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GUM-PRODUCING ORGANISMS IN SUGAR CANE

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The importance of the gummosis disease of sugar cane and the advance in our knowledge of the subject since the publication of the previous papers by the senior author have made a further study of the subject very desirable. The most important of these advancements is in our knowledge of the geographical distribution and of new species or strains.

HISTORY

This disease was reported first from Brazil by Dränert in 1869 but appears to have been known as early as 1863. It was reported next from Australia by Cobb who described the organism in 1894. He believed it to have been in the country as early as 1876. The wide separation of these two countries presented a very interesting problem in geographical distribution. Shepherd, of Mauritius, in his report for the International Survey of the Diseases of Sugar Cane (1932), said that it was probably introduced from Brazil to Mauritius on a shipment of seed cuttings in 1869 and from there to Australia in 1874. North, of Australia, in his report for the same survey, says that it was probably introduced into Australia from Mauritius in 1874. The next report was from Brazil in 1894, but it was less severe than in 1869, probably due to the use of resistant varieties. It was found in Puerto Rico by Matz in 1920 and reported the same year. It was discovered in St. Kitts of the British West Indies in 1925 and reported by Ballou in 1926. It was found and reported from Colombia by Chardón of Puerto Rico in 1926. It was found and reported in Guadeloupe of the French West Indies by Williams

[1] This is the third paper on the gummosis of sugar cane by the senior author. Early in 1932 the junior author began to cooperate in the work and has made the studies on the reaction of sugars to these organisms and also the serological studies.

(16) in 1929. It was reported from Dominica of the British West Indies by Ashby (1) in 1928–29; from St. Lucia of the British West Indies in 1929, and from Antigua of the British West Indies by Illingworth in 1930.

In addition to the above places it has been reported from Fiji Islands, Java, Borneo, Reunion and New Guinea. The report from Java by Groenewege in 1915 was proven to be a mistake by Wilbrink who published the results of her studies in 1920. The disease which was supposed to be gummosis was found to be what is now known as scald (*Phytomomas albilineans* Ashby). The reports from Borneo and New Guinea have not been confirmed.

The symptoms of the disease have been described by many students of the subject. The descriptions by the various authors are very harmonious. The senior author in his description which was published in 1928 makes the following statement:

> "The most reliable external symptom is the leaf streaks referred to by Matz and Cottrell-Dormer. However, this symptom is sometimes present on POJ canes, and occasionally on Uba and some other canes, although it is impossible to find any trace of gumming in the cut surfaces."

Again in a paper read before the plant-pathology section of the Fourth Congress of the International Society of Sugar-Cane Technologists (1932) (8) he said,

> "Although the presence of these stripes is the most important external symptom I am satisfied that it is not always reliable. I have found infected canes which did not show these symptoms and I have found these symptoms in canes which did not show internal gumming or discolorations."

Further studies in Puerto Rico on the symptoms of this disease following the publication of this first paper led to more confusion. The leaf symptoms appeared in several resistant varieties which did not show discolorations or gum in the fibro-vascular bundles of the stems or bacteria in the leaf stripes. Finally the senior author began referring to cases of this kind as "false gummosis". This phase of the problem was solved at the Fourth Congress of the International Society of Sugar-Cane Technologists which met in Puerto Rico, March 1932, when it was found that this "false gummosis" was the same as a disease reported by Dr. Wilbrink of Java in 1929 as "fourth disease" and by Martin of Hawaii in 1930 as "chlorotic streak." It was also learned that this disease had been noted by Bell of Australia who referred to it as "false scald".

The leaf symptoms of this fourth disease or chlorotic streak are very similar to the leaf symptoms of the gummosis. The streaks

may be somewhat broader than in gummosis and the boundaries may be somewhat wavy. As a rule the reddish dots which are characteristic of the gummosis do not develop in fourth disease or chlorotic streak, but there is a reddish margin on the older streaks.

The exudation of gum on the cut surfaces of the canes is the most reliable symptom of the disease. The diseased fibro-vascular bundles are usually discolored, the most common discoloration being red but a discoloration does not necessarily indicate the disease and the absence of gum on the cut surfaces does not necessarily indicate that the cane is free from the disease. During periods of very dry weather diseased canes may show discolorations and but little or no gum. During or following periods of wet weather the canes will show the gum in varying amounts, depending on several factors, such as weather conditions, length of time that the cane has been infected and variety of cane.

