The Journal of the Department of Agriculture
OF PORTO RICO

Published Quarterly: January, April, July and October of each year.
MELVILLE T. COOK, EDITOR

VOL. XIV APRIL 1930 No. 2

A NEW MECHANICAL METHOD FOR ARTIFICIALLY TRANSMITTING SUGAR-CANE MOSAIC

By FRANCISCO SEÍN, JR.

The study of sugar-cane mosaic transmission and its insect vectors started at the Insular Experiment Station in 1918 by Smyth and carried on by Wolcott and Chardon has been continued by the author since 1922 up to the present date.

For the purpose of obtaining data on how the virus of sugar-cane mosaic is introduced by the insect vector into the tissues of the healthy plant, a new method of artificial inoculation has been devised which due to its simplicity and efficiency has proved very helpful.

A very few notes on this new method and its operation were advanced in the Report of the Division of Entomology, Annual Report of the Insular Experiment Station of Porto Rico, 1927–1928. The Director of the Insular Station, Mr. R. Fernández García, has informed us that he reported it at the Third Congress of the International Society of Sugar Cane Technologists held in Java in June 1929.

Mr. Authur H. Rosendfeld, in the February, 1930, number of the *International Sugar Journal,* commenting on the Java Meeting of the International Society of Sugar Cane Technologists when refering to the paper on "Mechanical Transmission of Mosaic" by Miss G. Wilbrink writes that:

> "During the discussion of this paper it was brought out that Mr. F. Seín had developed a simple method for the mechanical transmission of sugar-cane mosaic at the Porto Rico Insular Experiment Station for use in the testing out of varieties as regards natural resistance to the disease. The device seems to be a very simple one, consisting of a number of pin points at the end of a small handle, and, with this device, Mr. Seín has succeeded in a great many cases in transmitting mosaic from an affected plant to an unaffected one simple by pricking the leaves of the affected plant first and then proceeding to do the same thing with the leaves of the healthy plant."

As stated above by the author, his purpose in devising a new method of artificial inoculation was the study of how the causative principle of the disease is introduced into the tissues by the insect

vector. The practical application of the new method in sugar-cane breeding work and in the study of the disease itself came up as secondary to the entomological problem which was contemplated. The reader will also become aware that the description of the method, the instrument used and the technique followed by the author are a little different from the description Mr. Rosenfeld has kindly advanced.

Since various mechanical methods for the artificial transmission of sugar-cane mosaic have been devised by previous investigators, and since there are divergent opinions on their efficiency, a review of the experimental work on this subject is desirable.

REVIEW OF THE LITERATURE ON THE TRANSMISSION OF SUGAR-CANE MOSAIC BY MECHANICAL MEANS

The mosaic disease of sugar cane was first reported from Java in 1890 by Dutch investigators who became convinced after unsuccessful attempts to transmit it artificially, that they were dealing with a bud variation rather than a disease. Kamerling (**9**), 1890, in Java reported successful infections obtained, we judge, by rubbing the lacerated leaves of healthy cane plants with the juice pressed out of mosaic plants. In the same publication Kamerling admits the occurrence of disease in his control plants and later Dutch investigators discredited his experiments. Kobus (**10**), van der Stok (**19**) and Wilbrink and Ledeboer (**21**), failed to produce the disease repeating Kamerling's experiments.

In America, Stevenson (**18**) was the first to work on sugar-cane mosaic. He began his studies at the Insular Experiment Station at Río Piedras, Porto Rico, in 1916, and continued them until 1919 but failed in all his attempts to transmit the disease artificially. Stevenson attempted to produce the disease by inoculating cane with pure cultures of several kinds of fungi; by hypodermic injections of the juices extracted from mosaic cane tissues; by placing the juice in holes made in the stalks; by inserting small bits of diseased tissue into various parts of healthy canes; and by rubbing the growing tips of the stalks after a diseased tip had been crushed in the fingers.

