THE JOURNAL
OF
THE DEPARTMENT OF AGRICULTURE
OF
PORTO RICO

VOL. III OCTOBER, 1919 NO. 4

THE YEAR'S EXPERIENCE WITH SUGAR-CANE MOSAIC OR YELLOW STRIPE DISEASE.

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In the JOURNAL OF THE DEPARTMENT OF AGRICULTURE AND LABOR for July 1919 (not published till January, 1920), Mr. J. A. Stevenson has given a summary of his studies on this disease, (for which he has proposed the name "Mottling"), made up to the time of his departure from Porto Rico in October, 1918. Active work has been in progress since that time on various lines connected with this investigation and it seems desirable at this time to make this report of further progress.

The present writer came to Porto Rico in August, 1918, commissioned by the United States Department of Agriculture to investigate this disease and with instructions to coöperate as fully as possible with both the Federal and Insular Experiment Stations and any other agencies or individuals engaged in any phase of its study. After a preliminary inspection of the situation it seemed best to divide the problem into a number of projects in which the different workers could interest themselves, thus avoiding duplication of effort and also centering attention at first on those phases of the problem that seemed to promise the most immediate practical results. The following projects or divisions of the general problem were outlined and the effort was made to get work started in each of them: 1st. A field survey to determine the present extension of the disease. 2nd. Methods of eradication adapted to recent outbreaks or cases of partial infection. 3rd. Methods of cultivation best adapted to badly diseased fields. 4th. Statistics of sugar production as affected by the

disease. 5th. Methods of natural or artificial infection. 6th. Resistance and immunity—variety studies. 7th. An ecological survey of the insect inhabitants of the cane fields with special search for possible carriers of the disease. 8th. Cage experiments with insects suspected as disease carriers. 9th. Morphological, histological and cytological studies of diseased cane. 10th. Studies on the nature of the disease and search for a causal organism. 11th. Chemical studies of diseased as compared with healthy cane. 12th. Soil studies: Effects on the disease of different soils, soil sterilization, special fertilizers or other topical applications. 13th. Relationship with other similar diseases: A comparative study of the mosaic diseases.

In the following pages these topics will be taken up in order and the results so far obtained discussed.

1st. Field Survey—Present Distribution of the Disease.

The disease has now (November, 1919), been found in nearly all parts of Porto Rico.[1] The Yabucoa valley is the only well-marked region of the Island where at least occasional cases have not been found. This, however, does not necessarily indicate the rapid invasion of new territory. Several of the recently located outbreaks in eastern Porto Rico give clear evidence that the disease had been present for at least two or three years. The rapid spread of the disease from one part of the Island to another that is indicated by the various published reports concerning it will have to be accepted with some caution for it is evident that it has often been present in the fields for long periods without attracting attention. On the other hand certain regions, especially along the south coast, which were carefully inspected two years ago and found free from it are now quite heavily infected. For some unknown reason infection seems to have been much more active on the south west than on the northeast part of the Island.

While the disease thus occurs in practically all parts of the Island its distribution is by no means uniform. Along the north coast from Bayamón to Barceloneta it occurs on every plantation and practically in every cane field, but as yet infection is only partial, running from 1 or 2 per cent up to 50 or 60 per cent, and even considerably higher than this in some of the upland fields among the limestone hills. The fact so often noted by Stevenson still holds that the disease is much

[1] A few cases have also been found in the Island of Vieques.

more abundant in these upland valleys than in the level lands near the sea. While the disease is now a commercial factor of importance in this district having caused very considerable losses in sugar yields during the past two years, there is still an abundance of healthy seed cane available, and as shown by the years experience at Central Carmen and Plazuela (see Bulletin 22) it is perfectly possible to control it at reasonable expense.

Farther west from Arecibo to Central Coloso below Aguadilla conditions are more serious. As shown by Mr. Figueroa's article on another page of this publication, yields of sugar have fallen off about 50 per cent in this district during the past three years. Even here, however, infection is not complete. With care good seed can still be selected from certain fields, and as shown by experience at Central Coloso (see Bull. 22) the disease can be controlled by methods of eradication if faithfully carried out. Cane planting has, however, been abandoned on large areas in this district, especially among the hills, as a consequence of the losses caused by this disease.

From Rincón around the west coast to San Germán infection is almost complete. Many fields are actually 100 per cent infected, the great majority are over 90 per cent diseased, and it is doubtful if any field can be found with as little as 50 per cent of sick cane. Much of the cane from this district goes to the big central at Guánica on the south coast, so that the published statistics do not fully show the facts in regard to sugar losses, but there can be no question that they have exceeded an average of 50 per cent. Most unfortunately, almost no healthy seed cane is available in this district. This will cause a still further falling off in the near future. The possibility of establishing seedbeds of healthy cane in this district has been discussed in another publication (Bulletin 22, pp. 15–16), where the opinion was expressed that though difficult this was not impossible. The suggestion was also made that for this district the planting of the resistant kinds discussed in Bulletin 19 might prove a more practical measure than attempts at eradication.

From San Germán eastward to Peñuelas the disease is also very prevalent. It has been spreading more rapidly during the past year in this district than in any other part of the Island, but there are still localities that have largely escaped so that some healthy seed cane is still available. The coast district near Guayanilla is as yet but little infected.

North of Ponce and in the neighborhood of Juana Díaz some

fields are badly diseased, but for the remainder of the south coast infection is still local and scattering and the disease can as yet hardly be said to have had any commercial effect.

The same may be said of the east coast and of the north coast east of Bayamón though severe local outbreaks occur at Trujillo Alto and in certain fields near Carolina.

Comparatively little cane is grown in the interior of the Island, but what there is is heavily infected as far east as Cayey. Outbreaks of importance also occur at Caguas and Juncos.

2ND. METHODS OF ERADICATION.

On arriving in Porto Rico last year scattered outbreaks of the disease were being reported in the eastern part of the Island in what was supposed to be clean territory. It seems obvious that such diseased plants should be at once destroyed to prevent further contagion without waiting for a more detailed study of the disease, and this advice was always given. In many cases it proved to be impossible to impress owners and managers with the gravity of the situation, but others responded immediately and did most effective work in cleaning up and dominating the disease. Secondary infection, the spread of the disease from infected to healthy plants, was often so active that at first it was feared that this method would not be effective in regions where infection was at all general, and it was only advised for isolated outbreaks. This method of controlling the disease was first suggested in print by Stevenson in the Spring of 1918 (REVISTA DE AGRICULTURA 1: 23, May, 1918). It had, however, been previously successfully practiced by Mr. Enrique Landrón, a cane grower in the hills back of Arecibo in a district where the disease was very active and destructive. It was also being followed with good results by Mr. José R. Aponte in the low lands of Arecibo near the Central Cambalache. Some eradication work had been done on the grounds of the Insular Station at Río Piedras, and Central Fajardo was carrying out a comprehensive eradication campaign. A study of these operations and continued field observations in all parts of the Island soon caused a change of view, and in November, 1918, Circular No. 14 was published strenuously advocating this method for controlling the disease in all parts of the Island, or at least in any region where healthy seed cane could still be secured. An active propaganda was undertaken among the cane planters in favor of this method and a considerable number of them