Within the past few years certain variations in the morphologic and physiologic characters of the organism have been reported which make further study desirable.

The writer sent cultures to Mr. M. C. Goldworthy of the University of California in 1927 who compared them with cultures sent from Australia by Mr. A. F. Bell for that purpose. Under date of June 27, 1927, he replied as follows:

"Your vascular types are different from those we have received from Australia. That is they behave differently on media. So far I have had no opportunity of comparing the cultures by the serological method."

Ashby (**2**) (1929) published the results of a study of this disease in the British West Indies in which he reported the finding of two organisms. He said:

"Plantings in peptone saccharose agar made with yellow ooze from the stalks of affected canes in St. Kitts yielded two types of colonies, these, the more rapidly appearing (3 to 4 days) being entire convex, glistening and, at first, colourless but later pale yellow and spreading: the later appearing colonies were entire, flat, deeper yellow, and the growth more restricted. The first type of colony yielded a straw or amber yellow (Ridgway) abundant slimy growth on slanted agar in tubes with a marked tendency to run down and accumulate at the bottom of the slope; after inoculating into milk a shallow clear zone formed at the top of the liquid in two or three days at tropical room temperature with a bulky indistinct clot apparently due to a labenzyme and the reaction became increasingly alkaline. The second more slowly growing type of colony which was yellow from the start yielded a restricted glistening aniline to primuline yellow growth on the agar with a compact slime showing little tendency to flow. In litmus and plain milk no change occurred in a week but there was gradual increase of alkalinity subsequently. As the organism in the first type of colony

showed cultural characters different from those of *B. vascularum* as described by Erwin F. Smith and as those of the second type were in agreement with his description the first type was discarded and attention given to the second.''

He secured cultures from North of Australia and made inoculations in cane in England which enabled him to compare the St. Kitts organism (second type) with Australian organism. The Australian type produced broader stripes, a withering of the heart and a rotting of the apical internodes. It was more severe than the St. Kitts second type. He said:

''The original cultures of the St. Kitts organism (second type of colony) gave rise to a glistening restricted growth on peptone-saccharose agar (saccharose 2.0, with peptone 0.5, dipotassium phosphate 0.05, magnesium sulphate 0.025, agar (bacto) 1.5, water 100.) between aniline and primuline yellow in colour (Ridgway); the slime was compact drawing out, in cultures which had attained their full growth, into elastic threads and showing little tendency to flow. The growth was opalescent in oblique light and gelatine (10 per cent.) of the same composition as the agar medium was slowly liquefied after two weeks. Lavender-colour litmus milk became gradually strongly alkaline and after two to three weeks at 23° C. began to clear from the surface with bleaching. After two to three months the milk had cleared with more or less suspended slime and restoration of the litmus colour but remained permanently strongly alkaline. If the milk carried a layer of fat a yellow growth developed on the surface and there was little deposit; in the semi-anaerobic conditions under the layer of fat, bleaching of the litmus was complete for a time, and it appeared that action on the milk was mainly by diffusion of metabolic products through the fat layer. In well-separated milk, surface growth was restricted to a yellow ring, there was active multiplication within the liquid and a good yellow deposit associated with partial bleaching and more rapid restoration of the litmus colour. It is doubtful if, at any time, a true clot was formed, the eventual clearing being apparently due to increasing alkalinity since cleared cultures yielded a bulky precipitate when acidified with hydrochloric acid. The organism which was actively motile in young liquid cultures by means of a single polar flagellum exceeded 0.5 microns in diameter and was from 1.0 to 1.5 microns in length occurring singly, in pairs and more rarely in short chains. Most of the isolations showed colonies similar to those of the original isolation but those from the leaf stripes of Uba were dry, flat, rough and pale yellow with wavy margins, the growth from these colonies on agar slants was at first similar but tended gradually to take on the glistening smooth deeper yellow character of the original form. Some of the cultures from the isolations caused, like the original, a slow liquefaction of gelatine but others showed no trace of liquefaction at room temperatures after three months. The action on milk varied in the rate of change but there was no true clot and all eventually cleared with persistent alkalinity.

