A Fusarium Disease of Cereus schottii,

by

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Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

in the College of Letters, Arts, and Sciences, of the

University of Arizona

1933

Approved: J G Brown
Major adviser

May 9th, 1933
Date

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INTRODUCTION

Besides the many species of Fusaria which have been reported as causing diseases of economic plants, there has been some work done on the relation of Fusaria to diseases of certain ornamentals. Fusarium conglutinans callistephi causes wilt in the china aster (1); Fusarium sp., a stem rot of aster (2); Fusarium dronthi Prell. and Delae., a stem rot of carnation (3); Fusarium sp., wilt of carnation (4); and Fusarium rot, caused by Fusarium oxysporum var. gladiolis, is an important disease of gladiolus corms (5). The cacti which are extensively used as ornamentals, are subject to various diseases. This paper deals with one of these diseases and the causal organism which has been found to be a Fusarium.

HOST

Cereus schottii, the host plant of the Fusarium studied, grows in Sonora (Mexico), Southern California, and Southern Arizona. The diseased plant from which the fungus was first isolated was brought from Sonora near Magdalena. An identical Fusarium was later obtained from

a plant growing in the cactus garden at the University of Arizona. Since the latter had been placed in the garden nearly a year before it showed signs of disease, it seems doubtful that it was infected when placed in the garden. Therefore, it appears safe to say that the disease occurs in the northern and southern limits of the area in which the host is found. No data are available as to whether or not the disease occurs in California.

SYMPTOMS

The diseased plant from which the Fusarium was isolated showed a necrosis of the rot type. A large, slightly sunken lesion covered the entire lower half of the stem. This lesion was fuscous* to fuscous-black in color with a narrow area of light green-yellow, 3 mm. in width, around the margin.

The interior of the infected part of the plant was colored fuscous to fuscous-black. All the tissues were involved, so it was impossible to judge what part of the plant was first attacked or the manner in which the parasite entered and made its way through the plant.

CAUSE

a. Isolation

In the fall of 1931 a Fusarium was isolated from a

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* Ridgeway, Robert.- Color standards and nomenclature.

plant of Cereus schottii (Plate I, Fig. 1) in the following manner. The plant was broken open, bits of the diseased tissue cut out with a sterile scalpel, and placed on slants of potato agar containing 2 percent dextrose. In most cases a pure culture of the Fusarium was obtained. Since plants of Cereus schottii were not available at this time, the pathogenicity of the fungus was then determined by inoculating various species of Opuntia, chiefly of the Engelmannii group.

Monosporidial cultures were then obtained in the following way. A thin suspension of spores was made and kept at a temperature just above the point of solidification. The agar containing spores was drawn up into a sterile capillary tube by means of a rubber syringe bulb. The contents of the tube, after being allowed to cool, were examined under the microscope and single spores separated from any others were marked. The pieces of glass tubing containing single spores were then cut out, dipped in 1-1000 mercuric chloride solution and placed on agar slants. The cultures thus obtained were used for all future work.

b. Reactions

1. Potato- 2 Percent Dextrose Agar, pH 7.2 - 28°C.

Mycelium was well developed. Aerial hyphae present, 3 to 5 mm. in height, of a loose, cottony appearance becoming slightly more compact and appressed with age;

pure white in young cultures, taking on a pale pinkish-buff color with age. Discoloration of the medium began in 7 to 10 days. In a 21-day culture, the medium was bordeaux to dahlia carmine. Hyphae within the medium were not colored. Blue-black sclerotia were found in 3-day and older cultures.

Lowering the $_{p}H$ to 5.6 produced the same results as those given above, except that growth was somewhat retarded. Potato- 5 percent and 10 percent dextrose agar, $_{p}H$ 7.2 were used with same results as given above, except that growth was slightly increased.

2. Autoclaved White Corn Meal, 28°C. (Plate II, Fig. 14).

The growth was much like that on potato-dextrose agar but slightly more compact in young cultures and never over 3 mm. in height. Medium was colored magenta to dahlia carmine, beginning on the 5th day and reaching bottom of plate in 12 days. The rate of growth on white corn meal was as follows:

Age of Culture	Diam. of Culture
24 hrs.	No perceptible growth
48 hrs.	1 mm.
3 days	10 mm.
4 days	13 mm.
8 days	19 mm.
12 days	32 mm.
16 days	45 mm.