were induced to give it a trial on a large scale. The results obtained from this work during the first season have recently been gathered together and published as Bulletin No. 22 of the Insular Experiment Station, to which the reader is referred for fuller details. Only the summary need be quoted here which states: "1st, it is considered proven that the cane mosaic or yellow stripe disease can be controlled by the method of eradication discussed in Circular No. 14, in all regions where a supply of healthy seed can still be obtained; 2nd. in regions of complete infection the establishment of healthy seed fields is necessary before a campaign of eradication can be undertaken. This is difficult but not impossible. Failure at one time may be followed by success at another under apparently identical conditions." The method of eradication referred to in the above publications consists in, 1st, planting healthy seed that has been carefully selected while the leaves are still attached. Attempts at selection after the leaves are cut are useless. 2nd, in the frequent inspection of the fields while the cane is young to pull out such cases of disease as may occur either from overlooked diseased seed pieces or from secondary infection. Of the two operations the second is really the more important, for if some bad seed is planted it is quickly detected and removed by these inspections, but the best of seed planted in an infected district and not carefully inspected and "rogued" will inevitably become contaminated through secondary infection. Inspection should begin when the young cane has made its third leaf and should be repeated two or three times a month until the cane closes. It is useless to attempt eradication in large cane except in the case of fields which are to be cut for seed. If large cane becomes infected it is usually best to wait until after it is cut and then clean up the young ratoons which should be treated exactly like plant cane. It is necessary to dig out and replant the whole stool if any of the stalks show the disease.

While it is comparatively easy and inexpensive to reduce the percentage of disease by this method to a point where it ceases to be a commercial factor, it must be admitted that complete eradication is very difficult. It is altogether probable that seed selection and the inspection of young fields will have to be continued as part of the accepted routine of cane growing. Fields will have to be protected from this disease just as they are now protected from weeds and grasses. It is not to be expected that this disease will ever be banished from Puerto Rico.

3rd. Methods of Culture Best Adapted to Badly Diseased Fields.

The unexpectedly favorable results from eradication, and the finding of immune and resistant kinds (see Bulletin 19) have greatly reduced the supposed importance of this topic. It is obviously unwise to continue cultivating diseased fields of the ordinary varieties with certain loss of from 20 to 50 per cent of yield when such loss can be cheaply avoided by the methods of eradication or by the substitution of immune or resistant kinds. The fact remains, however, that in many districts the fields are now heavily diseased, and even if the above facts were universally accepted and acted upon, which, unfortunately is far from being the case, it would still take some years before the present conditions could be radically altered. Meanwhile what sugar is made will have to come from heavily diseased fields so, during this transition period at least, the best method of treating them becomes a question of great and immediate importance. It is indeed fortunate that this crisis in the sugar industry of western Porto Rico comes at a time of such phenomenally high prices. Otherwise losses would inevitably be very severe. Now even half the normal yield of sugar may show a profit or at least avoid a disastrous loss. Circular No. 17 (issued in Spanish) entitled "Recomendaciones sobre el Cultivo de la Caña en Puerto Rico," was largely written as a contribution to this problem. It was, however, a study of the cultivation problem in general and its underlying idea was to show that by using improved agricultural methods cane can be grown not only at a less cost per acre but with the greater yields secured at a still greater saving in the cost per ton. With the continued rise in the price of sugar the immediate problem with diseased fields is not so much how to reduce costs as how to increase yields even at the expense of a reasonable increase in cost. Luckily, enough data is at hand to show that cane even when fully attacked by the mosaic will respond to increased applications of fertilizers, especially the nitrogenous fertilizers. Under present conditions, therefore, cane growers in heavily infected districts should largely increase their application of fertilizers. Instead of using 2 bags per acre, which at present is a common practice, they should use 4 bags, and on top of this a bag of sulfate of ammonia, or in the dry season nitrate of soda. As an example of yields that have been obtained from heavily diseased cane an instance can be cited on the irrigated lands of the south coast when a 20 acre field of *gran cul-*

tura (long-season plant cane) of the susceptible B–3922 variety gave 51 tons per acre last year though it was estimated as 90 per cent diseased. The same field in previous years before the disease appeared and under the same cultivation had given an average of 65 tons. Fields of 4- and 5-year Rayada ratoons on the north coast which were from 90 to 95 per cent diseased, when well cultivated and fertilized as above, gave last year as high as 20 and 25 tons of cane per acre though the year before with ordinary care and fertilizing they had only given 5 to 10 tons per acre. This shows that much can be done to increase yeilds even in heavily diseased fields by better cultivation (by which is meant stirring the land with implements, not mere surface hoeing) and by the heavily increased use of nitrogenous fertilizers. Under existing conditions these methods are certainly justified. The pressing problem of the moment is to provide a sufficient supply of cane to keep the mills of western Porto Rico grinding for the next two or three years, for it will take that length of time to dominate the disease situation there by the best of efforts either in eradication or the planting of resistant kinds.

4TH. STATISTICS OF SUGAR PRODUCTION AS AFFECTED BY THE DISEASE.

On another page of this publication Mr. C. A. Figueroa, inspector of agriculture with the Insular Department of Agriculture, gives interesting statistics showing the tons of sugar produced at each of the mills on the Island during the past three crops and the corresponding number of acres of cane harvested. The losses in sugar in the different zones in which he divides the Island agrees so closely with the percentage of disease present as to leave no doubt that this has been the determining factor. It is unfortunate that rainfall tables were not available in sufficient detail so that they might have been included also, this being the only important factor in crop production that is omitted. The severe drouth of the Summer of 1918 unquestionably reduced sugar yields. Field notes show that in August and September cane was suffering badly for want of rain in all parts of the Island, excepting in the Río Piedras-Loíza district on the northeast and the Mayagüez district at the west. In both of these districts local showers prevented serious damage. In the first of these districts (corresponding to zone 9 of the tables), where the disease only exists in a few scattered localities, the crop of 1919 was larger than that of 1917 and only slightly smaller than that of 1918. In the fully diseased Mayagüez district the loss was 32.4 per cent in 1918 and 39.4 per cent in 1919. The south coast district

(zone 5) lost 10.8 per cent. This was all chargeable to the drouth and to unseasonable rains during the crop which lowered sucrose and purity. The Arecibo district (zone 2) on the north coast suffered about equally from drouth, but here where the disease was abundant the loss reached 39.7 per cent. In the Arecibo district proper with the Aguadilla district omitted, where rainfall was more abundant, the loss reaches nearly 50 per cent. The difference between the losses from these two regions can only be chargeable to the mosaic disease. We are safe in concluding in a general way that when infection reaches an average of 60 to 80 per cent losses of sugar will be from 30 to 40 per cent.

5TH. METHODS OF NATURAL OR ARTIFICIAL INFECTION.

One of the most remarkable things in the history of this disease is the fact that so many investigators in different parts of the world have studied it for years without suspecting its infectious nature. It has been known in Java since 1892, but as late as 1910 in the comprehensive paper by Wilbrink and Ledebour (Archief V. de Java Suikerindustrie 18: 464–518) it is considered as an abnormal bud variation. No literature is at hand which shows any change in this view on the part of the Java pathologists. This view was at first accepted also by Mr. S. L. Lyon in the Hawaiian Islands, though he seems to have been the first to suspect its real nature for he soon characterized it as "an infectious chlorosis." Stevenson took up the study of the disease independently in Porto Rico in 1915 without suspecting its identity with the "Gele Strepenziekte" of Java. In fact, in his latest paper (JOURNAL DEPARTMENT OF AGRICULTURE OF PORTO RICO 4:3, July 1919,) he does not accept this identity as proven. In his earlier papers he confused the symptoms of the yellow stripe disease with those of root disease. Later he clearly recognized that he was dealing with a distinct specific malady, but he explained it as caused by degeneration or abnormal variation. It was not till the spring of 1918 (REVISTA DE AGRICULTURA DE PUERTO RICO 1: 18, May, 1918,) that he came to recognize it as an infection.