''The original culture from Australia gave rise on the agar to convex glistening almost colourless colonies of a fluid slimy consistency which became paler yellow and spreading. On agar slants the growth was abundant, opalescent and fluid slimy with a tint from straw to amber yellow: the slime tended to flow and accumulate at the base of the slant. On potato the growth was sulphur yellow

fluid and flowing off the surface but not so abundantly as the slime of *B. Malvacearum* E. F. Smith. Gelatine showed liquefaction under a week and a shallow clear zone formed at the surface of plain and litmus milk after three to four days at 23° C associated with the formation of a bulky indistinct clot apparently due to a lab-enzyme; alkalinity gradually increased becoming strong and persistent and the milk was completely cleared after two to three months of 23° C; the clearing appeared to be caused by peptonisation as no precipitation followed acidification with hydrochloric acid. Growth occurred as a yellow slimy layer on the surface of milk with fat present and absent. The organism was actively motile in young cultures and approximated in size to that of the St. Kitts form.

"The colonies which appeared in platings made from the inoculated canes were not uniform in type; some resembled those of the original culture, but they were as a rule, mingled with others showing a piled-up deeper yellow colour, a dark opaque nucleus, and little tendency to spread; others again were at first flat, but changed to the second type after a few days. Transfers from the different colonies to agar slants yielded, however, a similar pale yellow slimy fluid growth like that of the original form. All cultures from isolations behaved like the original culture in liquefying gelatine appreciably within a week and clotting milk in three to four days at 23° C. with gradually increasing alkalinity and in clearing it eventually but with marked differences in the rate of clearing as cultures from the second or 'piled' up type of colony were slower in action. The isolation from Australia, showed therefore, cultural characters similar to those of the first type from 'gummed' cane in St. Kitts, and it is believed now that they are probably identical. As the cultures made from the isolations out of the different varieties were essentially similar to the two types used for inoculation, and as no evidence was obtained that the one type could change into the other, it would appear that two yellow forms may cause Gummy Disease occurring in some instances together and which differ in cultural characters enough to be considered as distinct varieties of *B. vascularum*. The strain described by Erwin F. Smith resembles the second type from St. Kitts differing from some cultures of it only in not eventually clearing milk, a difference which appears to depend on the amount of alkali produced; the alkaline body is either ammonia or an amine as the vapour from boiling cultures turns red litmus paper blue."

In August, 1930, a gummosis disease was discovered on POJ 2878 at Jayuya, a point located in the center of the island and at an elevation of about 2,000 feet. This was especially interesting because this variety was supposed to be immune or highly resistant.

The cane was sent to the laboratory and put in a moist chamber, where it produced a typical gumming within 24 hours. Owing to the fact that this variety was supposed to be immune, the senior author and Mr. Pedro Richardson, Agronomist, visited Jayuya in order to make sure that it was POJ 2878. After a careful examination, it was decided that there was no doubt as to the variety. The symptoms were not quite typical. However, the gumming was so pronounced that it was detected by the foreman who was making cuttings for planting. The behavior of the organism in culture was typical of *B. vascularum*.

This outbreak was described by the senior authority----------- as follows:

"The cane was about seven months of age from date of planting and the infection was well over 75 per cent.

"The external symptoms were somewhat different from those described by the various writers on the subject. The early symptoms were not found but the late symptoms were very abundant. These symptoms consisted in a dying of the tissues in the leaves along the veins, thus producing elongated areas of dead tissue which frequently extended to the leaf margins. In many cases the margins of the leaves also were dead. When the tops of the canes were cut across, many of them showed a gumming, which was much more pronounced when the cuttings were kept in moist chamber for a few hours.

"The gum was not the typical honey yellow which has been described by several writers, but ranged from clear to creamy white or yellow, and in some cases was slightly tinted with honey yellow."

The further history of this outbreak is as follows:

A field test with healthy P.O.J. 2878, 2883, 2714, 2727, P.R. 801, 803, 807, 809, 820, 826, F.C. 916 and Guadeloupe 119 was started in which every third row was planted with infected P.O.J. 2878, so that every variety of healthy cane was in contact with an infected row. The rows were five feet apart and consisted of 50 stools each. The field of infected cane was plowed out.

At this same time cuttings of the same varieties were set in our greenhouse, and the young canes inoculated with cultures prepared in the laboratory. Some of P.O.J. 2878, 2883, P.R. 807, 809, 826, F.C. 916 and Guadeloupe 119 developed slight symptoms and the organism was recovered from them but in an attenuated form. After a time the new growths failed to produce symptoms and it was impossible to recover the organism.