Tower (**20**), 1919, of the Federal Experiment Station, Mayagüez, P. R., failed to transmit sugar-cane mosaic by crushing and rubbing mealy bugs from diseased cane on developing buds and shoots of healthy canes, and by forcing the crushed bugs into punctures made in the buds and shoots.

Earle (6), 1920, at the Insular Experiment Station, P. R., performed a very large number of experiments in which he exhausted his ingenuity in the attempt to transmit the disease artificially and to reproduce the work of the unknown, but suspected insect vectors. The experiments were performed in the open and thus there was always the possibility of error. The percentage of infections in his inoculated canes was exceedingly low but Earle believed that a portion at least of these cases were caused by artificial inoculation. But he comments: "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." Earle tried the following methods: rubbing or otherwise lacerating healthy leaves with diseased tissues; binding pieces of diseased tissue in contact with cut surfaces of healthy stalks; dropping bits of diseased tissue into the unrolled terminal leaf spindle and injections of the extracted juices by means of a hypodermic syringe. He also made a very interesting experiment in which a hypodermic needle was thrust into the soft tissue near the terminal bud of a diseased cane and then immediately inserted near the base of the unrolled leaf spindle of a healthy cane plant. There was no transmission in fifty attempts. This experiment although unsucessful is of interest to us because it is a departure from the attempt to imitate the work of a sucking insect. In a further experiment, where the attempt was to imitate the insect by extracting the juices from mosaic tissues and injecting them into healthy plants without exposure to the air, Earle was more successful. As this method was used later by Brandes and Bruner it is desirable to explain that upon the suggestion made by Mr. F. A. López Domínguez, Prof. Earle and Mr. E. D. Colón extracted the juice from the tender parts of mosaic cane plants by grinding with a pestle under a layer of mineral oil in a porcelain mortar. The juice was then taken up with a syringe, the needle of which was inserted through the oil layer, and injected immediately into healthy cane plants. With this method Earle was able to obtain five infections out of ten inoculated plants. On repeating the experiment two successive times, however, he was unable to obtain a single infection.

Matz (13), 1920, working also at the Insular Experiment Station, P. R., carried on an extensive series of complicated experiments in the endeavor to transmit sugar-cane mosaic artificially. His percentage of infection was extremely low and he concluded that "the exact method to insure takes is not known as yet". Matz tried the fol-

lowing methods: healthy and mosaic cane plants set in the same pots; healthy and diseased seed pieces split in half, a diseased half and a healthy half fastened together and then planted; healthy seed pieces watered with water in which diseased cane was allowed to stay for some time; and buds from healthy seed pieces inserted in diseased seed pieces and vice-versa, but no transfer of the disease took place.

Matz performed several experiments with juice pressed out of mosaic cane tissues exposed to the air. Healthy cane stalks in three pots inside the greenhouse were cut back leaving stumps about four inches above ground. There were one or more shoots about six inches high emerging from the base of the stumps. The juice was injected with a hypodermic needle into the stumps near the surface of the ground. The three shoots in one pot developed mosaic. The rest remained healthy. The experiment was repeated with twenty plants leaving twenty uninoculated as check. Two of the inoculated developed mosaic.

In another experiment ten cane plants were cut back, only a little above the growing point, five were inoculated in the cut surface of the top by injecting diseased juice with a hypodermic needle and five were left as checks. All remained healthy.

Next, twenty-five healthy stools in the greenhouse were cut back as in previous experiments and eight were inoculated with diseased juice and in addition, pieces of diseased cane were forced into small holes in the stems. All twenty-five plants remained healthy and *one of the checks developed the disease.*

The successful inoculations obtained in the first experiments were performed on cane more mature than that used in the last. To test this point eighteen seed pieces of mature Cristalina cane were cut to one or two eyes, twelve were inoculated near the base of the bud by boring a hole into the seed piece three-fourths inch deep and directly into it was pressed juice from diseased cane and six were inoculated in the same way with healthy cane juice. At the same time 35 Cristalina stools in a field that had just been cut were inoculated with juice in the stubble near the bases of sprouting buds. In both of these last two experiments not a single positive case developed. Matz remarks: "It was thought that by bringing in contact the cut ends of the vascular systems of diseased and healthy cane a transmission of the disease might take place. But no infection occurred". This is very interesting when compared with what Bonazzi reported in Cuba and Cook in Porto Rico later on.