Secondary Infection.

It has from the first been recognized by all workers with this disease that it was hereditary, that cuttings from diseased stalks quite uniformly produced diseased plants. The rapid spread of the disease in Porto Rico indicated clearly that there must also be a

secondary infection by which the disease was communicated from diseased to healthy plants. In fact, a careful reading of the records of field experiments in the Java literature shows that this secondary infection was also present there, though for some reason it was not recognized. On arriving in Porto Rico careful attention was given to this phase of the subject and, as will be seen by the following extracts from field notes, it was not difficult to abundantly demonstrate its occurrence and its importance in spreading the disease.

Extracts from Field Notes on Secondary Infection.

"Los Caños, September 9, 1918.—A field of spring-planted Yellow Caledonia is very interesting. Evidently a few pieces of diseased seed were planted. The stools springing from these are much dwarfed and the leaves are all clearly infested from the ground up. In every case these stools were clearly foci of infection, as they were surrounded by a number of more recent cases in which the top leaves were infected while the bottom ones were healthy and where the growth of the plant was but little or not at all checked. In these secondary cases often only one stalk in a stool was affected."

This was the first case in which secondary infection was clearly differentiated from primary or seed infection. The effects of the disease on the Yellow Caledonia are very strongly marked and there could be no doubt as to the correct interpretation of the facts. The same conditions have since been observed in literally hundreds of fields in all parts of the Island.

"October 19, 1918.—The above field has gone from bad to worse. There are now many more cases than were observed last month. It is doubtful if over 5 per cent of the seed was infected but fully 30 per cent of the stools are now diseased."

"Los Caños, October 31, 1918.—The attempt was made to clean up part of a small triangular field of Yellow Caledonia plant cane near the mill in order to try some inoculation experiments. The cane was about 2 feet high. A little over 11 per cent of the seed was found to be diseased and was pulled up. About six weeks later (12–16–1918) 27 per cent of the stools were found to be diseased in the part of the field from which the diseased seed had been removed, while in the remainder of the field 67 per cent of the stools were diseased, the one "roguing" seeming to have reduced the number of cases by 40 per cent. These figures serve to show how rapidly the disease was spreading by secondary infection at this time."

"Los Caños, August 23, 1919.—A field of March-planted cane from carefully selected seed which came up healthy and remained so for some time now shows numerous infections on the side next a diseased ratoon field. Most of these cases are recent, the cane leaves being entirely normal up to six feet or more. This illustrates the fact that large cane may become diseased. It also shows that secondary infection has been much more active during the past two months than it was earlier in the season when the cane remained comparatively healthy though equally exposed to the disease."

"Central Cambalache, September 7, 1918.—A count in a certain field of *gran cultura* cane near the pump house showed 6 per cent infection. The cane was then about one foot high."

"December 16, 1918.—A count at the same spot showed 50 per cent of infection."

"Central Cambalache, August 22, 1919.—Numerous cases of recent secondary infection were observed in large cane six and 8 feet high."

"Central Coloso, January 2, 1919.—All fields in this district that are planted without seed selection are heavily infected with mosaic, mostly running from 75 per cent to 100 per cent diseased. For the past two years this *central* has been paying attention to seed selection. Fields planted with selected seed are showing an average of only 25 per cent to 30 per cent of disease. On one-half of a large field of *gran cultura* planted with selected seed they have tried pulling up diseased cane. It has now been gone over three times. At the first pulling 12 per cent of disease was found and removed. At the third pulling only 3 per cent of disease was found. The cost of such pulling was 45 cents to 50 cents per acre. At this time this part of the field is practically clean; almost no disease can be found. The other half of the field from which no diseased cane was pulled now shows fully 30 per cent disease."

"August 2, 1919.—Another inspection showed but little change in the above situation."

"Yauco, January 28, 1919.—Examined a field of young cane next to town which is now about three feet high. Secondary infection has evidently been very active. Judging from the present condition of diseased stools, less than 15 per cent of the seed was infected. Now 85 per cent of the stools are infected and many of the cases are evidently very recent."

"Yauco, April 10, 1919.—A field was observed here some time ago that had been planted by 'breaking banks' in a recently cut cane field but without destroying all of the old stubble. At the time of this first observation the seed cane had all germinated and was apparently all healthy. Considerable disease was, however, showing on ratoons from the old stubble between the rows. At this date many of the diseased ratoons are still growing and the plant cane now shows from 15 per cent to 20 per cent of disease clearly caused by secondary infection."

"Santa Rita, Guánica, December 31, 1918.—Mr. Bourne, who is in charge of experimental work here, has shown me a field of young B–3312 cane from which he pulled up 6 per cent of diseased plants a month ago. According to his count it now has 11 per cent of disease while an adjoining field of this kind planted at the same time from the same seed but from which no disease cane has been pulled now shows 25 per cent of disease. The 11 per cent in the one case and the 19 per cent in the other evidently represented secondary infections."

The immunity experiment conducted at Santa Rita, Guánica, which has been fully reported in Insular Experiment Station Bulletin 19, gave one of the most convincing proofs of secondary infection. Thirty healthy seeds of each of 90 varieties were planted in early October, 1918. Every third row was planted with diseased

Rayada so that each kind was uniformly and completely exposed to infection. By December 31st all of these kinds excepting the immune Kavangire had developed from 50 to 100 per cent of disease. No more conclusive proof of infection than this could be possible.

The Means by Which Infection is Carried from One Cane to Another.

While nothing can be more certain than that this is an infectious disease, that the contagion is carried from sick plants to healthy ones, we so far knew nothing as to the means by which this is accomplished. On reading Stevenson's article on this disease published in *Phytopathology* (7:418–425, 1917), the idea at once occurred to the present writer that an insect carrier was involved, as is the case with some of the other mosaic diseases and with the Curley Top of the beet. A letter was written to Mr. Stevenson making the suggestion and asking if he had any field observations that would support it. Since coming to Porto Rico this question has been constantly in mind as it is of great practical importance. At times field observations have been made that seem to strongly support this hypothesis. For instance, at the Santa Rita immunity experiment when the disease was running so rapidly in December there was an unusual abundance of leaf hoppers of several species. They literally rose in swarms when walking through the young cane. Later when the disease had become so much less active the leaf hoppers had practically disappeared. Very few of them could be found. The aid of the entomologists was early invoked for help in the solution of this problem. Extensive cage experiments were tried with a considerable number of cane insects both here and at the Federal Station at Mayagüez. Professor Smyth gives an account of his work here in another part of this publication. The Mayagüez experiments will be reported in the Annual Report of that Station. Only 4 takes were secured by Professor Smyth out of 185 experiments. Under other conditions this might be accepted as proof that insects do sometimes carry the disease, but as the chance for accidental infection is always present in Porto Rico so small a percentage of takes can not be considered as conclusive. Professor Tower of the Federal Station reports no takes at all as the result of his experiments. The case therefore still stands as not proven.

The belief, however, remains that insect carriers of some kind are responsible for the spread of the disease. This would completely account for all of the observed facts and no other suggestion has been made that can do so.

To What Distance can the Contagion be Carried in Cases of Secondary Infection?