The field test was cut January 20th, 1932, and carefully examined by the writer. Neither symptoms nor gumming were found in any of the varieties, nor in the rows planted with infected cane. A few canes showed slight discolorations of the fibro-vascular bundles, but no gumming. They were brought to the laboratories and used for cultures. P.O.J. 2883 and F.C. 916 produced a gum organism which is not typical. The color of the gum is greyish, almost clear; some times tinted with yellow.

The greenhouse tests made by the writer with organism from P.O.J–2878 at Jayuya lead the writer to believe that P.O.J.–2878, P.O.J.–2883, P.R.–807, P.R.–809, P.R.–826, F.C.–916 and Guadeloupe 119 may be symptomless carriers although this has not been demonstrated. However, the studies during 1933 show that P.O.J.–2878 when inoculated with an extremely virulent strain of *B. vas-*

cularum will sometime develop leaf symptoms. It is the opinion of the writer that these varieties are so resistant as to be practically immune and that the experiments indicate that it is possible for a very highly resistant or apparently immune variety to be a carrier of this disease.

Cultures for P.O.J. 2878 were sent from Puerto Rico by the writer to Mr. A. F. Bell of Australia for comparison with the Australian organism. He replied as follows:

> "I desire to acknowledge receipt of your letter of 22nd October, also of four cultures. Of the latter three were apparently pure and one contained a yeast. The former were re-isolated and examined and compared with the organism of leaf-scaled, gumming, red stripe, and mottle stripe. Your organism is quite distinct from any of these four.
>
> "Parallel inoculations were made (in the transfer chamber) into cut shoots of Badila and from this small test your organism seemed much more virulent than the above four stains.
>
> "At the end of two days the lesions were about one-fourth inch in diameter and consisted in dark-red rings surrounding a water-soaked greenish or yellowish area. Later the centers became ashy coloured and the red ring surrounded by a yellowish halo.
>
> "I enclose a photograph of the lesions. The cultures and experimental material have now been destroyed. Our experience so far it that P.O.J. 2878 is highly resistant to gumming disease."

The senior author wrote another letter to Mr. Bell making inquiry as to the morphological characters of the Puerto Rican and Australian organisms. He replied (under date of March 30th, 1931) as follows:

> "Your letter of 10th February to hand. With reference to the organism you sent, this was quite distinct from our *B. vascularum* both culturally and morphologically. One considerable difference was that the organism received from you had flagella at both poles while the gumming organism has a singled flagellum only. The organism to which it bore most resemblance was that causing mottle stripe."

An examination of the photograph (Fig. 2) of this organism, made by Mr. Bell, shows that it is quite distinct from *B. vascularum*. The studies by Ashby and by the senior author emphasize the importance of more extensive studies on this disease and its cause or causes in different parts of the world.

1932 Studies

There was a severe outbreak of gummosis on the small island of Vieques in March and April 1932 and slight outbreaks in the vicin-

ity of Río Piedras and Canóvanas. The senior author made a special study of infected Cristalina from the island of Vieques on a very small mixed planting near Canóvanas and on mixed plantings containing Cristalina near Río Piedras during 1932. Vieques is a small island just east of Puerto Rico and has much less rainfall than Puerto Rico. Cristalina has been retained there as the variety of major commercial importance long after it gave way to other varieties in Puerto Rico. It is very susceptible to this disease and the infection is very near to 100 per cent. The rainfall was exceptionally high during the spring of 1932 and the disease was very evident. Most of the cuttings showed a high yield of gum.

Many cultures were made and studied. The results confirmed the opinions of the senior author which were published in 1928. There were many strains which varied in color and character of growth. These results are shown in table I.

TABLE I.

