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## THE EYE-SPOT DISEASE OF SUGAR CANE

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The eye-spot disease of sugar cane became serious in Porto Rico in 1922-23. Like most diseases which suddenly appear as menace to a crop it was referred to by the growers as a new and mysterious disease and was given several local names. Along the north coast it was frequently referred to as the Manatí disease because it was first found in the vicinity of Manatí. Along the south coast it was sometimes referred to as Santa Rita disease although that name was not used so extensively as Manatí. Fortunately these two names have gone out of use and the disease is now generally known as "eye spot", a name which is very generally used in other cane-growing regions of the world. The first studies made by the writer showed considerable variations in the spots and in size of the spores which led us to suspect that that spotting might be due to two-species of organism causing the disease but later studies have shown that this is not true.

Although the disease was at first referred to as new and mysterious, the studies made by the writer very soon showed that it is the common "eye spot" which is caused by a fungus (*Helminthosporium sacchari* Butler) and is well known in other parts of the world. It was first reported from Porto Rico by Johnston and Stevenson (8) in 1917, but it was no doubt present on the Island many years previous to that date. Its sudden appearance as a severe disease in Porto Rico was no doubt due to the growing of an increased acreage of susceptible varieties and to the climatic conditions in 1922-23 which were especially favorable to the growth of the fungus. In 1923-24 the disease was less severe, due to unfavorable conditions but it has been quite severe during the following years. The threatening character of this disease lead us to make studies which have been carried out during a period of four years and are recorded in this paper.

### HISTORY

The prevalence, wide distribution and general character of this disease give ample reason for believing that it is very old although

we have no authentic records previous to 1892 when it was first reported and described from Java by L. van Breda de Haan (5) who demonstrated that it was infectious and due to a fungus which he named *Cercospora sacchari*. A more complete account of the disease and a colored plate was published from Java in 1898 by Wakker and Went (18). In 1901 the disease was discussed in another publication from Java by Diekhoff and Hein.

In 1907 the disease was reported from Hawaii by Lewton-Brain (15) who stated that the disease had been known previously as "rust". In 1909 the disease was again reported from Hawaii by Cobb (3) who also published a colored plate which corresponds quite well to the disease as found in the greater part of Porto Rico, but his spore drawings are of *Helminthosporium*. In 1922 Larsen (9) reported the isolation of the fungus and the reproduction of the disease by spraying cane leaves with a spore infusion.

In 1913 it was reported and described from India by Butler (2) under the name of *Helminthosporium sacchari* who comments on previous work as follows:

"In Hawaii it has been briefly described by Cobb. From the figures published in Wakker and Went's well-known textbook of sugar-cane diseases, it appears probable that this fungus is really a *Helminthosporium* and not a *Cercospora*. A comparison of the two fungi has not been possible and could alone settle the question of their identity."

Butler's nomenclature is very generally followed by mycologists and plant pathologists. Butler reported that the disease had "not been found to damage the crop materially".

The disease was first reported from Porto Rico in 1917 by Johnston and Stevenson (8) who evidently did not consider it of much importance. It was next reported from Porto Rico by the writer (4) in 1924 in a preliminary paper as a disease which threatened to be of major importance.

The latest publication is a series of papers from Hawaii by Lee (7, 10-14, 16, 17) and others in which various phases of the disease are discussed. These publications will be referred to from time to time in this paper. In the first of this series Lee (10) calls attention to the fact that early publications indicate that the disease probably existed in Hawaii as early as 1854, that it was considered as serious and was known as "fire blight". This is 39 years earlier than the first authentic record by van Breda de Haan.

Although there is reason to believe that the disease is widely distributed throughout the sugar-cane-growing regions of the world the only reports of serious outbreaks are from Hawaii and Porto

Rico and the only important varieties that have proved, especially susceptible in Porto Rico, are D. 109 and F.C. 306. H. 109 has been introduced into Porto Rico but has not been grown extensively. It is less susceptible in Porto Rico than D. 109 and F.C. 303.

The preceding discussion includes references to the most important publications but there are many other brief references which show the wide distributions of the disease throughout the sugar-cane-growing countries of the world. These records show that the disease is known in Java, Hawaii, India, Philippines, Formosa, and Southern United States. It has been reported from West Indies, from Cuba, Jamaica, Santo Domingo, Barbados, Trinidad and Reunion.

#### SYMPTOMS

The eye spot is usually well defined and characteristic but is subject to many variations, depending on variety, age, severity of infection, climatic and possibly other factors. The disease occurs on the lower half of the blade, sometimes attacking the sheaths but rarely attacking the outer half of the leaf except in the case of young plants, when the entire leaf may be attacked.

The disease usually starts as very small reddish, occasionally black spots. If the variety is very resistant, this character may disappear with the growth of the plant. If the variety is susceptible, the spot increases in size and develops other colors. Most spots on susceptible varieties are red when young but usually develop black centers and yellowish borders. The arrangement of these colors and the elongated, pointed form have given rise to the common name "eye spot". However, the spots are not uniform in either shape or color. Any one of the colors may predominate. Sometimes the spot remains red until one-fourth inch in length before showing a black center. Sometimes the colors have rather definite outlines and sometimes they blend. Sometimes the spots have the definite eye form and at other times they take the form of long narrow black streaks of various lengths; usually one-fourth inch to 3 inches but occasionally more. Sometimes they are long, dark in color and of a rather irregular outline. Sometimes they unite, usually at the end. In young spots, the colors are usually bright and clear but as the spots grow old the colors become dull and almost disappear with the dying of the leaf. As the spots grow old, they usually develop ashy-colored centers as a result of a dying of the central tissues. In the case of the more susceptible varieties, such as F.C. 306 and D. 109, the entire leaf may dry, and in the case of severe outbreaks it is not uncommon for three-fourth or more of the leaves to

be killed. Entire fields become brown, except for a few green leaves at the tops of the plants. The spots may occur on any part of the lower half of the leaf from midrib to margin but do not occur on the midrib. In the case of severely infected young plants and seedlings the entire plant may be varying shades of red, the tips and outer leaves die and become brown very soon and in many cases the plant is killed. In some cases the spots are blunt, with a predominance of red and purple colors which darken with age. Sometimes the spots coalesce, become irregular and develop into blotches which may enclose small areas of apparently healthy green issues. The margins of these blotches are frequently yellow. These blotch characteristics were originally attributed to the so-called "Santa Rita" disease, but a more extensive study indicates that we have but the one species of the fungus. Therefore, the blotching must be considered one of the variations of the disease symptoms. Although the characteristic blotchings are most common on the south coast, they do occur in other parts of the Island and the writer has produced them under green-house conditions by heavy infection of the young canes. The top-rot symptom which is described in publications from Hawaii is rare in Porto Rico but the "fire blight" which is also described in the Hawaiian publications is quite common, especially on D. 109 when grown on the higher elevations. Many of these variations are no doubt due to varieties and to climatic conditions, but some of them may be due to soil conditions as previously referred to by the writer (4) or to the presence of other fungi which are more or less common on the spots.