3. Autoclaved Yellow Corn Meal, 28°C. (Plate II, Fig. 17).

Same as white corn meal. Rate of growth slightly differed from above from day to day, but the growth over a period of 16 days was the same as given for white corn meal.

4. Oat Meal Agar, 27°C. (Plate II, Fig. 16).

Same as above.

5. Steamed Rice, 28°C. (Plate II, Fig. 15).

Growth more compact, less fluffy than on potato-dextrose agar. Pale hortense violet color of medium appeared around the edge of the colony after 5 days. In 14 days color reached bottom of petri dish. Color of medium in 14-day culture, pale hortense violet to mulberry purple. In 60-day culture, the medium was discolored dull violet-black. Hyphae within the medium not discolored.

Rate of growth was slightly more rapid than that given for white corn meal. The growth after 16 days measured 5 cm. in diameter.

6. Nutrient Agar, $_{p}H$ 7.2, 28°C.

Aerial mycelium well developed, 3-5 mm. in height, of a loose cottony appearance. Mycelium was pure white in young cultures. With age growth appeared more compact, and pale pinkish-buff in color. No discoloration of the medium. Growth was slow on this medium as compared with the rate of growth on potato-dextrose agar,

yellow corn meal and white corn meal.

On nutrient agar, $_{p}H$ 5.6, growth was very slow and aerial mycelium not as well developed as at $_{p}H$ 7.2.

7. Steamed Potato Plugs, 28°C. (Plate II, Fig. 9).

Aerial mycelium was much more compact, less fluffy than on any of the media described above. Growth was raised only slightly above the surface of the medium and was rather slow as compared with that on potato-dextrose agar. On the fifth day, medium became discolored deep livid purple. By the 16th day the entire potato plug was colored deep livid purple, later turning dark violet-gray.

8. Autoclaved Watermelon Stem, 28°C. (Plate II, Fig. 13).

On second day the growth appeared as a small tuft of erect, delicate, white mycelium from the point of inoculation. In 10 days erect, delicate hyphae spread over the surface of the stem and sides of the test tube; mycelium more compact than on 2-day culture but never as much so as on potato-dextrose agar, corn meal, or rice. Growth more rapid than on any other medium except watermelon stem. Mycelium pale pinkish-buff with age.

9. Autoclaved Cotton Stem, 28°C. (Plate II, Fig. 12).

Same as above.

10. Czapek Agar, 28°C.

Growth slow as compared with potato-dextrose agar;

appearance of culture as given for potato-dextrose agar; discoloration of medium beginning on seventh day, light neutral red.

11. Two Percent Glucose Agar, 28°C.

Appearance of culture much like that on potato-dextrose agar but slightly more compact; height of aerial mycelium not exceeding 3 mm. In 10 days medium was discolored tawny, later turning to ochraceous tawny and orange cinnamon. Aerial mycelium was pure white in young cultures turning pale ochraceous buff with age.

12. Slices of Living Sweet Potato Root, 27°C. (Plate II, Fig. 8).

In 6 days most of surface was covered with white mycelium, not uniform in height, but ranging from 1 mm. to 5 mm. Mycelium turned light dove-grey with age. Rot of tuber produced, fuscous in color in 21-day culture.

13. Living Whole Potato Tuber, 27°C.

No growth.

14. Living Whole Sweet Potato Root, 27°C.

White mycelium from point of inoculation. After 5 days aerial mycelium took on a light dove-gray color. Fuscous rot of tuber produced.

c. Microscopic appearance of hyphae

Hyphae from the various cultures described were examined

in stained sections of a culture on potato-dextrose agar and in stained sections of the diseased stem of Cereus schottii. Sections were stained in Haidenhain's iron-alum haematoxylon and with light green in clove oil and saffranin. Hyphae were simply branched. The diameters of 25 vegetative cells from a culture on potato- 2 percent dextrose agar averaged 2.43 u. The average length of 25 such cells was 25.8 u.