No.	Source	Color	Growth on new cul.	Inoc. 6/20 Symptoms 6/24	June 18th Symptoms	July 1 Symptoms	July 18 Symptoms
1	Australia	Yellow	Poor	POJ-2878 none	Crist none	POJ-2878 none	None
2	Jayuya FC-916	White	Poor	POJ-2878 none	Crist none	POJ-2878 none	None
3	Vieques 6032	Canary Yellow	Poor	POJ-2878 none	Crist none	POJ-2878 none	None
4	Vieques Crist	Yellow	Good	POJ-2878 none	Crist none	POJ-2878 none	None
5	Vieques	Yellow	Fair	Very Slight	Crist none	POJ-2878 none	None
6	Vieques	Yellow	Good	Good	Good	POJ-2878 none	Slight
7	Vieques	Yellow	Good	Very Slight	Very Slight	Slight	None
8	R. P. Crist	Yellow	Good	Good	Good	18 in.	Slight
9	Jayuya POJ-2878	White	Poor	Very Slight	Slight	1½ in.	Slight
10	Vieques	White	Good	Slight	Very Slight	Outgrown	None
11	Vieques	Yellow	Fair	Slight	None	Outgrown	None
12	R. P. H.—109 slow	Yellow	Fair	Slight	Very Slight	Outgrown	None
13	R. P. H.—109 slow	White	Poor	None	Very Slight	Outgrown	None
14	R. P. H.—109 slow	White	Poor	Slight	None	None	None
15	R. P. H.—109 slow	White	Good	Slight	Very Slight	Outgrown	None

R. P. H.—109 = H 109 from Río Piedras.

The results given in this table confirm the opinion of the senior author expressed in 1928 that this species included a large number of strains which varied in color, growth and virulence.

1933 Studies

The first half of 1933 on Vieques was very dry as compared with 1932. The Cristalina and Rayada (a variety of Cristalina) canes were very heavily infected with *B. vascularum* but the exudation of gum was much less than in 1932. A large number of isolations were made and used for laboratory study. All the cultures used in 1933 were new and from Vieques canes except three: Nos. 1 and 2 were 1932 cultures which had lost their virulence but made excellent growths on agar. No. 23 was a culture sent to the senior author by Mr. A. F. Bell of Brisbane, Australia, late in 1932 and received in January, 1933.

The laboratory studies showed a large number of strains which possessed the following characters:

1. *Color*.—Various shades of yellow and milk white, while others were clear or sometimes clear and slightly tinted with yellow. A few were brownish. In many cases the first growth of a yellowish exudation was white when transferred to agar. Many strains changed color on the agar. The strains were grown on different media and at different pH but up to the present time the changing of colors has not been explained. The best growths were made in acid media but good growths were made on alkaline media as high as pH 9.6 although it was slower than on the acid media. Strains also changed from rough to smooth and smooth to rough without any apparent cause.

The variations in color were in harmony with those reported by the senior author in 1928. Cobb reported variations in color in 1905 but other writers did not give much attention to this phase of the subject. Possibly the material which they were studying did not show the extensive variations which are reported by the authors of this paper.

Some strains were extremely virulent while others were slightly virulent as shown by the leaf symptoms. Others did not produce leaf symptoms but grew in the canes as was shown by the exudations when the canes were cut and kept in a warm, moist chamber.

There was very little relationship between color and virulence although in general it may be said that the yellow strains were slightly more virulent than the others.

No experiments were made to determine temperature relationships but cultures that were put in incubators and refrigerators died in a short time.

Two series of inoculations were made and recorded in Table II. Other inoculations were made but the results were the same as shown in this table.

TABLE II.

No.	Color	
1	Yellow	No infection. Was virulent in 1932.
2	White	No infection. Was virulent in 1932.
3	White	No infection.
4	Almost clear	No infection.
5	White, yellow with age	Mild symptoms on Cristalina.
6	Yellow	Symptoms on Cristalina and H-109.
6	White	Symptoms on Cristalina and H-109.
7	Yellow	Symptoms on Cristalina and H-109 and POJ-2878.
8	White, yellow with age	Not infectious.
9	Clear, brown with age.	Not infectious.
10	White, brown with age	Not infectious.
11	White	Mild symptoms on Cristalina.
12	Whitish, almost clear..	Not infectious.
13	Yellow	Symptoms on Cristalina.
13	White	Symptoms on Cristalina.
14	White	Not infectious.
14	Yellow	Not infectious.
15	White	Very slight symptoms on Cristalina. Developing very slowly.
16	Clear, yellow tint	Mild symptoms on Cristalina and H-109.
17	Clear, yellow tint	Mild symptoms on Cristalina and H-109. Developing very slowly.
17	Yellow	Not infectious.
18	White	Not infectious.
19	White	Symptoms on Cristalina and H-109.
22	White	Symptoms on Cristalina and H-109 and POJ-2878,
23	Yellow	Very mild symptoms on Cristalina
24	Clear, white tint	Symptoms on Cristalina, H-109 and POJ-2878,
25	White	Symptoms on Cristalina and PR-803.
26	Yellow	Symptoms on Cristalina.
27	Yellow	Symptoms on Cristalina, H-109 and POJ-2878. Very slight symptoms on FC-916.
28	Light yellow	Symptoms on Cristalina, H-109 and very slight symptoms on M-28.
29	White	Symptoms on Cristalina.
30	Yellow (rough)	Symptoms on Cristalina and POJ-2878.
31	Yellow (smooth)	Symptoms on Cristalina. Slight symtoms on PR-803.
32	White	Symptoms on H-109.
32	Clear	Symptoms on Cristalina.
33	White	Symptoms on Cristalina.
34	Yellow	Symptoms on Cristalina.
35	Yellow	Slight symptoms on Cristalina.
36	Yellow	Symptoms on Cristalina.