In the field at Los Caños, where secondary infection was first clearly observed, the secondary cases were all clustered quite closely about the primary cases of seed-infected stools. In fact, most of them were in immediately adjoining stools. This seems to be the normal method of spreading, from an infected hill to those nearest to it. Instances have frequently been noted where a roadway or an irrigation ditch has acted as a fairly efficient but never as a complete barrier. Just east of the town of Bayamón there is a strip of pasture land with no cane fields for a width of perhaps half a mile. This has served as a barrier and has for three years prevented the disease from passing eastward.

On the other hand the disease is constantly appearing in new districts and at times under circumstances that make it highly improbable that diseased seed cane had been introduced. Of course in most cases new outbreaks are easily traced to diseased seed. At Central Fajardo at least two instances have been noted where a few isolated cases have been discovered in fields far removed from any other diseased cane and where no contaminated seed could possibly be traced. At Central Aguirre, too, a number of such scattered diseased stools have been found at points far distant from any known source of infection. As an instance, four diseased stools were found near together in the middle of a field near the mill. At that time no other diseased cane had been found within a number of miles of this place. A careful search of the field from which this seed cane came failed to show any sign of disease. While secondary infection thus usually takes place between diseased canes and those immediately adjoining it seems clear that at times the infection may be carried for very considerable distances.

Periodicity or Irregularity of Secondary Infection in the Same Locality.

It is a matter of common observation that at some times this disease spreads much faster that at others. Popular opinion seems to be that the spread is fastest in late summer and fall and less active in the spring. Such observations as have been recorded tend to confirm this view, but it is by no means proven that there is any such periodicity in the irregularity of infection. The point needs further study since it might have an important bearing on the time for attempting to establish seed fields in infected territory.

In August it was noted at both Cambalache and Los Caños near Arecibo that secondary infection had recently become quite active

in spring-planted fields that had largely escaped contagion earlier in the season.

The most remarkable instances of irregularity in the spread of the contagion is that recorded in Insular Experiment Station Bulletin 19 on the immunity experiment at Santa Rita. Healthy seed planted in early October was quite fully infected by the end of December, showing unprecedented activity in infection. That planted early in December never became over half infected, nine of the varieties escaping entirely, while that planted the last of December had in April only developed 6 and 8 per cent of disease. It must be remarked that this last was not interplanted with diseased cane like the others, but on the side adjoining diseased old cane it had only developed 8 per cent of disease, showing a most remarkable falling off in virulency from the condition in the same field in November and December.

Difference in the Activity of Secondary Infection in Different Localities.

Since this disease first attracted attention in Porto Rico a marked difference has been noted in its behavior in different localities. Stevenson in his various reports has frequently called attention to the fact that it always seems to spread faster among the hills of the interior than in the open level lands near the sea. That this condition still prevails has been confirmed by hundreds of observations made during the past year. At least for the whole extent of the north coast it is rare to find a field near the sea that is heavily infected, but back in the valleys among the limestone hills it is equally rare to find one that is not so infected. Even when the seed infection has been about the same the disease has spread much more rapidly among the hills. This is not so marked on the west coast, where practically all of the fields are now heavily infected.

In a general way the spread of the disease by secondary infection has been much more rapid and alarming in the territory west of a line drawn from Arecibo or Barceloneta to Ponce than it has been at any point east of that line. No cases have been observed in eastern Porto Rico where entire fields have been quickly involved, as happened at the Santa Rita immunity experiment and in the attempted seed field plantings at Los Caños and Florida. Secondary infection has occurred in all districts, but in the eastern part it has involved comparatively few plants at any one time.

It is a curious fact that in the propagating house at the Insular Experiment Station no secondary cases were observed for many

months, although healthy and diseased plants were growing side by side for three years. It is only during the last six months that a few such cases have appeared. In some cases diseased and healthy cuttings were planted in the same pot and grew with their roots and leaves intermingled for over a year with no transmission of the disease taking place. Again, diseased and healthy plants have been grown in the same wire netting cage in the open grounds with no development of secondary cases even when the cage was heavily colonized by sucking insects.[1]

When the method of natural infection is once learned these facts can doubtless be easily explained, but at present no theory can be offered that will account for them.

Artificial Inoculations.

The different mosaic diseases which have been investigated present very marked differences in the ease with which they may be produced artificially. At the time that these investigations were begun, (August, 1918,) only one successful inoculation experiment had been reported with the cane mosaic or yellow stripe disease, that by Dr. Kamerling in Java in 1902.[1] Later investigators in Java had been unable to corroborate this result since, according to Wilbrink and Ledeboer,[2] all subsequent attempts at inoculations had failed. Stevenson, too, in his various papers on this disease reports only failures in his attempts at inoculation. Since inoculations with diseased cane juice had given such unsatisfactory results the attempt was made to try out other methods by which the disease might be conveyed, the results of which are given in the notes on the following 21 experiments. It will be noted that three of these experiments, Nos. 1, 2 and 12, consisted in rubbing or otherwise lacerating healthy leaves with diseased tissue which is the successful method for conveying the bean mosaic. No cases resulted. Experiments 3, 5, 10 and 11 consisted in binding pieces of diseased tissue in contact with cut surfaces of healthy stalks. Out of 11 such attempts one was successful (see No. 5). In experiments 4, 6 and 8 bits of diseased tissue were dropped into the inrolled terminal leaf spindle so as to lie in contact with unwounded young tissue. Out of 60 attempts four positive cases resulted. In experiment No. 6, three out of five attempts were successful, the highest proportion in any of these experiments, yet the same method used on a large scale at Arecibo (No. 8)

[1] Since the above was written secondary infection has developed in some of these cases.
[1] Am. Rept. Kagok Proefstation, Java, 1902.
[2] Med. Het Proefstation, Java, 1910.

completely failed. In experiment No. 7 a hypodermic needle was thrust into the soft tissue near the terminal bud of a diseased cane and was immediately inserted near the base of the inrolled leaf spindle. There was no result from 50 attempts. Experiment No. 9 was the only one made with diseased juice exposed in the open air. It was intended as a check on the following experiments, no positive results being expected on account of the failure of this method that had been so often reported. As a matter of fact two out of seven attempts developed good cases, the one in a little over three weeks, the other in between four and six weeks.

Since the disease was spreading rapidly in the fields by secondary infection and since insect carriers seemed to be the only logical explanation of this spread the attempt was made to visualize any possible differences in method between this hypothetical inoculation by insects and the previous attempts at artificial inoculation. Since young cane tissue, and to a less extent cane juice turns brown quickly when exposed to the air it seemed possible that this oxidation might affect the vitality of the mosaic virus, and that a sucking insect flying from a diseased to a healthy plant and again feeding might regurgitate a minute quantity of the diseased juice without having exposed it to the air. To test this idea the attempt was made to extract juice from diseased cane under oil to avoid exposing it to the air. In experiments 13 and 14 the technique was faulty, still one case developed in No. 4 after only two weeks incubation. In experiment 15 a satisfactory juice was obtained which remained clear and absolutely colorless under the protective oil covering. Of the ten inoculations in this experiment five developed typical cases of disease within four weeks time, and the basal suckers also showed the disease, demonstrating the fact that the entire plant had become infected. However, experiments 20 and 21 which were designed to exactly duplicate this one gave no positive cases.

Experiments 17, 18 and 19 are sufficiently explained by the notes under each. The peculiar differences in behavior of the inoculated plants in 18 and 19 can only be explained on the supposition that the virus from the diseased bits of tissue in the test tubes had propagated in the protected healthy juice and that it produced local lesions in the leaves of the plants into which it was injected even though no cases of disease were induced. The same effect was observed to a marked degree in experiment 20 and to a less extent at various other times. These observations seem to indicate that the virus may cause

temporary local lesions even when the disease does not become generalized so as to affect the entire plant.