The disease attacks the lower half of young leaves of growing cane while they are unrolling. The spores are apparently carried by the wind and fall into the tops of the cane, and since the arrangement of the leaves is especially favorable for the collection of moisture, the conditions are most favorable for the growth of the fungus.

The *ring spot* of sugar cane is due to an entirely different fungus (*Leptosphaeria sacchari* J. van Breda de Haan). It is possible that this disease might be confused with the "eye spot" by inexperienced observers, but a little study will enable any one to distinguish the two diseases in most cases. This spot occurs on the outer half of the older leaves. The spots are much broader and shorter than the eye spots and are usually not more than two or three times as long as wide, more or less irregular in outline, and tending to unite, so that in many cases the outer-one-fourth or one-half of the old leaves are entirely dead. The ring spots are brownish or reddish but the colors are never so bright as in the eye spots. Gray dead centers develop

very early so that in a very short time the spots show as large gray areas surrounded by a narrow zone of brown, black or reddish tissue.

#### LOSSES

It is extremely difficult to estimate the losses due to a disease of this type with any degree of accuracy, but it is very evident that the losses must be considerable in the cases of the severe outbreaks. Since the greater part of the manufacture of sugar is in the leaves of the plant, the destruction of any considerable part of the leaf must necessarily reduce the working power of the plant proportionately. And since the disease attacks the leaf so early in its development, the areas that are destroyed have practically no part in the manufacture of sugar or in furnishing elaborated foods for the growth of the plant. Therefore, the greater the destruction of the leaf tissue, the greater the reduction in growth and manufacture of sugar. The writer has seen very large areas of B. 109 and F.C. 306 in which 80 per cent of the leaves were entirely dead long before maturity. Furthermore, the disease frequently kills canes varying in height from a few inches to four feet. In one field of F.C. 306, fifty-seven per cent of the canes were killed. In the case of very susceptible varieties, many young canes of ratoon growths are killed. Seedlings are frequently destroyed in great numbers. The writer has seen outbreaks in the seedling flats which resulted in the death of many hundred seedlings, and Mr. Robert L. Davis of the Porto Rico Agricultural Experiment Station writes that in the case of one variety "only 250 out of 1,106 survived". When young canes are killed, they are very frequently attacked by the rind disease (*Melanconium sacchari* Massae) which finishes the work of destruction, although it is primarily a saprophyte.

#### THE ORGANISM

The organism was described by Butler (2) from India in 1913 as follows:

"The infected leaves first show small red spots, which spread rapidly, chiefly in the longitudinal direction and, especially towards the tip of the leaf, may run together to form long streaks. The center of the spot soon changes to a dirty straw color, around which the margin remains red for a time and then changes to dark brown. The spots occur equally on the midrib, where they may be confused with those caused by the leaf form of *Colletotricum falcatum* and on the thinner part of the leaf. When numerous, they cause death of the leaf tissues beyond the limits of the spots; the tip of the leaf often withers completely and there are many long withered strips down the margins.

"The sporophores are stout, erect, rather rigid hyphae, which arise from the peripheral cells of the stromata (Pl. VI, Fig. 4). They are usually unbranched,

3 to 10 septate, dark greenish-brown below, paler above and several times bent or 'geniculate'. Spores are produced at each bend and at the apex, the lowest being due to the spores being always apical at first and being then pushed to one side by continued growth of the sporophore from just below the insertion of the spore. The sporophores are 100 to 190 microns long, by 5.5 to 7.5 microns broad.

"The spores (Pl. VI, Fig. 5) are borne single and readily fall off. They are cylindrical or long elliptical in shape, with very thick walls, and divided into from 4 to 11 compartments by broad thick partitions. The colour varies from olive green to brown and the size from 35 to 60 microns long, by 8.5 to 12 microns broad.

"*Helminthosporium sacchari* Butl. n. sp. Maculis amphigenis, elongatis, initio, rubris, dein avellaneis, vel stramineis ac ferrugineo-marginatis,  $3-25 \times 2-6$  mm.; caespitulis minutis, atris; hyphis fertilibus erectis simplicibus, 3-10-septatis, geniculatis, olivaceo-brunneis, apice pallidioribus,  $100-190 \times 5.5-7.5$  microns; conidiis aerogenis, cylindraceis vel oblongo-ellipticis, utrinque rotundatis, 3-10 septatis, carssissime tunicatis, olivaceo-brunneis,  $35-60 \times 9.5-12$  microns."

The description given by Johnston and Stevenson reads as follows:

"Hyphae dark, cobwebby, arising from the center of an elongated brown spot on the leaf-blade; sporophores more or less erect with single terminal spores; spores several septate with very thick walls, rounded at both ends,  $32-90 \times 9-14$  microns, on conidiophores 120-160 mm. long."

The writer measured several hundred spores (Fig. 1) the minimum being  $22 \times 6.6$  u while the maximums were  $92 \times 13$  u and  $88 \times 15$  u. The average was  $58.7 \times 11.1$ . The septa ranged from 3 to 11. The minimum sporophore (Fig. 2) was 44 u while the maximum was 337.5 u. These measurements were made from spores and sporophores from leaves of 23 varieties of cane which had been in moist chamber 24 hours. It was impossible to correlate the measurements with the varieties of cane.

The size of the spores appear to depend primarily on humidity. Both in the field and laboratory, under dry conditions, the spores were few and large while under moist conditions they were many and small. However, in the laboratory the spores formed in moist chamber during the first 24 hours were usually larger than those for any succeeding 24 hours.

The young sporophores are more or less straight and the spores are borne apically. Possibly it would be better to say that the youngest spore is borne apically. Following the formation of the first spore, there is a new growth starting just back of the point of attachment which extends forward and at a slight angle. A new spore is formed at the tip of the new growth and another new growth is started. This occurs repeatedly and results in a geniculate spo-

rophore with scars at the angles. The attachment of the spores is so delicate that very few are seen in place.

#### LABORATORY STUDIES

The spores are very brittle and fall from the sporophore very readily. Therefore, it is difficult to find them on leaves fresh from the field unless the leaves are collected early in the morning and handled carefully. However, they can be secured in great abundance on leaves kept in a moist chamber for 24 hours. However, the spores produced each succeeding 24 hours are likely to be smaller than the preceding. Furthermore, the spores grown in culture are very generally smaller than those found in nature. The spores are borne more abundantly on the lower than on the upper surface of the leaf. The sporophores vary greatly not only in size but also in each group or cluster. When single they were usually longer and more spores are produced than when in groups. They are geniculate, a single spore being produced from the convex side of the bend or angle. The spores originate from the apex, a new growth starts just below the spore and is forced slightly to one side, causing the first bend. This is repeated and results in alternate right and left bends.