Nos. 1 to 23 inoculated 5/5/33. Nos. 24, 36, inoculated 5/19/33. Time for appearance of leaf symptoms one to three weeks, occasionally longer. Nos. 1 and 2 were 1932 culture which lost their virulence. No. 23 from Australia. All others were 1933 cultures.

METHODS OF INOCULATIONS

Several methods of inoculation were used as follows:

1. Pricking the cultures into young cane or leaves with a needle. This was successful but slow and the percentage of takes less than with the other methods.

2. Cutting of the tops of the canes almost down to the growing points and the immediate application of the organism in agar. Then covering the mass with a pad of wet cotton. These inoculations were made late in the afternoon so that the agar would remain moist as long as possible. In some cases distilled water was poured on the cotton one or more times during the following day. The inner leaves push upward within 48 hours and if the variety is very susceptible white streaks will be found running downward. Varieties that are practically immune will not show these streaks. Sometimes streaks

three or four inches in length, will develop in highly resistant variety. However, they do not lengthen after the first few days and the organism dies.

3. Cutting a small hole into the spindle above the growing point and the insertion of the organism from an agar culture. Within ten days or two weeks the injured parts of the leaves pushed out and unrolled. The presence of the organism could be determined by the development of white streaks in the leaves running up and down from the point of injury. The variations in time depended on the resistance of the variety and the virulence of the strain in the culture.

4. The inoculation of the organism from agar cultures into the stem below the growing point. This method was successful but slow. A high percentage of gum pockets resulted.

5. Removing the upper half of an advanced cane so as to force the development of side shoots. When the side shoots are well advanced cut holes in the old cane and insert agar containing the organism.

6. The insertion of pieces of diseased cane into slits in the spindle or cane. The results were practically the same as when agar cultures were used but more uncertain.

7. Inoculation of seed cuttings by the insertion of the organism from cultures in holes cut in the seed pieces. This method was slow and the results irregular.

The results of the inoculations not given in the Table II may be summarized as follows:

Rapid-growing canes respond to inoculations much more readily than slow-growing canes.

Canes inoculated with a culture may show negative results at one time and mild symptoms at another time. The symptoms may develop more slowly in some cases than in others although the inoculations are made from a single culture and on one variety. Some strains do not produce leaf symptoms but do produce gum in the canes and in cultures.

When an infected cane is cut across and placed in a warm, moist chamber, the gum oozes out on the cut surfaces, sometimes in such great abundance as to cover the entire cut area. Two or more colors may emerge from a single piece. When the gum is transferred to agar plates, it makes a very rapid growth. Sometimes all the colors persist and sometimes the deep yellow makes a clear growth. Some of the clear growths from yellow gum become yellow later and some remain yellow. The yellow strains show a great tendency to produce modifications of yellow and sometimes become clear or white. Some

strains grow much more luxuriantly than others. Some strains are much more virulent than others. Some strains that produce gum in the cane and in culture do not produce leaf symptoms. Positive results may be obtained from cultures of all colors and all tints but in general the yellows are more virulent than the other colors.

Bacteriological Study

Twenty-seven cultures collected in 1932 and previous to that date were studied and classified as follows:

Group I.—Is composed of five cultures isolated from the island of Vieques. They produced an abundant canary-yellow growth in twenty-four hours. They are bacilli arranged parallel and side to side, motile and show no spores nor capsules. The colonies are homogeneous, entire edged, straw in color, stain negative to Gram, growth in broth is turbid, agar stroke is slow, confluent, smooth, viscid and opaque and show no change in litmus milk. It does not ferment dextrose, lactose, dulcite, manite, maltose, sucrose, xylose or arabinose; does not produce hydrogen sulphide nor indol; the V.P. and M.R. reactions are negative; does not reduce nitrates, has no odor and emulsify very poorly. This group of organisms is identical with the cultures of *B. vascularum* isolated in Australia that we had previously studied.