Tests with Sudan III and osmic acid on hyphae taken from a culture on autoclaved white corn meal showed oil globules to be numerous in most cells. A negative test for glycogen was given with potassium iodide. Cells which had most of their protoplasmic contents and become extensively vacuolate were common in all cultures, only a thin layer of protoplasm remaining around the cell wall in some cases. Swollen hyphal cells were numerous in all cultures. Some of these cells closely resembled chlamydospores but they showed only a single wall.

d. Reproduction

Microconidia and macroconidia (Plate III, Figs. 5,6,7) were found in cultures. Microconidia, both ovoid and sickle-shaped, were numerous in young cultures on all the media used. In the first cultures examined, non-septate spores were produced almost exclusively on all media. When septate spores were transferred from sporodochia, which occurred occasionally, cultures producing septate spores were

obtained. Microconidia were not borne in sporodochia or pionnotes.

Macroconidia were produced in sporodochia which were confluent in nature. Conidial masses were pale vinaceous-pink. Macroconidia were gradually attenuate at both ends, usually uniformly curved throughout; apedicellate, or with the foot poorly developed. Macroconidia were 3-5 septate.

Non-septate chlamydospores, both terminal (Plate III, Fig. 4d) and intercalary (Plate III, Fig. 4e), were found in cultures on potato-dextrose agar, autoclaved potato plugs, corn meal, rice, and autoclaved stems. Although chlamydospores were found without difficulty, they were never abundant. The average diameter of ten such spores, measured through the widest part of the spore, was 15.2 u. Cells differing from true chlamydospores in that they had only a single wall were very numerous in all cultures. (Plate III, Fig. 1).

Blue-black sclerotia were abundant (Plate III, Fig. 8). In cultures 30-days old on potato- 2 percent dextrose agar numerous sclerotia were found imbedded in the medium. In cultures 70-days old on autoclaved rice and corn meal, sclerotia were so numerous that the remaining medium appeared blue-black in color. Sclerotia varied in size up to 1½ mm. in diameter.

SPORE MEASUREMENTS*

Medium	0-Septate	1-Septate	2-Septate	3-Septate	4-Septate	5-Septate
White corn meal 10-day	93 % 5.2-14.5 X 2.5-4 u Av.,9.7 X 2.3 u	6 % 15.5-16.5 X 3-4 u Av.,15.3 X 2.3 u	1 % 15.5-16.5 X 3-4 u Av.,15.5 X 3.5 u			
White corn meal 43-day	14 % 8-12 X 2-3 u Av.,9.8 X 2.8 u	18 % 8.5-9.5 X 2.5-3.5 u Av.,8.8 X 2.7 u	60 % 8-11 X 2.5-3 u Av.,9.6 X 26 u	8 % 9-12 X 2.8-3u Av.,10 X 2.5u		
Potato-2 % dextrose agar pH 7 20-day	83 % 4.2-11.5 X 2-3 u Av.,9.9 X 2.7 u	4 % 9-14.2 X 2.5-4 u Av.,11.5 X 3.8 u	13 % 8-14.8 X 3-4 u Av.,12.2 X 3.5 u			
Potato-2 % dextrose agar pH 7.2 63-day	4 % 9-12.5 X 2.8-3.9 u Av.,11.8 X 3 u	10 % 9-15.5 X 2.8-4 u Av.,14.8 X 3.6 u	4 % 9-16.8 X 3.2-4 u Av.,15.2 X 3.6 u	21 % 11.5-16 X 3-4 u Av.,15.5 X 3.5 u	9 % 14.2-17.2 X 3.2-4 u Av.,15.2 X 3.5 u	52 % 17.2-21.4 X 4-4.2 u Av.,19.9 X 4.15 u
Autoclaved cotton stem 32-day	5 % 10.5-13.5 X 2.5-3 u Av.,12 X 2.7 u	16 % 9-16 X 3.1-3.5 u Av.,11.9 X 3.1 u	7 % 10.5-16 X 2.8-3 u Av.,14 X 2.9 u	72 % 11.5-16.5 X 2.5-3.5 u Av.,14.5 X 3 u		
Autoclaved watermelon stem 51-day	5 % 11-12.9 X 2.8-3.5 u Av.,12.5 X 3.2 u	6 % 9-16 X 2.8-4 u Av.,15.3 X 3.9 u	4 % 9-16.2 X 3.4-4 u Av.,14.2 X 3.4 u	15 % 11-16 X 3-4 u Av.,15.4 X 3.9 u	8 % (50 spores) 14.2-15 X 3-4 u Av.,14.4 X 3.9 u	62 % 16-17.2 X 4-4.2 u Av.,16.3 X 4 u