The spores germinate very freely in water (Fig. 4) usually with a single tube and usually from one end of a spore. If grown on a piece of glass, the tube twists around and grows in various directions (Fig. 6) but if grown on the leaf it advances but a short distance and then penetrates at a point between the veins or fibro-vascular bundles without regard to the presence or absence of stomata (Figs. 7 and 10). Spores will germinate on and penetrate either surface of the leaf, but inoculation experiments have shown that the upper surface is more susceptible than the lower surface. This was demonstrated by applying agar containing spores at various points along the surfaces of the leaf or along the entire surface. This film of agar was removed after 48 hours. These experiments were repeated a number of times, and the plants kept in a greenhouse. The spots were always more numerous on the upper than on the lower surface. Very young leaves are susceptible from base to tip on both surfaces, but as they grow older they gradually lose their susceptibility, beginning with the tip; the upper surface remaining susceptible longer than the lower surface. The upper surface of a reasonably young leaf is susceptible nearer the tip than the under surface of the same leaf. The sporophores will also germinate and are probably as important in spreading the disease as the spores.

The penetration of the leaf by the germ tube is very quickly followed by a discoloration and formation of a spot (Figs. 7 to 10). In some cases the spot can be detected by the unaided eye within 30 hours after inoculation. This spot increases in size, the greater growth being lengthwise and between the larger fibro-vascular bundles. It appears to be rather difficult for the fungus to penetrate the fibro-vascular bundles, although this does occur.

The germ tube appears to be able to penetrate at any point between the fibro-vascular bundles or veins. It was not seen to penetrate immediately over or under a fibro-vascular bundle, nor into a stomata. The first growth of the mycellium after penetration is in the parenchyma tissues and is either inter- or intra-cellular (Fig. 12) and the septa are comparatively few. The spots increase in size, especially in length with age, the discolorations usually extending beyond the range of the fungus. The mycelium becomes more and more abundant and the septa more numerous. It frequently acquires the power to penetrate the fibro-vascular bundles (Figs. 13 to 15), but age and the resistance of the hard walls of the cells of the fibro-vascular bundles results in the formation of masses of fungus cells resembling sclerotia, usually just within the epidermis and next to the fibro-vascular bundles (Figs. 9 and 11). The resistance exerted by the hard cell walls of the fibro-vascular bundles appear to be the stimulus which leads to the formation of septa and the sclerotia-like growth. This growth breaks, through the epidermis and forms sporophores. It also frequently produces a growth which spreads over small areas of the surface of the leaf (Fig. 11). The sporophores were also seen growing out through stomata.

A careful study was made of the structure of the leaves of a large number of varieties and a comparison of the most resistant with most susceptible varieties indicated that the varieties with numerous, close fibro-vascular bundles were more resistant than the varieties in which the fibro-vascular bundles were rather widely separated by large quantities of parenchyma tissue. This is in harmony with the large amount of mycelium in the parenchyma as compared with the fibro-vascular bundles.

#### INOCULATION EXPERIMENTS

The inoculations were made in both greenhouse and field for the purpose of determining the relative susceptibility of the many varieties. Inoculations are easily made either by the use of spore infusions applied with an atomizer or by pouring the infusion into the tops of the cane plants. The simplest and easiest method, was the



application in the top of the cane by means of a glass tube. When the spore infusions are applied to the base of the young leaves, infections can be obtained in very nearly 100 per cent of the cases, but if applied to the old leaves or tips of leaves the results are usually negative. The best time for inoculation is late in the afternoon, probably because the plants retain the moisture during the night better than under the bright sunshine of the day. It is not necessary to injure the surface of the leaf. Infections may be obtained at any season of the year but the plants respond more readily during the cooler months of November, December, January and February and March, which is the season the disease is most abundant in the fields. During the warm, summer months, infections are easily obtained, but under conditions here at the Insular Experiment Station there is very little spread of the disease from the infected to the neighboring plants. All varieties can be infected, but in the most resistant varieties the spots can not be seen without close study. Uba may be considered most resistant, and close examinations made 48 hours after inoculation showed minute black spots. Varieties do not always respond in the same manner even though the experiments are made in the same place and as nearly as possible under the same conditions. This is well shown in the following tables:

TABLE I

## GREENHOUSE INOCULATION

The groups are arranged in order of susceptibility, the most susceptible first

D. 433	F.C. 214
F.C. 306	S.C. 12(4)
D. 109	B.H. 10(12)
P.R. 460	P.R. 328
B.H. 10(12)	P.R. 329
B. 67	B. 3412
D. 109	H. 109
Ba. 11569	D. 1135
P.R. 433	P.R. 492
	P.R. 440
B. 6450	Cristalina
D. 109	P.R. 333
P.R. 729	Uba
Ba. 6032	Badila
D. 448	P.O.J. 105

TABLE II  
GREENHOUSE INOCULATION

The groups are arranged in order of susceptibility, the most susceptible first

F.C. 306	P.R. 440
D. 109	P.R. 328
	P.R. 492
H. 109	Yellow Caledonia
P.R. 433	B. 67
Ba. 11569	P.O.J. 105
D. 448	P.R. 440
D. 109	B.H. 10(12)
Ba. 6032	S.C. 12(4)
P.R. 329	Cristalina
F.C. 214	B. 3412
	P.R. 333
D. 1135	Badila
B.H. 10(12)	Uba

In Table I it will noted D-109 was used in three and B.H. 10(12) in two different containers with different results, although the containers were separated by spaces of less than 10 feet.

It will also be noted that the results in the two tables are slightly different. This is especially true in the case of B. 67. However, F.C. 306 and D. 109 can be depended on as very generally showing the greatest susceptibility.

TABLE III  
PLANTS KILLED IN GREENHOUSE AS RESULT OF HEAVY INFECTION

These plants were grown in 5-gallon oil cans and four buds planted in each can.

	First record	Second record	Third record
D. 117 -----	all	—	—
Cristalina -----	5	1	1
Ba. 6032 -----	1	2	all
D. 448 -----	2	—	all
D. 433 -----	1	—	—
B.H. 10(12) -----	—	4	—
B. 6450 -----	—	1	2
P.R. 711 -----	—	1	1
Badila -----	—	1	—
Ba. 11569 -----	—	1	—
F.C. 306 -----	—	1	—
P.R. 328 -----	—	1	—
B. 3412 -----	—	1	1
H. 109 -----	—	1	—
P.R. 460 -----	—	—	2
P.R. 433 -----	—	—	1
P.R. 704 -----	—	—	1
P.R. 440 -----	—	—	1

In comparing the results given in Table III with the results of Tables I and II it will be readily seen that the varieties showing the greatest leaf infection in Tables I and II did not show the highest mortality. However, the number of plants used in Table III was so small that no definite conclusions can be drawn from this experiment. Furthermore, the field observations showed a greater mortality in FC-306 than in any other variety.