The prompt production of diseased suckers from the base of infected stalk shows that the entire stalk must become diseased at about the time that it first becomes evident in the terminal leaves. The leaves formed before this time, however, do not show the disease but remain normal in color until they dry up. Secondary infections in the field can usually be distinguished from seed infection, for in the latter all the leaves will be affected and the growth usually stunted while in the former the basal leaves remain normal and for a time at least growth is but little checked. Then, too, in seed infection all the stalks in the stools are involved; in secondary infection at first only one or a part of the stalks show the disease. After cutting the cane all of the ratoon sprouts from an infected stool will show the disease; that is, all of those having organic connection through the old stubble. Without this organic connection two plants, one healthy and the other diseased, may grow in close contact with their roots intermingled for months or even for years without any transference of the disease.

In interpreting the above results it must be borne in mind that in practically all parts of Porto Rico there is more or less danger of natural infection. The results of all inoculation experiments made here must always be subject to more or less doubt from this cause. As a matter of fact two natural cases appeared in that part of field No. 11 where most of these experiments were made, and several others occurred in other parts of this field. In the experiments in this field where inoculations with diseased juice or diseased tissue were made in 54 stalks, 12 of them developed the disease. Several hundred stalks were included in the area where only two cases developed from natural infection. This proportion is so much smaller that we are forced to conclude that at least a portion of these 12 cases were caused by artificial inoculations. The fact remains, however, that the successes were much less frequent than the failures, that the best results could not always be duplicated, and that the successful transfer of the disease is dependent on some factor or factors as yet absolutely undiscovered.

Inoculation Experiments.

September 12, 1918.—Insular Experiment Station greenhouse, cane plants in pots.

1. Five stalks. Young leaves rubbed vigorously with diseased leaf (as in method of conveying bean mosaic). No results.

2. Five stalks. Young leaves rubbed with tissue from near the tip of diseased stalk. No results.
3. Four stalks. Cut with slanting cut and wedge of diseased tissue inserted. No results.
4. Five stalks with bits of diseased tissue dropped into the inrolled leaf spindle of terminal bud.

On October 21 one plant in this lot was showing symptoms of mosaic and by October 26 it was a clearly developed case.

January 12, 1919.—Insular Station field No. 11, Yellow Caledonia ratoons.

5. Two stalks. Made slanting cut on side and pushed in a wedge-shaped "graft" made from the tip of a diseased cane, covered with waxed paper and tied firmly.
6. Five stalks. Dropped bits of diseased tissue in inrolled leaf cylinder of terminal bud.

On January 31 one of the "grafts" in experiment 5 was still alive the other was dead.

February 27, 1919. One of the stalks in lot 5 had developed a good case. The other remained negative.

February 11. One of the plants in lot 6 show the disease.

March 13. Two more plants in lot 6 have developed the disease and the first one shows diseased suckers at the base. The other two plants remained negative.

October 31, 1918.—Central Los Caños, Arecibo. Plant cane of Yellow Caledonia, stalks about 2 feet high.

7. Fifty stalks inoculated with needle punctures through the inrolled leaf spindle just above the terminal bud. The hypodermic needle was first thrust into the soft tissue near the tip of a diseased cane and then into the stalk to be inoculated.
8. Fifty stalks. Diseased tissue dropped into the inrolled leaf spindle.

Two or three cases of mosaic developed in each of these lots but as natural secondary infection was active fully as many cases developed in the adjoining untreated rows. The result was thereafter negative.

January 31, 1919.—Insular Station field No. 11. Yellow Caledonia ratoons.

9. Inoculated 7 canes (about 2 feet high) with hypodermic

needle, using juice from diseased cane pressed out by hand laboratory mill. The needle was thrust into the leaf spindle above terminal bud.

Februar 23, 1919. One of these cane has developed mosaic.

March 11, 1919. One more case has just developed the disease. The remaining 5 stalks remained negative.

January 20, 1919. Insular Station greenhouse.

10. "Inarched" a diseased and a health cane (both growing in pots) by cutting away about one-third of each cane for a distance of 3 inches and binding the exposed surfaces together.

The canes lived for some months but the healthy cane did not contract the disease.

January 31, 1919.—Insular Station Field 5. Spring plant cane about 7 feet high with well-developed stalks, variety P. R.-271.

11. Prepared 4 stalks by cutting out a block of cane one inch long and one-quarter to one-third inch thick with a bud in center. The space was filled with a similar block with bud in center cut from a diseased cane which was firmly tied in place and well covered with waxed paper.

These diseased blocks remained alive for some weeks but no cases resulted.

January 31, 1919.—Same field as above.

12. Two canes inoculated by placing a diseased leaf in contact with a healthy one and boring the point of a penknife through the two leaves so as to blend the tissues. No results.

January 31, 1919.—Insular Station. Field 11. Yellow Caledonia ratoons.

Since freshly cut cane tissue and cane juice oxidizes quickly with change of color when exposed to the air it was thought that this oxidation might destroy the contagion. The attempt was made to protect the juice from air by crushing pieces of cane in a morter which was partially filled with olive oil. It was difficult to get out the juice in this way and it seemed to emulsify to some extent with the oil.

13. Ten stalks were inoculated with the mixed oil and juice.

On February 8 these plants showed extensive yellow

oil-soaked areas both above and below the needle pricks. These shaded out into mottled areas and stripes looking much like incipient cases of the disease. Some of these stalks finally died from the effect of the oil but none of them developed mosaic.

February 1, 1919.—Insular Station Field 11. Yellow Caledonia ratoons.

14. Ten more stalks were inoculated with juice pressed out under gas-engine cylinder oil in a mortar. This did not emulsify but so little juice was secured that water was added in order to suck it into the needle without oil.

February 15. One typical case had developed and was photographed. The other nine remained negative.

15. February 8, 1919. Prepared juice from diseased cane without exposure to air by taking bits of the cane in strong pincers and holding them under gas-engine cylinder oil in a beaker while pressing out the juice.

Inoculated 10 canes about 2 feet high, Yellow Caledonia ratoons, field 11, with this juice by inserting the hypodermic needle into the leaf spindle just above the terminal bud.

Also inoculated 7 canes in same field by inserting the needle into the midrib of young leaves. These last gave only negative results.

March 7. Five of the ten canes inoculated in the leaf spindle on February 8 now show pronounced cases of mosaic. In three of them basal suckers are also showing the disease thus demonstrating that the entire plant quickly becomes infected.

16. February 9, 1919. With the oil protected juice prepared yesterday 3 inoculations were made in the leaf spindle of terminal bud in spring-planted P. R.-271 cane 6 feet high in field 5 C. When the new leaves developed conspicuous yellowish areas appeared both above and below the needle pricks. For some time they were regarded as incipient cases but these symptoms finally faded out and no infection followed.

17. February 12, 1919. In order to test the possibility of propagating the mosaic infection outside of the cane plant, juice from healthy cane was expressed under cylinder oil to protect it from oxidation. This juice was pipetted to test

tubes in which half an inch of oil had been placed and thus protected from contacted with the air the tubes were sterilized in the autoclave. When cool bits of tissue cut with flamed scalpel from near the growing point of diseased cane were forced under the oil in one series of tubes and corresponding pieces of tissue from healthy cane were placed in another series as checks.

The juice remained bright and clear in both series for many weeks, the only difference noted being that the bits of healthy tissue mostly sank to the bottom of the tubes while most of the bits of diseased tissue floated between the juice and the oil.