*Measurements are for 100 spores except as indicated.

e. Identification

By comparison with the descriptions (6,7) of the section Elegans of the genus Fusarium, it is evident that the fungus belongs in that group. The fungus described in this paper agrees with these descriptions in the presence of both microconidia and macroconidia; in the occurrence and size of terminal and intercalary chlamydospores and blue-black sclerotia; in the degree of development and color of aerial mycelium and in the discoloration of media. However, 3-septate conidia are given in the descriptions as typical for this group while 4 and 5-septate conidia were found to predominate in two groups of 100 spores measured in this study.

Within the section Elegans, the fungus falls close to Fusarium oxysporum but there are certain differences which appear to be of sufficient importance to set it apart from F. oxysporum, as a variety. The fungus agrees with F. oxysporum in the following characteristics. Conidiophores are typically much branched (Plate III, Figs. 2 and 3). Sickle-shaped microconidia are gradually attenuated toward the apex, nearly cylindrical and broadest in the middle half of the length. Macroconidia are borne in sporodochia. Conidial masses are pinkish-buff in color. Aerial mycelium is white and well developed, of medium height, 3-5 mm., and discoloration of the substrate varies through shades of red and purple. Both terminal and intercalary chlamydospores are

present, and also blue-black sclerotia.

Characteristics which do not agree with Wollenweber's (6) and Sherbakoff's (7) descriptions of F. oxysporum are as follows. The non-septate microconidia measured, taken from a culture on potato- 2 percent dextrose agar, pH 7.0, averaged 9.9 x 2.7 u (4.2-11.5 x 2-3 u) as compared to 8 x 2.9 u (4.5-12 x 2-4 u). One-septate spores averaged 11.5 x 3.8 u (9-14.2 x 2.5-4 u) compared to 16.5 x 3.4 (11-23 x 2.5-4.1). Two-septate spores averaged 12.2 x 3.5 u (8-14.8 x 3-4 u) compared to 18.5 x 3.9 u (17-26 x 3-4.2 u). Three-septate spores averaged 12.2 x 3.5 u (8-14 x 3-4 u) compared to 27.5 x 4 (15-39 x 3-4.7 u).

Four-septate conidia occur only rarely, according to the descriptions of F. oxysporum, previously mentioned, never making up more than 3 percent of the number of spores measured, while 8 and 9 percent measured in two groups of 100 each in this study were 4-septate spores averaging 14.4 x 3.9 (14.2-15 x 3-4) and 15.2 x 3.5 (14.2-17.2 x 3.2 -4) respectively, compared to 36.7 x 4.43 u (34.5-60 x 4.3 -4.5 u). In the descriptions, 5-septate conidia make up 1 percent of the spores measured while in the two groups of 100 spores here studied the 5-septate spores predominated, the average size being 16.3 x 4 (16-17.2 x 4-4.2) and 19-9 x 4.15 (17.2-21 x 4-4.2 u) as compared to 35 x 4.4 (no minimum and maximum given).

The spores of the fungus studied were either apedicellate or had the foot typically less well developed than is characteristic of F. oxysporum. There is also a marked difference in the parasitism of the two organisms. The Fusarium studied does not infect the white potato.

Therefore, because of the minor differences between F. oxysporum and the organism described in this paper,- the differences in spore size, the poor development of the foot and the difference in parasitism- it would seem that the organism might be classified as a variety of F. oxysporum.

f. Effect upon the host.