TABLE IV

## INOCULATION MADE ON PLOT WITH OVERHEAD IRRIGATION

Records made every week for six weeks

xxxxx = very severe; xxxx = severe; xxx = medium; xx = slight; x = very slight

Rank	Variety	First record	Second record	Third record	Fourth record	Fifth record	Sixth record
1.	F.C. 306	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
2.	D. 109	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx	xxxxx
3.	H. 109	xxxx	xxxx	xxxx	xxxx	xxxx	xxxx
4.	P.R. 433	xxx	xxxx	xxxx	xxxx	xxxx	xxxx
5.	P.R. 460		xxxx	xxxx	xxxx	xxxx	xxxx
6.	P.R. 328	xx	xxx	xxx	xxx	xxx	xxxx
7.	B. 3412	xxx	xxx	xxx	xxx	xxx	xxx
8.	P.R. 440	x	xxx	xxx	xxx	xxx	xxx
9.	B.H. 10(12)		xx	xx	xx	xxx	xxx
10.	B. 6450					xxx	xxx
11.	P.R. 792	x	xx	xx	xx	xxx	xxx
12.	P.R. 329	x	xx	xx	xx	xx	xx
13.	F.C. 214	xx	xx	xx	xx	xx	xx
14.	D. 433	x	x	x	x	x	x
15.	B. 67					xxxxx	xxxxx
16.	Cristalina					xxxxx	xxxxx
17.	P.R. 726					xxx	xxx
18.	Ba. 6032					xxx	xxx
19.	Ba. 11569					xx	xx
20.	Badila					xx	xx
21.	D. 448						x
22.	D. 1135						
	P.R. 492						
	D. 117						
23.	Uba						

Tables V, VI and VII are made from a field at the entrance of the Insular Experiment Station. The land is low, the humidity high and the temperature favorable during the winter months. The disease appeared here in advance of any other place on the Insular Experiment Station and was more severe.

TABLE V

xxxxx = very severe; xxxx = severe; xxx = medium; xx = slight; x = very slight

Rank	Variety	First record	Second record	Third record
1.	F.C. 306	xxxxx	xxxxx	xxxxx
2.	D. 109	xxxx	xxxx	xxxx
3.	P.R. 329	xxxx	xxxx	xxxx
4.	B. 3412	xxxx	xxxx	xxxx
5.	D. 448	xxxx	xxxx	xxxx
6.	F.C. 214	xxx	xxx	xxxx
7.	D. 433	xxx	xxx	xxxx
8.	B.H. 10(12)	xxxx	xxx	xxx
9.	H. 109	xxx	xxx	xxx
10.	P.O.J. 36	xxx	xxx	xxx
11.	M. 36	xxx	xxx	xxx
12.	P.R. 440	xxx	xxx	xxx
13.	Cristalina	xx	xx	xxx
14.	Yellow Caledonia	xxx	xx	x
15.	D. 1135	xx	xx	xx
16.	P.R. 492	xx	xx	xx
17.	P.O.J. 228	xxxxx	---	---
18.	Ba. 6032	xxxx	x	---
19.	P.R. 460	xxx	x	---
20.	D. 117	xxx	x	---
21.	B. 67	xx	x	---
22.	P.R. 328	xx	x	---
23.	P.R. 729	xx	x	---
24.	B. 6450	x	x	---
25.	P.O.J. 234	xx	---	---
26.	Ba. 11569	xx	---	---
27.	S.C. 12(4)	xx	---	---
28.	Uba	---	---	---

Table V is a composite made up from studies following inoculation. The first reading was made 4 days after inoculation and the second two months later and the third one month after the second.

A comparison of Tables IV and V shows that although F.C. 306 and D. 109 are the most susceptible and that Uba is the most resistant, the other varieties do not occupy the same positions in the two tests.

TABLE VI  
NATURAL INFECTION IN THE SAME FIELD

December 8th, 1926

F.C. 306	xxxxx	P.O.J. 36	xx
Ba. 6032	xxxxx	D. 433	xx
D. 109	xxxx	D. 1135	xx
P.R. 329	xxxx	P.R. 492	xx
F.C. 214	xxxx	P.R. 460	xx
D. 216	xxxx	B. 67	xx
P.R. 328	xxxx	P.R. 729	x
H. 109	xxxx	P.O.J. 234	
B.H. 10(12)	xxx	P.O.J. 228	
M. 36	xxx	D. 117	
P.R. 440	xxx	Ba. 11569	
B. 6450	xxx	Uba	

TABLE VII  
NATURAL INFECTION IN THE SAME FIELD

January 14th, 1927

F.C. 306	xxxxx	Cristalina	xx
D. 109	xxxxx	P.R. 729	xx
Ba. 6032	xxxxx	B.H. 10(12)	xx
Y. Caledonia	xxxxx	D. 433	xx
H. 109	xxxx	D. 448	xx
P.O.J. 36	xxx	P.R. 492	x
M. 36	xxx	P.R. 460	x
B. 3412	xxx	B. 67	x
P.R. 440	xxx	Ba. 11569	x
P.R. 328	xxx	B. 6450	x
P.R. 329	xxx	P.O.J. 228	trace
F.C. 214	xxx	P.O.J. 234	trace
		D. 1135	trace
		D. 117	trace
		Uba	0

A comparison of Tables V, VI and VII shows that although the varieties do not occupy exactly the same relative positions, F.C. 306 and D. 109 are most susceptible and that Uba is the most resistant.

TABLE VIII

This field was part of the property of Central Cambalache. It had been planted to varieties for the purpose of studying resistance of varieties to mosaic but became infected with *Helminthosporium sacchari*. This field is located on low land, near the coast.

Variety	Severity	Remarks
F.C. 306	XXXXX	
D. 109	XXXXX	
P.R. 329	XXXXX	
D. 448	XXXXX	
B. 3405	XXXXX	
B. 3412	XXXXX	
P.R. 662	XXXXX	
P.R. 433	XXXXX	Very few living leaves.
B. 67	XXXXX	Leaves yellow, long narrow streaks.
Rayada	XXXXX	Apparently less damage than on others.
H. 109	XXXX	
D. 625	xxx	
B. 208	xxx	
P.R. 545	xxx	No great damage.
S.C. 12(4)	xx	Many small spots. No great damage.
Ba. 11569	xx	Many small spots. No great damage.
P.R. 328	xx	
P.R. 414	xx	
P.R. 440	xx	
P.R. 561	xx	
D. 117	xx	Very small spots.
B. 3696	x	Very few small spots.
Cristalina	x	
L. 511	x	Very few spots.
P.R. 417		Occasional spot.
P.R. 492		Occasional spot.
P.R. 433		Occasional spot.
B.H. 10(12)		Occasional spot.
P.R. 260		Occasional spot.
Uba		Practically immune.

TABLE IX

In another record at a different date, from the same field; also a grouping with reference to age, purity and sucrose content of the varieties. The field data was taken by the writer. The information on age, purity and sucrose was furnished by Mr. Pedro Olivencia of Central Cambalache.

