate culture: conidia abundant in concentric circles. Hyphal masses may be present.
Type, S 21.

Var. IV. Tube culture: conidia trace to few, only at the top of the slant. Hyphal masses very abundant.
Plate culture: conidia abundant in concentric circles. Hyphal masses may be present.
Type, S 6.

Var. V. Tube culture: conidia very few, if present visible only microscopically and not as pustules. Hyphae abundant, not in hyphal masses.
Plate culture: conidia present in concentric circles in center of colony, hyphae more abundant toward the periphery.
Type, S 11.

Var. VI. Tube culture: conidia very few, if present visible only microscopically and not as pustules. Hyphae abundant, never in definite hyphal masses.
Plate culture: conidia as in tube cultures. Hyphae abundant, forming concentric circles. No hyphal masses.
Type, S 27.

The differentiating characteristics of the varieties of *S. americana* are given in condensed form in Table XX, using the symbols as above.

S 22 is the type for both var. I and the species *S. americana*. This is one of the commoner varieties. For instance, strains S 36, from a rotting peach; S 4 and 22 from peach apothecia; S 50 and 51 from rotting apples; S 2 from an old peach mummy; and S 28

from a rotting plum were all of var. I and collected in Maryland. S 14 and 19, from apples from the State of Washington; S 45 from a California apricot; and S 41 from a New Jersey peach canker were also of var. I. This shows well the wide distribution of closely similar types of *S. americana* and also the wide host range.

A number of varieties of *S. americana* may however be present on the same kind of fruit in the same orchard. Thus in a single

TABLE XX.

Condensed presentation of characteristics of varieties of Sclerotinia americana.

	Type strain	Tube cultures			Plate cultures		
		Hyphae	Conidia	Raised hyphal masses	Hyphae	Conidia	Raised hyphal masses
Var. I.....	S 22	tr.	++++	—	tr.	++++	—
Var. II.....	S 13	++ to ++++	++ to ++++	—	+	++++	—
Var. III.....	S 21	++ to ++++	++ to ++++	++ to ++++	++	++++	— to ++
Var. IV.....	S 6	++++	tr. to +	++++	++	++++	— to ++
Var. V.....	S 11	++++	—	—	++	++ to +++	—
Var. VI.....	S 27	++++	—	—	++++	—	—

peach orchard at Havre de Grace, Maryland, a collection of rotting fruits by Dr. J. B. S. Norton included *S. americana* var. I, II and III (S 36, 34 and 35).

Var. IV, V, and VI are apparently of less common occurence. Of these, S 11 of var. V deserves especial mention. This was isolated some years ago by Dr. R. A. Jehle from a New York peach canker. This strain showed, in both series of oxidase tests, the highest activity of any of the strains tested, which is of interest in relation to Wormald's conclusion that the wood-inhabiting *S. cinerea* forma *mali* was stronger in oxidase activity than forma *pruni*. On the other hand, S 40 and 41, from New Jersey peach twig cankers, were of the common varieties I and II.

The association of different disease phenomena or different hosts with the varieties of *S. americana* can scarcely be accomplished with the information at hand. More is known of the relation between *S. cinerea* and *S. americana.* Wherever diseases caused by *S. cinerea* have been studied, as in Oregon (*15*), England (*41*), and Russia (*42*), the injuries described differ from the brown-rot of fruits and the blighting of blossoms familiar in this country on stone fruits, by causing greater injury to the wood of the trees and by the production of conidial pustules not soon after infection as with *S. americana,* but after a considerable interval, often during cool weather. The species thus not only differ morphologically and physiologically, but their effect on the host appears sufficiently distinct to necessitate separate treatment from a pathological viewpoint.

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