Group II.—This group consisted of twelve cultures isolated from "Cristalina" cane in Vieques and from "F.C.–916" cane in Jayuya Some produced a whitish-gray growth while others had a lemon-yellow growth. They are bacilli arranged side by side, parallel or end to end, motile, have no spores nor capsules. The colonies are homogeneous, entire edged, and straw in color. They are negative to Gram stain, grow slowly in broth with slight turbidity. In agar the growth is slow, confluent, raised, smooth, viscid and opaque. There is no change in litmus milk, no acid is produced in arabinose, xylose, dextrose, lactose, dulcite, manite, maltose and sucrose. They produce no hydrogen sulphide and no indol; the V.P. and M.R reactions are negative; they do not reduce nitrates, do not produce any odor and have a poor emulsificability.

Group III.—Is composed of ten cultures isolated from "P.O.J.-2878" at Jayuya and "Cristalina" cane from Vieques. The growth of some of the cultures is absolutely colorless and in others is enamel white. They are bacilli which are found singly or in short chains, motile, form no spores nor capsules and are negative to Gram stain. The colonies are granular, straw in color and have entire edge. The

growth in broth is slight turbid with filament and at times a ring adherent to the tube is formed. The agar stroke is filiform, elevated, smooth and translucent or white. They do not ferment dextrose, maltose, manite, xylose, sucrose, arabinose or dulcitol; they do not produce hydrogen sulphide and the V.P. and M.R. reactions are negative. They do not reduce nitrates, have no odor and have poor emulsificability.

The cultures studied differ in pigment production but their sugar fermentations are similar. In 1933, eighteen cultures have been studied, ranging in color from yellow, white, creamy, lemon-yellow, grayish white to colorless. Thirteen of the cultures are bacteriologically similar to the description given in Group I of the 1932 cultures, that is, they are Gram negative organisms, motile, do not ferment any of the sugars tested, do not produce hydrogen sulphide, have no change in litmus milk, do not produce indol, do not reduce nitrates, the V.P. and M.R. reactions are negative, they have no odor and have very poor emulsificability.

TABLE III.

FERMENTATION REACTION, 1933 CULTURER

Culture B	Isolated from	Color	Glucose	Manite	Lactose	Xylose	Sacchnrose	Maltose	Dulcite	Arabinoses
23	Australian culture	Yellow								
24	Cristalina	White								
25	Cristalina	White								
26	Cristalina	White	+	+		+	+	+	+	+
27	Cristalina	Yellow								
23	Cristalina	Creamy yellow								
29	Cristalina	White								
30	Cristalina	Creamy yellow								
31	Cristalina	Creamy yellow								
32	Cristalina	Colorless								
32 c	Cristalina	Yellow								
33	Cristalina	White	+	+		+	+	+	+	+
34	Cristalina	Yellow								
35	Cristalina	Grayish white								
36	Cristalina	Grayish white								
37	Cristalina	White	+	+		+	+	+	+	+
38	Cristalina	Yellow	+	+		+	+	+	+	+
39	Cristalina	White	+	+		r	+	+	+	+

There are five cultures labeled 26, 33, 37, 38 and 39 which range from white to yellow color and which are absolutely different from the others macroscopically. They are bacilli arranged singly, motile, form no spores nor capsules. The colonies are finely granular, straw in color and entire edged. They are Gram negative. The growth in broth is heavy and granular; agar stroke is filiform, elevated, smooth and whitish in color. They produce slight acidity and coagulation in milk. They produce acid and gas in glucose, manite, xylose,

sucrose, maltose, dulcite and arabinose. They do not ferment lactose. They produce hydrogen sulphide; indol is negative; V.P. and M.R. reactions are negative; they have no odor and emulsify very poorly. These cultures produce a gummy substance but it is still questionable to our mind if they produce true gummosis.