Variety	Severity	Remarks	Age in months	Purity	Sucrose
B. 3412	xxxxxx	Most severe of all	12	86.37	15.35
P.R. 433	xxxxx	Most leaves die	11	91.19	16.87
B. 67	xxxxx	Most leaves very yellow	12	90.63	17.22
P.R. 329	xxxxx		12	88.70	15.79
P.R. 662	xxxxx		11	90.58	17.80
F.C. 306	xxxxx		12	79.01	13.54
B. 3405	xxxxx		12	80.70	12.28
Rayada	xxxxx		12	83.46	15.61
D. 448	xxxxx		12	90.40	19.12
P.R. 545	xx	No great damage	12	83.38	14.68
B. 208	xx		12	86.90	14.40
P.R. 440	xx	Many small spots	12	88.49	15.00
P.R. 561	xx	Many small spots	12	79.18	13.92
P.R. 414	xx		12	86.90	13.61
P.R. 328	xx	Many small spots	12	84.88	15.11
P.R. 569	xx	Very small spots	12	90.40	17.37
H. 109	xx	Many small spots			15.51
S.C. 12(4)	xx	Many small spots	12	87.30	13.85
P.R. 412	x	Resistant, slight infection	12	72.08	12.63
Cristalina	x	Resistant, few small spots	11	89.22	15.48
D. 117	x	Resistant, many small spots	15	84.13	15.06
B. 3696	x	Resistant, very few small spots	12	88.24	15.31
L. 511	x	Very resistant	14	86.50	15.65
B.II. 10(12)	x	Very resistant	12	76.91	13.85
D. 433	x		15	81.29	14.49
P.R. 260		An occasional spot	12	86.02	15.70

A comparison of the Tables VIII and IX is not possible because they do not record exactly the same varieties but they do show somewhat the same relationship of hosts to the disease.

Table IX also shows a grouping with reference to purity and sucrose, but these figures are only approximately correct as other analyses where no field studies were made have given different results. These variations in purity and sucrose content are due to factors which do not come within the scope of this paper. They indicate that there may be some relationship between susceptibility and resistance to purity and sucrose content. However, it also appears that there are other factors, such as climatic conditions and structure which are more important.

## VARIETAL SUSCEPTIBILITY AND RESISTANCE

A study of these tables and our notes on field observations shows very definitely that F.C. 306 is the most susceptible commercial variety grown in Porto Rico. D. 109 is a close second. H. 109 can be placed third in the list but is much more resistant than D. 109. Uba is the most resistant variety grown on the Island. The records on the other varieties show more or less variations but they can be grouped as follows:

F.C. 306	P.R. 440
D. 109	D. 117
H. 109	
	S.C. 12(4)
D. 433	D. 1135
P.R. 460	P.R. 492
B.H. 10(12)	P.R. 333
B. 6450	Cristalina
Ba. 6032	Badila
D. 448	
F.C. 214	P.O.J. 105
P.R. 329	P.O.J. 36
Yellow Caledonia	P.O.J. 228
	P.O.J. 234
B. 67	M. 36
P.R. 729	
P.R. 328	Uba
B. 3412	

In general it may be said that the disease is so severe on F.C. 306 and D. 109 under most conditions in Porto Rico and that we cannot recommend their use. H 109 is more resistant but cannot be recommended. B.H. 10(12) and S.C. 12(4) which may be looked upon as the leading varieties in Porto Rico at this time are fairly resistant under most conditions in Porto Rico, but the writer has seen several severe outbreaks of the disease on B.H. 10(12) and a few severe attacks on S.C. 12(4). Although Uba is so resistant that for a time the writer considered it practically immune, one field was found in which this variety was heavily infected and a few fields in which there was a slight infection.

A comparative study of the structure of the leaves of a large number of varieties was made and it was found that resistance was correlated to a greater or less degree with structure, although this may not be the only factor involved. Varieties in which the fibrovascular bundles of the leaves were closely placed were more resistant than those varieties in which they were widely separated by parenchyma tissue. This is in harmony with the fact that the fungus



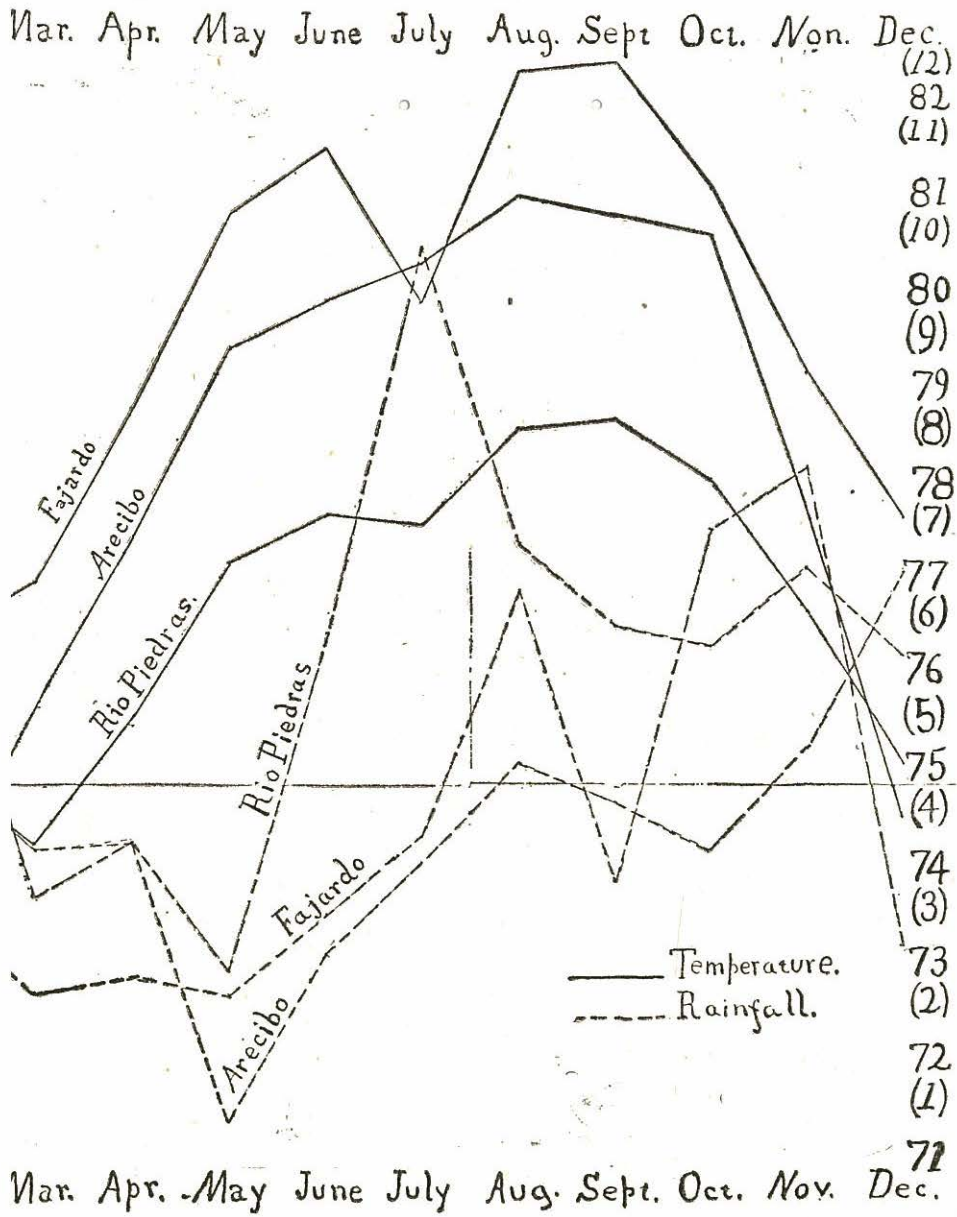


TABLE X

Temperature and rainfall, based on figures given in Table XI. The temperature indicated in degrees F. The rainfall in inches by the figures in parentheses

penetrates between the veins of the leaf and makes its first growth and in fact most of its growth in the parenchyma tissue.