Inoculation of plants of Cereus schottii and prickly pear were made by cutting a small slit in the sterilized surface of the stem with a sterile scalpel and inserting a bit of monosporidial culture. Within ten days a pronounced swelling appeared around the point of inoculation (Plate II, Figs. 4 and 5). The raised area was discolored fuscous with a light green-yellow margin. In six days the lesion had become sunken and had doubled in size (Plate II, Fig. 2). After three weeks, some of the plants had become fuscous or fuscous-black almost throughout, while others had checked the progress of the organism by developing several rows of cork cells between the lesion and the normal tissue (Plate II, Fig. 3 and Plate III, Fig. 9). These

thick-walled cork cells originated in the parenchyma.

Upon examination of plants which did not develop protective tissue the vascular system and parenchyma adjacent to it were found to be discolored the entire length of the stem. Stained sections of tissue at some distance from the point of inoculation showed no hyphae present. These observations led to the conclusion that the fungus was excreting a substance or substances toxic to the living plant cells, and that the toxic excretion was carried through the plant in the vascular system.

To further investigate this condition, a culture of the Fusarium was grown in nutrient broth containing 2 percent peptone (8). After 8 days, the broth was filtered so that all spores of the fungus were excluded and the filtrate examined under the microscope to be certain of this fact. A cut end of a stem of Cereus schottii was then immersed about 1½ inches in the filtrate and allowed to remain there for 8 days. The stem was then cut longitudinally and examined. The first ½ inch of stem above the cut surface was discolored fuscous throughout. Above this region only the vascular bundles were discolored a light brown. This discoloration of vascular tissues extended to the apex of the plant.

A cross section of the stem in the region of inoculation showed discoloration throughout. Sections of tissue in this region stained with Haidenhain's iron-alum

haematoxylon showed the presence of both intercellular (Plate III, Fig. 10) and intracellular (Plate III, Fig. 11) hyphae. In the older part of the lesion, cells had entirely lost their protoplasmic contents and cell walls were badly broken down. Some sections showed only hyphae of the fungus and fragments of cell walls.

SUMMARY

1. A Fusarium was isolated from a plant of Cereus schottii which showed necrosis of the rot type. Inoculations proved the fungus to be pathogenic. Monosporidial cultures were then made.

2. The Fusarium was found to belong to the section Elegans and to fall close to Fusarium oxysporum in that group. It differs from F. oxysporum in having smaller macroconidia, a higher percentage of 4 and 5-septate spores, in having spores with the foot less well developed than is characteristic of F. oxysporum, and in its pathogenicity. For these reasons the fungus is tentatively classified as a variety of F. oxysporum.

3. When plants of Cereus schottii were inoculated with the Fusarium, symptoms were produced which were identical with those shown by the naturally diseased plants and the fungus was re-isolated.

4. Inoculated plants of Cereus schottii showed discoloration of the vascular system and adjacent cells. Discoloration of tissues extended beyond the cells containing hyphae of the fungus as shown by stained sections. This fact led to the conclusion that a substance (or substances) toxic to the cells of the host was being excreted by the fungus. The Fusarium was grown for 8 days in nutrient broth. The broth was then filtered and the cut end of a stem of Cereus schottii immersed in the filtrate. The results of this experiment substantiate the belief that a toxic substance was excreted which affected the living cells of the host.

ADKNOWLEDGMENTS

The writer wishes to express sincere appreciation to Dr. J. G. Brown, Head of the Department of Plant Pathology, University of Arizona, for his advice and assistance in this work. The writer is also indebted to Mr. M. M. Evans, Research Assistant in the Department, for his help with photographs appearing in this paper.

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DESCRIPTION OF PLATES*

Plate I.

Fig. 1. Plant of Cereus schottii from which the Fusarium was isolated.

Fig. 2. Plant of Cereus schottii 10 days after inoculation showing fuscous lesion, with light green margin.

Fig. 3. Same plant 24 days after inoculation. Corking off of lesion has begun.