18. March 9, 1919. Inoculated 10 canes in the Yellow Caledonia ratoons, field 11, with juice from one of the tubes prepared on February 12 in which a bit of diseased cane was suspended and 10 more from one of the check tubes
19. which contained a bit of healthy tissue.

On March 15 it was noted that a number of the plants in the first series were showing local discoloration near the needle punctures but that no such discoloration could be noted in the check series.

On March 26 trifling local discoloration was noted in two plants in the checks series the remaining 8 showing only dried-down needle pricks. Five of the ten in the first series, on the contrary, showed mottled discolored areas three or four inches in extent about the needle pricks and they were noted as incipient cases. Three of the plants showed slight local discoloration only, while the other two were intermediate, but it was thought at the time that they would develop good cases. As a matter of fact, after being visible for some weeks the color finally faded out from all of these areas and no cases resulted in either series, but there can be no question as to their different behavior.

March 21, 1919.—Caledonia Ratoons, field 11.

20. Inoculated 10 canes with oil-protected juice from diseased cane (prepared as on February 8) injected into leaf spindle just above the terminal bud.

On March 29, it was noted that nine of the above inoculations showed pronounced local discolorations in the neighborhood of the needle pricks. These discolored areas

presented much the appearance of the true mosaic and in some cases they could be traced for three or four inches above and below the needle pricks. As the successful inoculations made in this same manner on February 8 had also shown these preliminary symptoms it was confidently expected that nine positive cases would result. However, after remaining visible for some weeks the discolorations finally faded out and no infections followed.

21. March 21, 1919. Six inoculations were also made with the same oil-protected juice in young ratoons in pots in the greenhouse. No cases resulted.

The results of inoculation experiments made at this station by the pathologist, Mr. Julius Matz, will be found on another page. He also had only occasional successes in communicating the disease. Details of the cage experiments with insects suspected as being carriers of the disease are given by the entomologist of the Station, Mr. E. G. Smyth, at another place in this publication. The verdict here will simply have to be "not proven."

The situation under this heading may be summarized as follows:

1st. Sugar-cane mosaic is hereditary, being uniformly carried in diseased cuttings and always appearing in plants grown from them.

2nd. Secondary infection exists in nature and is often responsible for the rapid spread of the disease to previously healthy cane. Ordinarily it is nearby stools that are thus affected, but occasionally the disease seems to be carried for long distances. Secondary infection is more active in some localities than in others. It is also more active at some times than at others in the same locality. Insect carriers of the disease have been suspected, but so far this is not proven.

3rd. Successful artificial transfers of the disease have been made by various methods but the results have not been uniform and complete failure often results.

6TH. RESISTANCE AND IMMUNITY—VARIETY STUDIES.

The importance of this topic was early recognized and field observations were made on the behavior of the varieties to the disease at all opportunities. A few notes on the supposed resistance of certain kinds had been published by Stevenson and by Cowgill. It was, however, the finding of an apparently immune variety, the Japanese [1] Kavangire, in the experimental plots at the Federal Station

[1] Since the above was written the publication of a paper on this variety by Dr. Cross of the Argentine Station at Tucuman shows that this is a north Indian cane but that it has never been cultivated in Japan.

at Mayagüez, that focussed attention on this phase of the problem and indicated the necessity for an immediate comprehensive study of varietal resistance. Evidently plots for this purpose would have to be located in a diseased district and would have to be so planted as to subject each kind to an equal chance for infection. It was at first proposed to the Federal Station at Mayagüez that they make such a planting. No land for the purpose being available, however, an arrangement was made with the Central Guánica for putting in such an experiment at their trial grounds at Santa Rita under the supervision of Mr. Bourne, who was then in charge of their experimental work. A total of 171 kinds were planted in rows of 30 seed pieces each and every third row was planted with diseased Rayada in order to secure a uniform chance for infection. The results of this experiment were published in Insular Station Bulletin 19, where full details are given. They may be summarized by saying that the full immunity of the Kavangire cane was proven. Convincing proof of periodicity or irregularity in the activity of the infection was secured. From this cause 9 of the kinds failed to become infected. Of the remainder 40 varieties were clearly more susceptible than the Rayada; that is, they showed greater injury when attacked by the disease; 42 kinds were listed as about equal to Rayada in this respect; while 73 kinds made a somewhat better showing than the Rayada. The bulletin, however, fails to call attention to the fact that the Rayada rows are all from infected seed and that this constitutes a heavy handicap in comparing them with the other kinds, which were all secondary infections. If the experiment is continued another year the Rayada will make a decidedly better showing in the ratoon crop, which will practically all come from infected stubble. Of these 73 kinds 24 were listed as being especially resistant, or perhaps the better word would be tolerant, to the disease since, though fully infected, their growth was but little affected. Of these the best in order named were given as Java 56, Java 234, and G. C.–1313 (Guánica Central seedling). The name of the first-mentioned and most promising of these kinds needs a further word of explanation. In a footnote on page 6, Bulletin 19, the statement is made that "this is the J.–36 of the Argentine but is not the true J.–36 of Java." This was said because of the description by Noel Deerr (Cane Sugar, p. 41) of Java 36 (P. O. J.) which calls for a green cane. The finding of the very full description of this variety by Jesweit (Med. V. Proefs. V. Java-Sukerindustrie Series 1917 (No. 12, p. 6) shows conclusively that this is our cane and that its name is Java 36

Java. It is one of Kobus' seedlings having the North India variety (P. O. J.), the initials standing for words meaning Proofstation East Chunnee as staminate parent and the Black Cheribon (Louisiana Purple) as pistilate parent. The description of the green variety quoted by Deerr properly belongs to Java 36 (Bouricius), a cane belonging in a different series of seedlings and from different parentage. The careless use of the initial J. to indicate any seedling cane from Java is an error, since several numbered series of seedling canes have been produced in Java. "J–228" and "J–234" of Bulletin 19 should also be written J–228 (P. O. J.) and J–234 (P. O. J.), since they, too, are seedlings by Kobus from the same parentage. The resistance of these kinds clearly comes from the North Indian ancestry. The Japanese varieties also all came originally from northern India. Observations on another Japanese cane, the Zwinga or so-called "fodder cane" of the southern States, indicate that it, too, is immune to the mosaic, though it has not been subjected to such convincing tests as the Kavangire, which it closely resembles but from which it may be easily distinguished.

7TH. ECOLOGICAL SURVEY OF THE INSECTS INHABITANTS OF CANE FIELDS.

The importance of this topic was based on the supposed existence of some insect carrier for the mosaic disease. Field observations on cane insects have been made wherever possible and many scattered notes have been made, but the personel has been lacking for a comprehensive study of this subject. The paper on cane insects on another page by Mr. Smyth brings out many new facts and serves as an important contribution to the subject. It is indeed remarkable that the minute spring tail, which is so exceedingly common on cane leaves and which is responsible for so much of the minute spotting which is often confused with the mosaic, should never before have been recorded as a cane pest. This emphasizes the need for work in this interesting field aside from the chance discovery of a carrier for the mosaic.

8TH. CAGE EXPERIMENTS WITH INSECTS SUSPECTED AS DISEASE CARRIERS.

Much painstaking work was done on this topic both here at the Insular Station by Mr. Smyth and at the Federal Station at Mayagüez by Mr. W. V. Tower. The details of part of this work appears on other pages of this publication. Very unexpectedly, no results

at all were secured at Mayagüez and the cases of disease following colonization with insects here were so few as to be within the possibility of accidental or natural infection. The case therefore is not proven. This, however, does not preclude the possibility that an insect carrier or insect carriers exist. In fact, this hypothesis is the only one so far suggested that will account for the observed facts in the spread of the disease.