A limited study was made of the roots but no injuries were traced to *H. sacchari*. Cultures of the organism were applied to the roots of growing cane in the greenhouse but we failed to find any evidence that the organism attacks the roots.

#### CLIMATIC FACTORS

Very early in our studies on this disease, it became evident that there were three very important factors in its severity: variety, temperature and moisture. The question of variety has already received consideration in the preceding pages.

Although the disease is much more severe in Porto Rico during the winter than during the summer months, it can be found at all times during the year. Fortunately, the U. S. Weather Bureau has excellent rainfall and temperature records for the three points where we made our studies. Tables X and XI are made from these records and is an average for 1922 to 1926 inclusive (except for the temperature record at Río Piedras which covers only three years). Hydrographs were placed at two points on the property of Central Camalache; one near the coast where the records contained in Tables VIII and IX were made and the other in the hills. The later instrument was removed to the Granja and was broken by the wind storm that swept over the Island July 23rd, 1926. We have no records of value from this instrument. Two instruments were placed on the property of the Fajardo Sugar Co.; one in the locality of high humidity and the other in a locality of low humidity, a hydrothermograph was placed in the field on the Insular Experimental Station Farm from which the records of Tables V, VI and VII were made. All these records have been of value in our studies, but the hydrograph records have not been satisfactory as we had hoped for when the work was started.

TABLE XI  
SHOWING AVERAGE RAINFALL AND TEMPERATURE OVER FIVE  
YEARS (1922-26)

Except for Río Piedras, where the temperature covers three years only

	AVERAGE RAINFALL											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Río Piedras---	4.65	5.17	4.35	4.44	3.10	6.55	10.56	7.50	6.63	6.44	7.26	6.35
Arecibo-----	4.24	6.94	3.88	4.40	1.50	3.24	4.21	5.20	4.82	4.38	5.38	7.22
Fajardo-----	2.75	3.71	2.84	3.01	2.83	3.68	4.48	6.99	4.07	7.65	8.29	3.33
	AVERAGE TEMPERATURE											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Río Piedras---	73.1	75.1	74.4	75.7	77.3	77.8	77.7	78.7	78.8	78.2	76.8	75.2
Arecibo-----	73.7	73.9	75.8	77.6	79.5	80.0	80.4	81.1	80.9	79.7	77.9	74.7
Fajardo-----	77.1	76.5	77.1	78.9	80.9	81.6	82.0	82.4	82.3	81.2	79.3	77.8

The compilations from the U. S. Weather Bureau records (Tables X and XI) show a gradual decline in temperature from September to January and then a gradual rise until June. The coolest months are November to February inclusive and the warmest months are May to October inclusive. Of course the temperature was slightly lower during the night and somewhat higher during the day than is shown on the chart. The disease is most severe during November, December, January and February, and March the period of lowest temperature. The average rainfall for the same period is also shown on this chart. It will be readily seen that the greatest rainfall with one exception coincides fairly well with the period of low temperature. Our studies indicate that a low temperature and a high atmospheric humidity are most favorable for the development of this disease but it is very evident that the disease is more sensitive to variations in temperature than to variations in rainfall.

The thermograph records at Río Piedras showed the lowest temperature during the winter months and the highest during the summer, the most noticeable being about November 1st which was also the date of a rapid increase in the eye spot. The hydrograph showed a higher humidity during the summer months than during the winter months.

The hydrograph at Arecibo was placed near the ocean, where it was under the influence of a wind current from the ocean practically all the time, and showed a slightly higher humidity during the summer months. This is in harmony with the rain fall records although the ranges at Arecibo are not so pronounced as at other points. The average temperature records are lowest from December to March inclusive, the period of greatest severity for the disease.

At Fajardo, the range of lowest rainfall according to the U. S. Weather Bureau reports was from December to June inclusive and the lowest temperature from December to March inclusive, which was also the period of greatest severity for the disease. However, there was one record at Fajardo that was especially interesting. At one point (Paraiso) the rainfall ranged from 1.20 inches in March to 3.20 inches in October greater than the average for nine years for the Central zone. The disease was always more severe at this point than at two other points (Fortuna Centron and Santa Rita) where the local rainfall coincided quite well with the average for the Central zone.

However, a paper on "Relation of Temperature to the Growth of the Eye Spot Fungus" by Halma and Fawcett (6) was published in *Phytopathology*, August 1925, which has been valuable to us in

our work; according to the studies of these workers the fungus makes its most rapid growth under laboratory conditions at about 84° F. It grows more slowly at both higher and lower temperatures, making no growth at temperatures above 95 and very slow growth at 56 degrees.

We do not have the equipment for extensive experiments on either temperatures or humidity, but the results obtained with the one thermograph here at the Experiment Station and a study of the disease in connection with the U. S. Weather Bureau records indicate that the organism makes its best growth with an average outdoor temperature ranging from 73 to 77 degrees F. which is lower than the optimum temperature in the laboratory experiments with the organism in Hawaii. Of course it must be remembered that Weather Bureau records are not made in cane fields and that temperatures vary with local conditions and character of plant growths within short ranges of distance.

We have not done any experimental work for the control of this disease. The F.C. 306 and D. 109 which are our most susceptible varieties are gradually giving way to the more desirable canes, such as B.H. 10(12) and S.C. 12(4) which are much more resistant to the disease and this is solving our problem of control at this time. The future development or introduction of more valuable canes which are susceptible to the disease may force studies for the control of the disease in the future.

#### SUMMARY

1. The "eye spot" disease which is caused by *Helminthosporium sacchari* Butler is serious on certain varieties of sugar cane in Porto Rico.

2. It is most severe on F.C. 306 and D. 109 with H. 109 as a poor third. The Uba and P.O.J. canes are most resistant.

3. The susceptibility varies with leaf structure, the higher the fiber content the greater the resistance. However, there appears to be some slight correlation with sucrose content.

4. The young leaves are more susceptible than the old leaves; the bases and upper surfaces more susceptible than the tips and lower surfaces.

5. The spores and sporophores germinate readily in water and penetrate the leaf without regard to the stomata. The greatest mycelial growth is in the parenchyma tissues. The spores are borne more abundantly on the lower than on the upper surface of the leaf.

6. The disease is most severe during the cool, wet months of the

year. Temperature appears to be the most important environmental factor.