Serological

Two rabbits were inoculated intravenously with cultures 32 c and 33, respectively, and two potent antisera were obtained. By direct agglutination cultures 23, 24, 25, 27, 28, 29, 30, 31, 32 and 32 c, 34, 35 and 36 agglutinated to different titers with antiserum 32 c, while they did not agglutinate at all with antiserum 33. The same was true of cultures, 33, 26, 37, and 39, which agglutinated with antiserum 33, but did not agglutinate at all with antiserum 32 c. This demonstrates that 13 cultures are antigenically alike, one of these strains, 23, being a known gummosis strain isolated from Australia. The other five cultures, i.e., 33, 26, 37, 38 and 39, are antigenically different from the first 13 cultures studied.

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EXPLANATION OF PLATES

The photographs were not made on the same scale but the measurements were practically the same in all cases.

FIG. 1.—*B. vascularum*. Photographed by W. Cottrell-Dormer of the Bureau of Sugar Experiment Stations, Brisbane, Australia.

FIG. 2.—The organism from the gumming cane (P.O.J.–2878) at

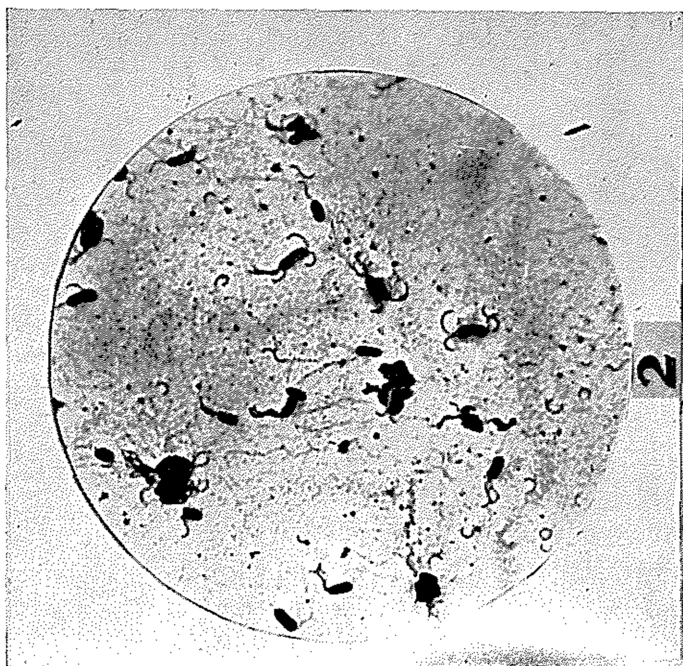
Jayuya, Puerto Rico. Photographed by A. F. Bell, of the Bureau of Sugar Experiment Stations, Brisbane, Australia, from a culture sent to him by the senior author.

FIG. 3.—*B. vascularum* from white culture from Cristalina from Vieques.

FIG. 4.—*B. vascularum* from yellow culture from Cristalina from Vieques.

NOTE.—Numbers 3 and 4 photographed by Mr. Brewer and Dr. Artschwage of the U. S. Department of Agriculture.

PLATE XXI



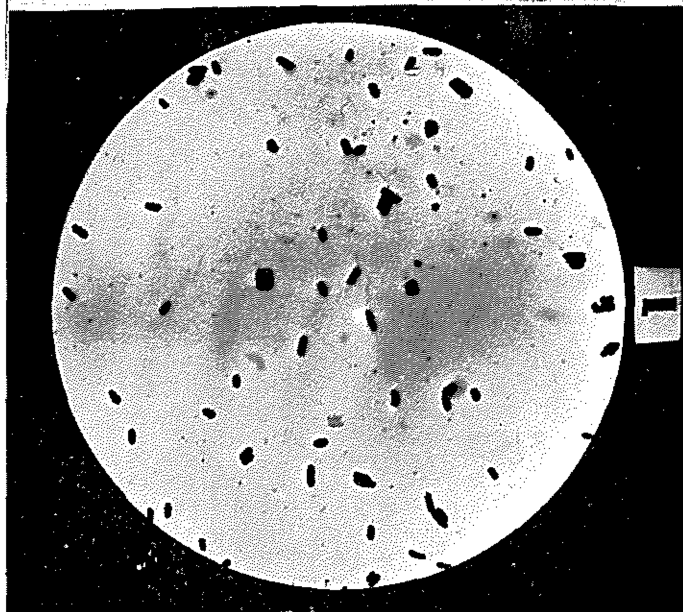


PLATE XXII