9TH. MORPHOLOGICAL, HISTOLOGICAL AND CYTOLOGICAL STUDIES OF DISEASED AS COMPARED WITH HEALTHY CANE.

A reading of the literature of this disease is sufficiently convincing that this subject is in need of study. The statement is found that infected leaves contain less chlorophyll than normal ones, but it is not clear whether this is because less is being elaborated by the chromatophores, as in etiolation from shade, or whether these bodies themselves are lacking. In discussing the stem cankers Stevenson says (Ann. Rept. 1917, p. 47):

> "Penetration of the tissues is never very deep, hardly more than from one to two millimeters at first, and is often limited to a few layers of cells only. The affected tissues are red, but not different in shade or other characteristics from similar effects produced by other causes. There are no other internal symptoms except as noted below."

In the paragraph to which this last remark seems to refer he adds:

> "In addition to the stunting or dwarfing of the stools there is a shrinking of the internodes of the individual stalks. This is especially pronounced in what might be determined third-phase cases or those in the last stages of the disease. Such stalks are almost completely lacking in juice, the limited amount of pith tissue formed being of a rubbery consistency."

This practically completes our previous knowledge of conditions within the diseased plants, so that the paper by Mr. Matz on another page of this issue constitutes a decidedly new contribution. The present writer has followed Mr. Matz's work with great interest and has seen his preparations. While the study is a preliminary one and no sweeping deductions are as yet to be drawn from it, the interesting fact remains that certain cells or groups of cells in the parenchyma of diseased stalks are filled with a peculiar granular protoplasmic substance. These plugged cells can be detected in very young tissues. They may occur at any point within the center of the stalk, and are also found in the leaves and leaf sheaths. The

cankers are formed by the final breaking down of these abnormal areas. A somewhat similar condition has occasionally been found in injured discolored tissues of canes that were free from the mosaic disease, but this abnormal condition can be distinguished from the one under discussion. These groups of abnormally filled cells at least furnish a physical basis for the disease, and that is something which has heretofore been lacking. The appearance of the abnormal material filling these cells is so much like that of a plasmodium that eager search was made for some indication of swarm spores or other fruiting bodies, but for a long time without result, the only change noted being that in the older tissues the granular appearance of the plasma became more marked as though it were becoming multinucleate. At length in an old cankered stalk that had become partially dried by lying two or three weeks in the laboratory it was observed that the entire plasmodium had broken up into minute, irregular, rod-shaped bodies, some of which showed X and Y forms. These minute rods were motile, revolving slowly on their axes so that the whole mass was clearly agitated but there was little active movement of translation. Taken alone, these bodies would unquestionably be called bacteria, but ordinary bacteria are not formed from a plasmodium that exists in that form for weeks and months. A similarity to the parasitic genus *Plasmodiophora* among the slime moulds is clearly suggested, but these motile bacteria-like bodies are very different from the regular globose spores of that genus. Perhaps the nearest parallel is furnished by the nodule-forming organism of the *Leguminosæ,* where the first stage is a zoogloea mass within the young root cells, but this very early breaks up into the irregular rods that in shape and behavior closely resemble those of the organism under discussion. For the present it seems best to withhold any positive statement as to its true systematic position. No causal relationship with the mosaic disease has as yet been proven, but at least the presence of this peculiar organism seems to serve as a diagnostic character of importance and one that has heretofore been overlooked.

10TH. STUDIES ON THE NATURE OF THE DISEASE AND SEARCH FOR A CASUAL ORGANISM.

The close relation of this topic to the last one is easily recognized. The nature of the disease has also been discussed in the paragraphs on natural and artificial infection where we have seen how persistently it has been regarded as a degeneration, bud variation or

abnormality. Nothing can be more clearly proven than that it is an infection, so these earlier views now have only an historical interest.

The proper name to be applied to this disease demands a moment's discussion. The Dutch investigators in Java have called it "Gele Strepenziekte," or as literally translated by Hawaiian writers, "Yellow Stripe Disease."[1] This is clearly the earliest name applied to it in scientific literature and if priority is insisted on it must be recognized. Unfortunately, it is misleading since in the great majority of cases no striping effect is produced. Stevenson's name of "Mottling disease" is much more truly descriptive. In its Spanish form, "Matizado" it has come to be the universally recognized term for it in Porto Rico. The present writer is responsible for adding still another name, "Sugar-Cane Mosaic" (Insular Station Circular 14:6), but he has always used it as a descriptive phrase in connection with one or both of the other names, intending by so doing to convey some idea of its general nature and relationship. The mosaic diseases are an obscure class of poorly understood disorders on which there has come to be a considerable literature. While there are well-marked differences among them they seem to have much in common. Whichever term we may prefer as the specific name of this cane sickness the fact will remain that to the best of our present knowledge it is a mosaic disease.

The real nature of this class of disorders has been the subject of much discussion. Very divergent views have been held regarding them and it must be admitted that even at the present day pathologists are by no means in full accord regarding them. A few years ago it was the fashion to ascribe them to an abnormal secretion of enzymes produced in some inscrutible manner by a change in the internal functions of the plant. They were considered functional diseases. It is easy to understand that sudden changes in environmental conditions might induce functional disorders. It is well known, in fact, that this is the case. Many such invironmental diseases are known but none of them are contagious. No satisfactory explanation has ever been given of how a disease may be conveyed from a sick plant to a healthy one except by means of living parasitic organisms. The advocates of the above theory have therefore always sought to minimize the evidence of infection and to account for the

[1] Stevenson even in his latest paper, *Journ. Dept. Agric. of Porto Rico,* III (No. 3), July 19, is unwilling to admit that the identity of this with the Porto Rican disease is proven. The fact that so many outbreaks in different parts of the world can be traced to importations of Java seed cane together with the internal evidence from the descriptions and illustrations in the Java literature leaves no possible doubt in the mind of the present writer that Lyon is absolutely correct in considering them as identical.

spread of these diseases on the ground of inherited predisposition or, in other words, by degeneration and abnormal bud variation. As we have seen in the foregoing pages, the evidence of the infectiousness of this cane disease is overwhelming. It is equally convincing in regard to all of the other mosaics that have been studied. Recently this has lead to the rather wide acceptance of the idea that they are caused by ultramicroscopic parasites—the hypothesis held to-day by human pathologists to account for contagious diseases such as smallpox and various others, for which no parasites have yet been discovered. It is certainly true that no ordinary bacteria or fungus hyphae can be found in the diseased cane tissues except those that are clearly secondary in very old cankers, and no such organisms can be cultivated from them by ordinary laboratory methods. It can be safely affirmed that the mosaic diseases are not caused by ordinary bacteria nor by filamentous fungi. Of course, we know that chemical atoms and molecules are far too small to be visible under the microscope. There is nothing impossible in the conception of living bodies so small, that like the atoms and molecules we can only know them by their effects. On the other hand, we may have the alternate conception of a naked-celled amoeboid parasite not so small but so similar in structure to the other protoplasmic contents of the plant cells that it has so far escaped detection. A living virus seems to be necessary in order to account for the spread of infectious diseases. When we cannot demonstrate one we are forced to imagine one. It is not yet proven, that the plasmodium-forming organism referred to under the last heading as having been uniformly found by Mr. Matz in mosaic disease tissue, is the real cause of the sugar-cane mosaic though the evidence so far points strongly in that direction. If this proves to be so this will be a case of a different kind where a comparatively large and conspicuous organism has been overlooked by a long series of pathologists simply because it is a strict parasite of an unusual kind and one that cannot be grown on ordinary culture media.