The case of infection in his checks that we have specified is one of the several reported by Matz.

Smyth (**17**), 1920, at the Insular Station also performed twenty-seven experiments which consisted in crushing the juices of mosaic cane tissues into the leaf tissues of healthy cane plants, using very young, vigorous plants, and seventeen experiments which were attempts to transmit the disease by spreading quantities of finely-cut-up diseased tissue, either in juicy or dry condition, over the healthy plants and over the soil immediately surrounding them. All these forty-four tests gave negative results.

Lyon (**12**), 1921, in Hawaii, conducted numerous experiments in attempts to convey the disease by artificial means. The media most employed were juices extracted by pressure from various parts of affected canes, but more particularly from tissues adjacent to the growing point of the stem. Inoculation was attempted by applying these juices externally to all parts, and introducing them internally at various points in the stem, eyes and spindle with a needle-syringe. In no case did the disease appear on a high percentage of treated canes or canes from treated cuttings, and in every experiment it appeared on such a number of shoots in the checks as to invalidate the evidence of artificial infection. Most of these experiments were conducted under open field conditions and in a restricted area where the disease was at the time present in nearby canes. In the few experiments where canes grown in tubs and carefully isolated in a glass house were employed, only negative results were obtained.

Brandes (**1**), in 1920, performed his well-known experiments at Washington, D. C., in which he showed by eleven successful inoculations that mosaic could be transmitted artificially but under great difficulties. Bruner (**4**) remarks: "since Brandes' limited experiments were not repeated under the same or more natural conditions it is not sure that the same results could be duplicated."

In Brandes' experiments, "virus" was obtained for artificial inoculation by two methods. Cell sap from young leaves, designated as virus No. 1, was obtained by grinding the young, tightly rolled leaves of diseased Rayada cane in a food chopper and straining through several thicknesses of cheese cloth. It was used undiluted for inoculation immediately after being prepared. Virus No. 2, consisted of cane juice from the youngest joints, including the growing point. To prevent oxidation this was pressed out under a mineral oil (Nujol) in a specially designed press. This also was used undiluted as soon as prepared. Inoculations were made in a compartment of a fumigated greenhouse separated from all diseased material

and protected by every means from accidental infection. The results of these inoculations show that where the virus No. 1 was used, one plant out of ten developed mosaic when the "youngest leaves were inoculated by numerous needle pricks." No more explanation as to Brandes' technique is given, but presumably the virus was smeared on the leaf surface and pricked in with the needle. Where virus No. 2 (extracted under oil) was used, presumably in identical manner, none of the ten plants developed the disease. Where ten plants were inoculated by injecting ½ cc., of virus No. 1 into the growing point with a hypodermic syringe, two out of ten developed mosaic, and where virus No. 2 was used in the same manner, eight out of ten became diseased. Brandes infers from these results that the sugarcane mosaic virus is highly infectious only when exacting demands in the matter of favorable conditions are satisfied. It is considered as proved, however, that the cell sap of diseased plants is infectious when introduced in the proper manner.

Bruner, (4), 1922, in Cuba published the results of his transmission expermients conducted, as he says "since we consider it necessary to be able to transmit the disease artificially before we can study it more thoroughly." Bruner repeated experiments made by Earle and Brandes. Twelve cane plants were inoculated by injecting juice from mosaic cane tissues extracted under oil. Castor oil was used and a few cubic centimeters of distilled water were placed in the bottom of the press before adding the oil: all inoculated plants remained healthy. Thirteen cane shoots were inoculated with juice obtained as in the previous experiment except that Nujol was used instead of castor oil: two developed the disease, or fifteen per cent. Ten plants were inoculated with juice obtained as in the last experiment but three injections performed in each plant: three of the inoculated plants became mosaic, or thirty per cent.