Figs.4-5. Prickly pear (Engelmannii group) showing swelling and beginning of discoloration as result of inoculation. Fig. 4 illustrates the profile of the swelling and Fig. 5 is a surface view.

Plate II.

Fig. 6. Apex of plant of Cereus schottii 3 weeks after inoculation showing discoloration. Cork tissue was not developed.

Fig. 7. Cross-section of plant in Figure 6 with discoloration of vascular system and tissues ad-

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* The original magnifications are given here.

jacent to it.

Fig. 8. 6-day culture on slice of living sweet potato root. X 2.

Fig. 9. 6-day culture on steamed potato plug. X 1.8.

Fig.10. 6-day culture on steamed carrot plug. X 1.8.

Fig.11. 6-day culture on steamed watermelon plug.

Fig.12. 6-day culture on steamed cotton stem. X 2.

Fig.13. 6-day culture on steamed watermelon stem. X 2.

Fig.14. 6-day culture on autoclaved white corn meal, 27°C. X 2.8.

Fig.15. 6-day culture on autoclaved rice, 27°C. X 2.8.

Fig.16. 6-day culture on oatmeal agar, 27°C. X 2.3.

Fig.17. 6-day culture on yellow corn meal, 27°C. X 2.3.

Plate III.

Fig. 1. Swollen hyphal cells from a 52-day-old culture on white corn meal. X 2410.

Fig. 2. Part of a conidiophore from a 40-day-old culture on potato- 2 percent dextrose agar. $_{p}$H 7.0. X 2410.

Fig. 3. Conidiophore from a 104-day-old culture on white corn meal. X 2410.

Fig. 4. Group of chlamydospores: c and d from a 42-day culture on potato- 2 percent dextrose agar- $_{p}$H 7.0. X 2410; e-chlamydospores in a chain from a 42-day-old culture on steamed cotton

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stem. X 2410.

Fig. 5. Group of spores from a 51-day culture on white corn meal. X 2410.

Fig. 6. Group of spores from a 104-day culture on white corn meal. X 2410.

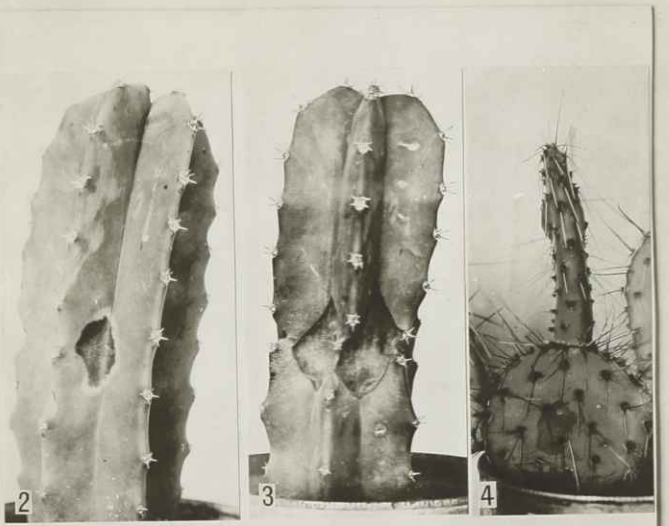
Fig. 7. Group of spores from a 90-day culture on potato-2 percent dextrose agar, pH 7.0. X 2410.

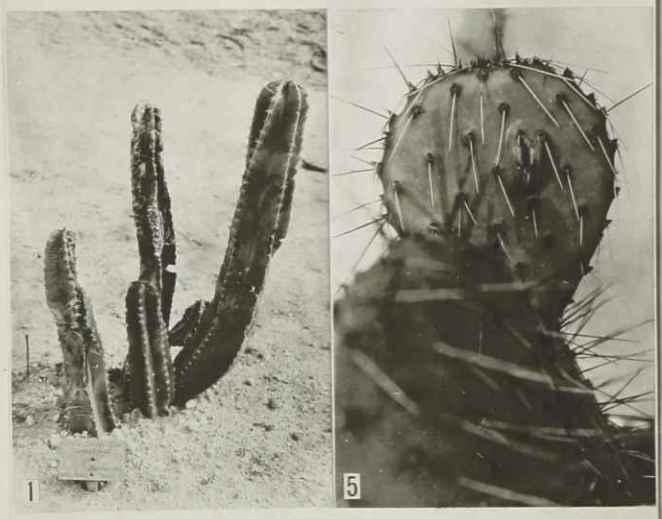
Fig. 8. Section of a sclerotium. X 2410.