7. The most satisfactory control for Porto Rico at his time consists in the use of resistant varieties.

The writer wishes to express his thanks to the Hon. C. E. Char-dón, Commissioner of Agriculture of Porto Rico; Mr. Rafael Me-néndez, Director of the Insular Experiment Station at the time the work was started; Mr. F. A. López Domínguez, present Director of the Insular Experiment Station; Dr. O. L. Fassig of the U. S. Weather Bureau in San Juan; Mr. R. A. Veva of the Fajardo Sugar Company; Mr. Pedro Olivencia of the Cambalache Sugar Company and many others for their assistance in this work.

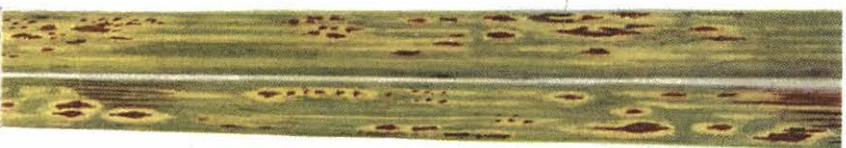
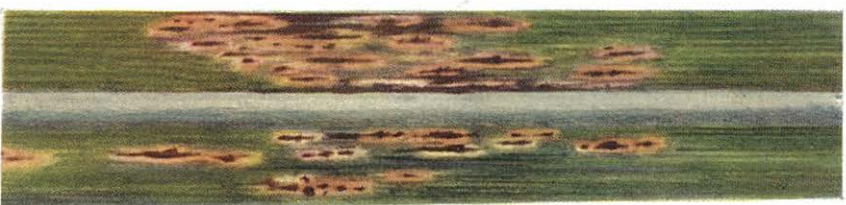
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## EXPLANATION OF PLATES

- Fig. 1.—Spores of *Helminthosporium sacchari*, showing a variety of sizes and variation.
- Fig. 2.—Sporophores of *H. sacchari*, showing variations.
- Fig. 3.—Sporophore and spore.
- Fig. 4.—Germination of spores.
- Fig. 5.—Germination of sporophore.
- Fig. 6.—A spore germinating on a glass slide.
- Fig. 7.—Germinating spores on leaf of sugar cane. No discolorations.
- Figs. 8 *a* and *b*.—Germinating sporophores on cane leaves. *a* shows the first trace of the formation of a spot. In *b* shows the dotted line indicates the size of the spot which lies between two fibro-vascular bundles.
- Fig. 9.—A diagram of the lower margin of the leaf showing mycelium masses on the surface and also next to the fibro-vascular bundle. (Cross section.)
- Fig. 10.—A germination spore on a leaf. One branch of the germ tube has penetrated the leaf and a spot is forming.
- Fig. 11.—Showing the massing of the fungus near the lower surface of the leaf, the breaking through the epidermis, the growth on the surface and the formation of the sporophores. (See fig. 9).
- Fig. 12.—The inter and intra-cellular growth of the mycellium.
- Figs. 13-15.—The growth of the fungus in the cells. The large circles in 14 and 15 are tracheary tubes.