Thirty plants were divided in two groups and the plants in each group given two injections. Fifteen were inoculated immediately after extracting the juice exposed to the air: three became mosaic. Fifteen others were inoculated immediately after extracting the juice under Nujol oil: three developed the disease. Checks were kept in all these experiments. In no case did mosaic appear in the checks. From his experiments Bruner concludes that undoubtedly sugarcane mosaic can be transmitted artificially but that there is yet to be found a method which will produce a constant high percentage of infections since there is some factor that influences them which is not known.

Bruner performed also an experiment similar to the one performed

by Earle, thrusting rapidly a fine hypodermic needle through a freshly cut mosaic cane leaf so that it would penetrate directly into the central nerve (or near to it) of the healthy leaf against which it was held in contact. This was done with the purpose of introducing the virus of the disease into the tissues of the healthy plant with the minimum waste of time and exposure to the air. In this manner, three leaves were inoculated in each plant; one tender, one mature and one old leaf, making three perforations in each leaf. Check plants were identically treated omitting only the diseased leaf.

Kind and condition of the plant: The plants inoculated were of the Cristalina variety, old ratoons about 1½ meters high in a field where infections of cane mosaic had never been observed.

Number of inoculated plants: 100 shoots in 23 stools
Date on which inoculated: December 6, 1920
No indications of infection: January 7, 1921
March 9, 1921:
Stool No. 4 out of 4 shoots, 3 are mosaic
Stool No. 5 out of 4 soots, 1 is mosaic
Stool No. 18 out of 10 shoots, 4 are mosaic

All the check plants remained healthy. As further precaution against error, all the other stools in the field were examined and no infection was found in them. Bruner concludes that the eight plants inoculated became diseased as a result of the introduction into them of the infectious agent of mosaic when the perforations were made with the needle. Bruner, however, does not take into consideration that the virus of sugar-cane mosaic moves from one shoot to another in the same stool. This has been reported by Earle (**6**) and by Menéndez Ramos (**16**) and confirmed by the writer in unpublished experiments. Thus, there may have been only one successful inoculation in each stool.

Kunkel (**11**), in Hawaii, reports an experiment on mosaic transmission performed December 8, 1921. Undiluted juice pressed from the leaves and upper joints of diseased Lahaina cane was rubbed into wounds made on the leaves of six healthy Striped Tip cane plants. The leaves were wounded by crushing them between finger and thumb. The wounded tissue was inoculated by rubbing it with a small piece of absorbent cotton saturated with the diseased juice. Since no infections were obtained the inoculations were repeated December 26 and January 4, 1922. On April 7, five of the six plants inoculated had mosaic.

Fawcett (**7**), 1925, in Argentina has reported some very interesting experiments. His first attempt to transmit sugar-cane mosaic

by wetting the leaves of healthy cane shoots fifty centimeters high with virulent cane juices failed, but upon repeating the experiment with five cane shoots ten to twenty centimeters high they all developed mosaic. At the end of three weeks the inoculated shoots showed well-marked symptoms of mosaic while eleven similar shoots growing close to them and left as checks remained healthy. Fawcett's paper is published in the Spanish translation and it is not clear whether the virulent cane juices were merely placed on the leaves or whether pieces of cotton wet with the juices were attached to the leaves. In either case, however, no perforations were made or the leaf surface broken up in any way. As an explanation, Fawcett says:

"The other well-known disease: tobacco mosaic, is transmitted easily by contact. The application of sap from diseased plants to healthy plants is sufficient to produce infection. To determine whether sugar-cane mosaic can be transmitted in this manner, juices were extracted from mosaic cane leaves by cutting them into bits, adding a little distilled water, grinding and extracting by pressure."

It is evident that Fawcett attempted to transmit sugar-cane mosaic like tobacco mosaic and it is most remarkable that he was able to obtain 100 per cent infection with shoots ten to twenty centimeters high after having failed utterly with shoots fifty centimeters high. No one else has being able to transmit sugar-cane mosaic in this manner.