Fig. 9. Host tissue: dead parenchyma cells (a), cork cells (b), cork cambium (c), and normal cells (d).

Fig.10. Introcellular hypha. X 750.

Fig.11. Hypha in intercellular space. X 750.





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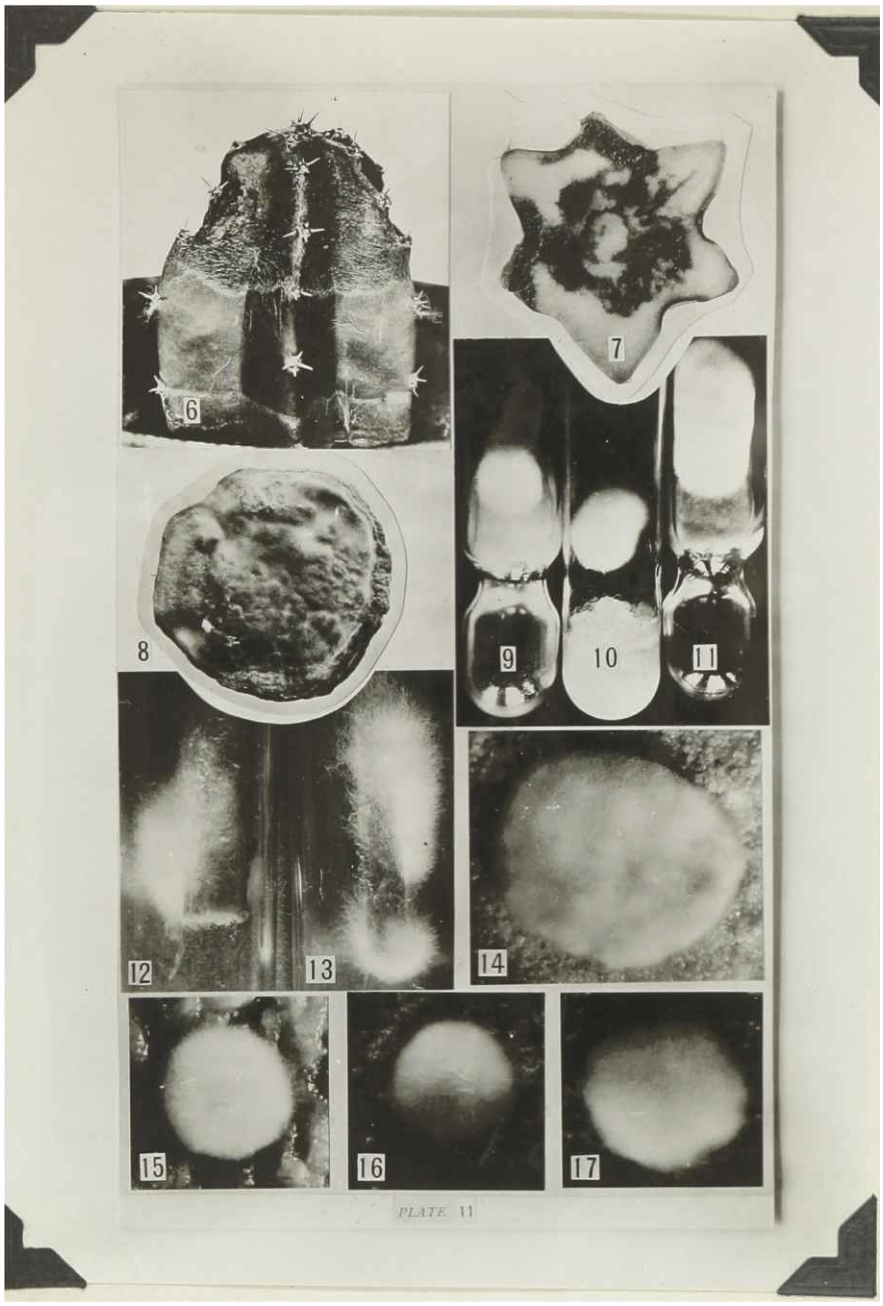