*Drawn by Mario Brau*

PLATE I

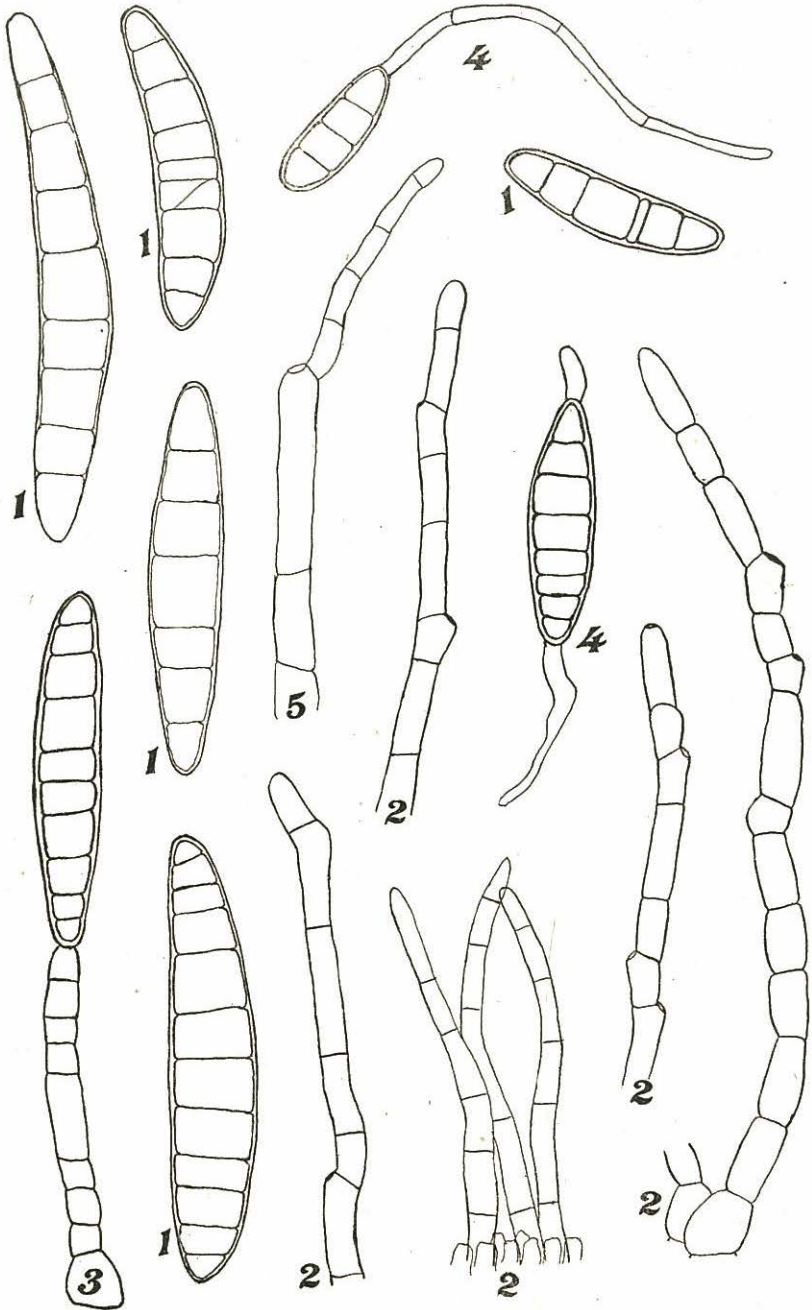




PLATE II

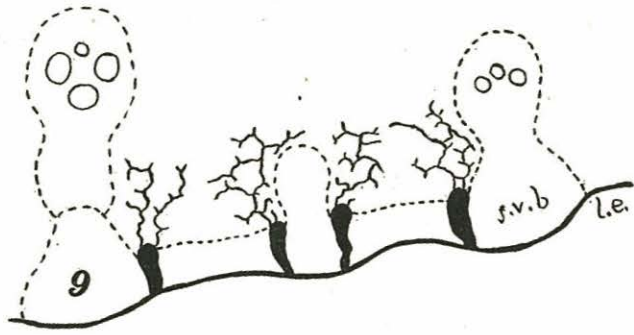
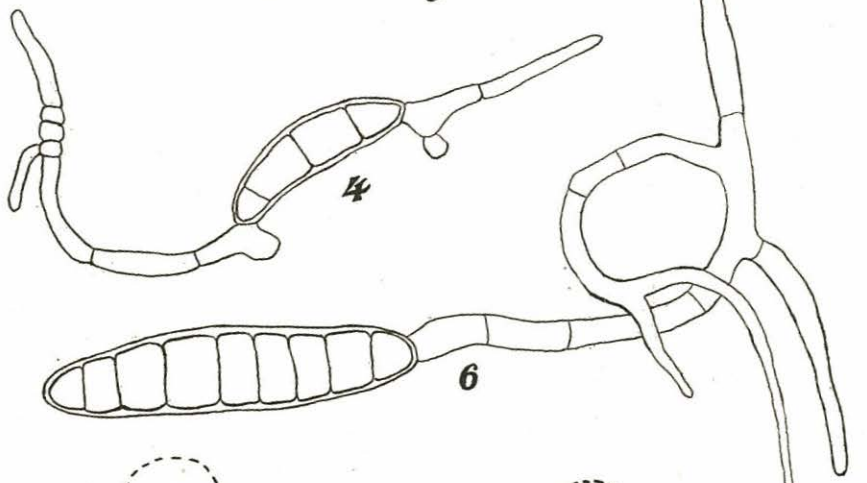
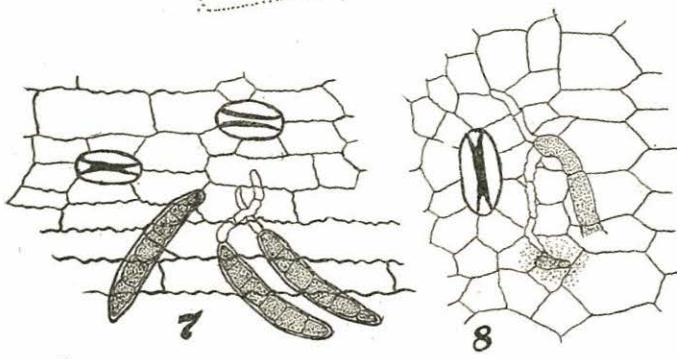
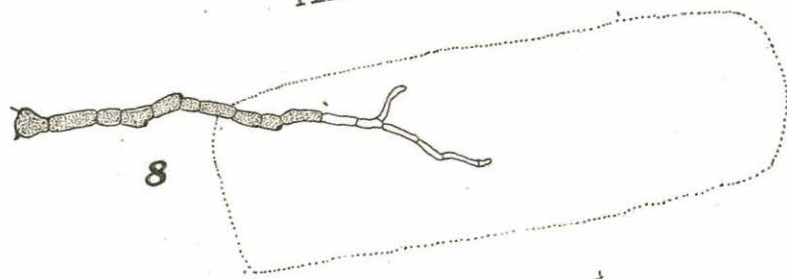


PLATE III

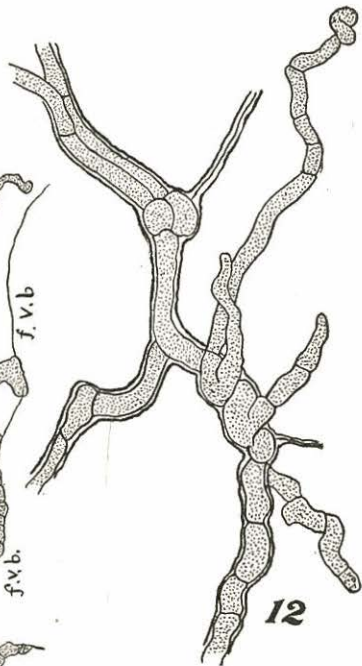
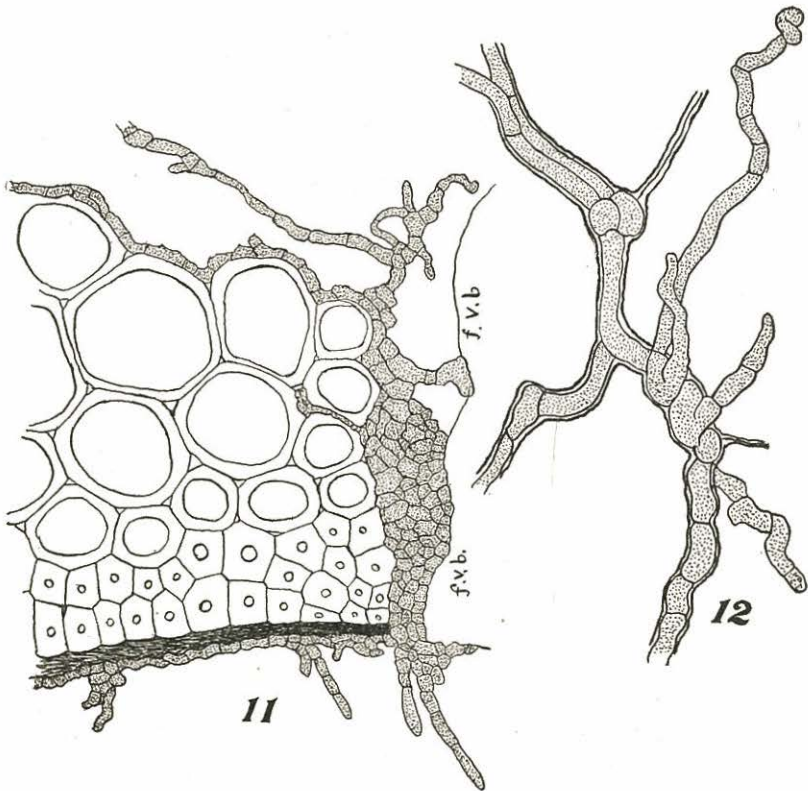
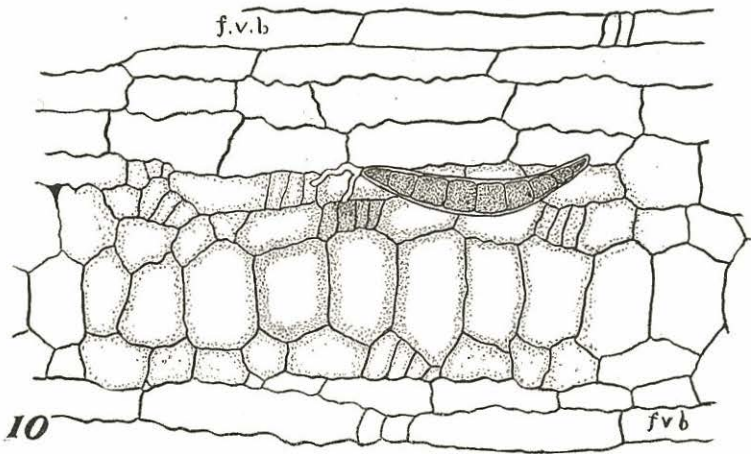


PLATE IV