In another experiment, Fawcett inoculated the virulent juices into the tender leaves in the spindle a short distance above the growing point. He inoculated eleven shoots 10 to 20 centimeters high leaving twelve similar shoots untreated as checks. Between one and two cubic centimeters of virulent juices were inoculated into each shoot. Forty days later, seven out of the eleven inoculated shoots had developed mosaic and all the rest remained healthy.

On repeating the experiment, Fawcett inoculated five small shoots leaving similar shoots as checks. Twenty-four days later three out of the five inoculated shoots had developed mosaic. The others remained healthy.

It is very surprising to see that Fawcett should have obtained a higher percentage of infections when the virus was merely placed on the leaves rather than when it was injected. Fawcett, however, does not consider it significant and he adds:

"The effect of injections is essentially the same as that of the application to the leaves differing only in that the virus is placed in contact with the surface of the tender leaves that have not yet expanded which undoubtedly

are more sensitive than the expanded leaves whose epidermis has already hardened.''

According to Fawcett, then, the virus of sugar-cane mosaic is capable of passing through the epidermis of the leaves and does not require the breaking of the epidermis to reach the tissues inside. We know of no other experimenter who has obtained similar results.

Bonazzi (**5**), 1926, Director of the Chaparra Experiment Station of Cuba published the following method for transmitting sugar-cane mosaic which he used on seed pieces of one bud each:

A hole is bored diagonally into the node, immediately above the leaf scar and as close as possible to the bud, without thereby impairing this tissue. For this work a 3 mm. sterile cork-borer is used, the small core thus resulting being kept to seal the wound at the site of inoculation. In the hole thus prepared a small piece of fresh apical bud tissue, obtained from an infected cane, is introduced and crushed by means of a sterile glass rod. The hole is closed by means of the cork-borer core and the wound finally, hermetically sealed with a small cotton plug soaked in melted paraffin. Planting is done soon after inoculation. The infected tissue for inoculation should be removed from a freshly exposed apical bud only a few seconds before use, the cut being renewed for every prelevation of a new inoculum, in order to avoid undue aeration.

Dr. Bonazzi reports a high percentage of infection in the treated seed pieces and none in the checks but he does not state whether these results were constant upon repeating the experiment. Neither does he explain what is meant by ''questionable infections'' five of which he reports among his treated plants.

Working at the same time at the Insular Station in Porto Rico as Dr. Bonazzi in Cuba, but independently, Dr. M. T. Cook devised and tested a method for artificially transmitting sugar-cane mosaic which he has informed the author is capable of producing a high percentage of infection in susceptible varieties. The method is somewhat similar to that of Bonazzi. A one bud seed piece is cut out of a healthy stalk leaving the bud in the center and part of the internodes at each end. One end is then hollowed out by removing with a knife the spongy tissues down as far as the bud or close to it. About half of the seed piece is thus emptied out into a hollow cylinder. A plug is prepared from the uppermost joints of a mosaic cane stalk by removing the hard outer covering and forced into the healthy cylinder. The grafted seed piece is then planted.

McRae (**14**), 1927, and McRae and Subramanian (**15**), 1928,

in India have transmitted cane mosaic artificially by the following method: the cell contents of mosaic leaves were crushed out, then placed on leaves of healthy plants and pricked in with a sterile needle, controls having distilled water pricked in. In no case did the latter show any trace of disease, but mosaic was transferred from leaf to leaf or leaf sheath to leaf sheath in different canes; from various canes to maize and sorghum, but not to the lesser millets. This method seems to be the same used by Brandes, even though neither he nor McRae and Subramanian specify the kind of needle used. When inoculating from leaf of Co–213 to leaf of Co–213, McRae was able to obtain 8 infections out of 13; 8 out of 16 when inoculating from the same leaf to leaf sheath of the same variety of cane; and 16 out of 16 when inoculating from leaf of Red-Mauritius to leaf of Co–213. In no case did the controls produce mosaic markings. In contrasts to McRae's success it is interesting to remember that Brandes was able to obtain only 1 infection out of 20 inoculations using probably the same method.