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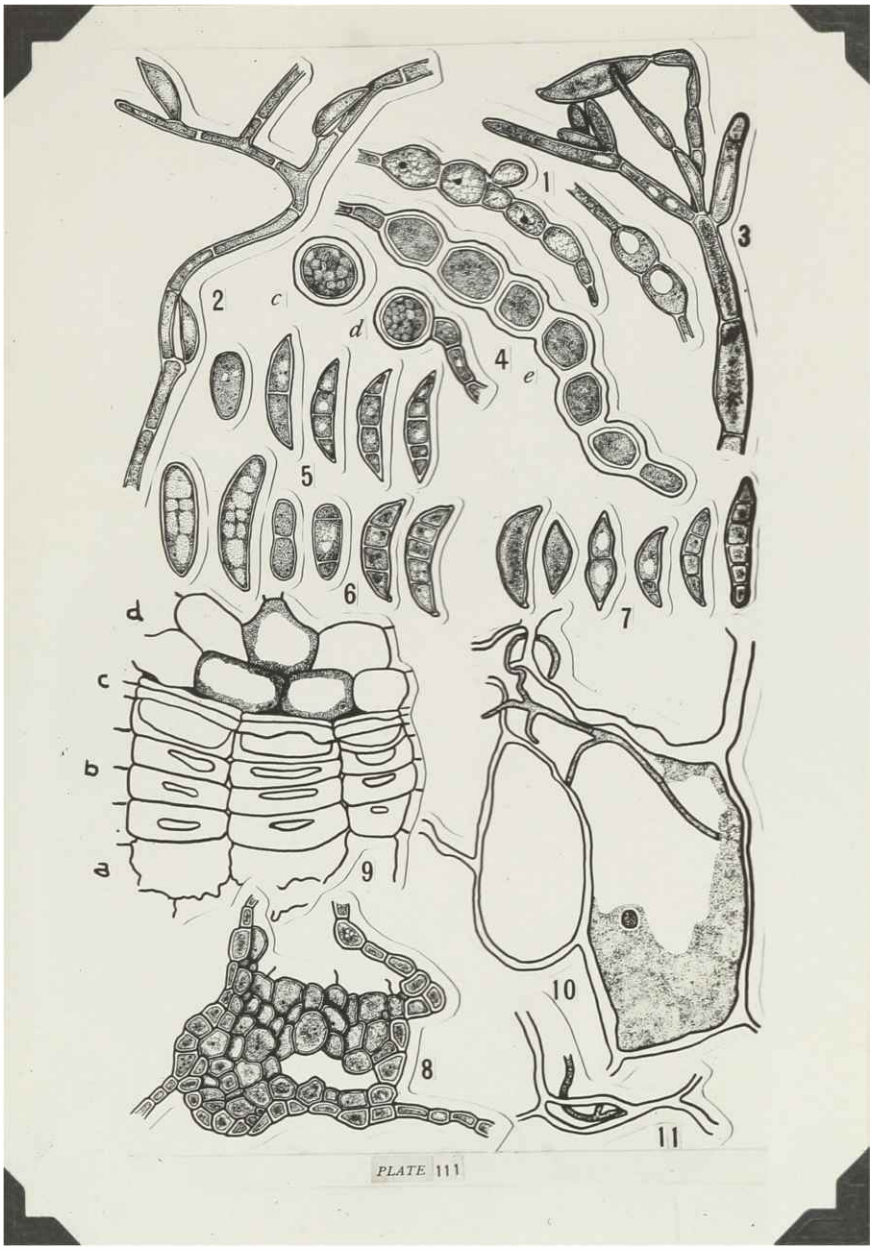

PLATE 111