The general symptoms of this disease have been described so often that it seems unnecessary to repeat them here. Mr. Matz's paper shows that the lesions leading to the formation of cankers when near the surface of the stalk are also deep seated, and on their final collapse leave internal cavities which account for the light weight and lack of juice in the infected canes. These studies also show that the stuffed parenchyma cells that constitute the earlier stages of

these lesions can be detected in the very young tissue of the stalk and also in the young tissues of the leaf sheaths. In many varieties the more superficial ones may be detected with a hand lense in the very young and still soft internodes. This paper also shows that in the discolored areas of the leaves there is a lack not only of chlorophyll but of chloroplasts. These points should be added to the descriptive diagnosis of the disease. The fact should also be restated in this connection that in cases of recent infection the disease often appears in basal suckers within a few days of its appearance in the young terminal leaves although the matured leaves farther down on the stalk never develop the diseased symptoms. This shows that the infection has really invaded the entire stalk and the growing point of all the buds almost simultaneously.

Attention should be more forcibly called to other leaf spottings and discolorations that may be confused with the mosaic symptoms. In the course of these investigations the fact has developed that cane foliage is often attacked even in the unrolled bud spindle, by great numbers of several species of minute insects and mites which cause very considerable damage through the minute spotting of the leaves. Singularly enough, this damage has escaped the attention of the entomologists and some of the species are listed as cane insects for the first time in Mr. Smyth's paper on another page of this issue. Later these minute discolored specks often become invaded and enlarged by one or another facultative fungus parasite. We have a considerable literature on cane leaf spots as caused by fungi but there is little in print to show that in practically all cases the inciting cause of the spotting was the puncture of some minute insect. This however is the fact. In the later stages when invaded by fungi these leaf spots are sufficiently different from mosaic and there is no danger of confusing them. Many times, however, especially in old fields where the foliage is yellowish from root disease and bad cultivation, this minute insect spotting on the young unrolling leaves is sufficiently like the first indications of incipient mosaic infection to be very confusing. The mosaic disease, if present, however, very soon shows itself unmistakably and there is seldom any practical difficulty in distinguishing it. In examinations to determine the presence of mosaic disease attention should always be given to the youngest leaves, and especially to those not fully unrolled.

In all of his writings on the subject Stevenson has insisted on a three-year phase for this disease and he only describes the cankers

as occurring in the third or final stage. The present writer has been unable to confirm this view. The response of different varieties when attacked by the disease is so different that no general statement of this kind is possible. For instance, in the Santa Rita immunity experiment the Yellow Caledonia variety which was attacked by secondary infection soon after germination, developed serious stem cankers within six months and a number of the attacked stools were actually killed before the final inspection at the end of ten months. This was an extreme case but it is only one of many that show that the three-year phase idea is untenable. There is, however, usually a well-marked difference in the effect on the plant between primary infection from a diseased seed piece or diseased stubble and cases arising from secondary infection. In the former cases, except with the very resistant kinds like Java 36 (P. O. J.), which are scarcely affected by the presence of the disease, there is a pronounced dwarfing and all of the leaves on all of the shoots are equally affected. In secondary cases there is at first but little dwarfing and only one or a few of the stalks in the stool are involved. It is true that the course of an invasion by the disease has often resulted in what amounts to a three-year phase. In the first year a few scattered cases have appeared, perhaps by secondary infection but only too often from the criminally careless use of infected seed for replanting previously healthy ratoons. By the second year these cases have spread quite widely by secondary infection, but being secondary cases with a semiresistant variety like Rayada the damage has been comparatively slight. This would correspond to Stevenson's secondary phase. The following year with a considerable percentage of cases from diseased stubble the dwarfing effect would be much more obvious and losses of weight from cankered stalks would be much greater. With susceptible varieties like Otaheite, Cavengerie and Caledonia, this may end the productive life of the field, but as seen on page 9, fields of Rayada are known that though fully diseased for years at the fifth cutting gave as high as 20 tons of cane per acre in response to better cultivation and fertilization. That many diseased fields became valueless after the third year is freely admitted, but that the disease presents any approach to a three-year cycle must be strenuously denied.

11TH. CHEMICAL STUDIES OF DISEASED AS COMPARED WITH HEALTHY CANE.

The papers by director Colón and by Mr. López on other pages

of this issue are contributions to this subject. While the investigations have so far only been of a priliminary nature, they show no very striking chemical changes as a result of the presence of the disease. The earlier published statement that the diseased cane was very objectionable in the mill can refer only to those extreme cases where the cane became badly cracked. It seriously affects the quantity of the juice rather than its quality. Very much more than half of the cane ground this last year at all of the mills from Arecibo westward was diseased. The statistical report on sugar manufacture in Porto Rico issued by the Department of Finance gives for the 1919 crop not only the tons of sugar produced at each of the mills but the number of tons of cane ground. By averaging these figures for eight of the mills in the worst infected district we find that it required 9.88 tons of cane to produce a ton of sugar. At five representative mills from the eastern district where there was little or no disease the average was 9.32 tons of cane to the ton of sugar. So small a difference as this could easily be accounted for by differences in mill equipment and extraction or by weather conditions in different parts of the Island.

12TH. SOIL STUDIES; EFFECTS ON THE DISEASE OF DIFFERENT SOILS, SOIL STERILIZATION, SPECIAL FERTILIZERS, TOPICAL APPLICATIONS.

But little has been done under this heading. Field observations in all parts of the Island demonstrate that soil conditions have nothing to do with the spread of the disease. It is to be found on all types of soil on which cane is planted. Emphatically it is not a soil disease. On the other hand, good soils, abundant nitrogenous fertilizers and good cultivation while they will not ward off the disease will increase yields of cane that has become diseased. Many planters believe that liming the soil has some effect in preventing the disease. So far we have no exact facts in support of this theory.

An experiment was planted with the kind coöperation of Russel & Co. to test the effect of lime and sulfate of iron in combination with different fertilizers as follows:

Plot 1 at rate of 400 pounds tankage per acre.
Plot 2 at rate of 800 pounds tankage per acre.
Plot 3 at rate of 1,200 pounds tankage per acre.
Plot 4 check, no fertilizer.
Plot 5 at rate of 800 pounds tankage and 400 pounds sulfate of potash.
Plot 6 at rate of 800 pounds tankage and 400 pounds sulfate of amonia.
Plot 7 at rate of 800 pounds tankage and 800 pounds acid phosphate.

These plots were cross divided by three bands one of which received at rate of 4 tons of lime per acre, one 500 lbs. iron sulfate and the other no application. The intention was to have these plots planted with diseased Rayada seed cane. By some accident the seed selected was only about half diseased so that the cane came up very irregularly infected. The soil, too, developed unexpected irregularities in fertility before the fertilizers were applied. It is not expected, therefore, that this experiment will give conclusive results. At this writing it can only be said that the heavy applications of fertilizer have given a very heavy growth, but no very specific results can be noted from the different treatments. These plots will be cut and weighed when fully mature.[1]

13TH. RELATIONSHIP WITH OTHER SIMILAR DISEASES—A COMPARATIVE STUDY OF THE MOSAIC DISEASES.

Nothing has been done under this topic.

[1] A later inspection indicates a deleterious effect from the sulfate of iron and no appreciable effect from the lime.