No other method for artificially transmitting sugar-cane mosaic has been published up to date and the following statement by Hadden (8), 1928, sums up the situation: "Sugar-cane mosaic may be transmitted artificially by hypodermic injections, needle punctures, grafting, etc., but only with great difficulty and many failures."

Recently, at the Third Conference of the International Society of Sugar-Cane Technologists, held in Java, 1929, Dr. G. Wilbrink presented a paper to prove that sugar-cane mosaic can be transmitted by means of the knife used in cutting cane seed for planting. Dr. Wilbrinks's experimental plants, however, were exposed in the open to natural infection by *Aphis maidis* and the percentages of infection obtained were exceedingly low. It is claimed that the inoculum was carried and deposited on the cut surfaces of the healthy canes when they were sectioned later with the same knife. We have made a few tests of this method with complete failure and Matz (13) failed with a similar one. It seems rather improbable that it can produce infection.

This leaves as the most desirable and successful, the method used by Brandes and by McRae and Subramanian. This method, however, has not been tested under Porto Rican conditions.

A TEST OF THE METHOD USED BY BRANDES AND BY MCRAE AND SUBRAMANIAN

All the information given by Brandes (1) as to his technique is as follows: "Virus rubbed with fingers on unbroken surface

of young leaves. Youngest leaves inoculated by numerous needle pricks."

All that McRae and Subramanian (**15**) say is: "In the infection experiments given in Table VI juice was crushed from mottled leaves and immediately pricked into leaf-sheaths and stems of plants known to be free from mosaic disease."

Neither Brandes nor McRae and Subramanian specify what size of needle was used in making their inoculations. In testing their method under Porto Rican conditions we used a row of two-hundred cane plants of the variety SC–12(4) set in a plot at considerable distance from other mosaic cane plants. The plot was practically free from weeds, but of course, being in the open, a certain amount of error from infestation by *Aphis maidis* was to be expected. To obtain the virulent juice for inoculation, tender mosaic sugar-cane leaves of the same variety were cut up into bits with scissors, pounded in a porcelain mortar, the ground mass tied up in a piece of muslin and the juice extracted under a hand press. All the utensils were carefully washed and the juice was collected in a clean beaker and used immediately after extraction.

One hundred shoots each in a different plant were inoculated leaving every other plant in the row as checks. To perform the inoculation, the mature, expanded leaves were bent down and some stripped off exposing the tender parts of the central spindle of tightly rolled young leaves. A little of the virulent juice was then placed on the spindle with the finger tip and as the drop of liquid slid downward it was pricked in with a No. 0 Asta black insect pin. Three drops were placed on each spindle and numerous needle pricks made. The inoculations were made April 25, 1929. Up to May 18, 1929, 52 out of the 100 inoculated shoots had developed mosaic and by June 25, 1929, when the experiment was closed, the number of infections had increased to 56. As the mosaic pattern became clearly marked, the infected plants were pulled out of the row to prevent natural transmission. In spite of this precaution, however, one case of secondary infection appeared in the checks.

DISCUSSION

There can be no doubt that sugar-cane mosaic can be transmitted by pricking the virulent juices into the young leaves. It is nevertheless very likely that were we to repeat the experiment, the results would vary. The extraction of the virulent juices is a troublesome procedure and since the virus seems to be readily

destroyed or rendered non-infectious by exposure to the air the results would depend on the rapidity and care with which the work was done.

For the purposes we had in mind, the percentage of infection obtained by pricking in the virulent juices was not sufficiently high. The method itself was too troublesome for routine field work.

The direct pricking in of the inoculum from mosaic to healthy tissues had been attemptted by Earle (**6**) and by Bruner (**4**) with failure or low percentages of infection, but the instrument used by these investigators seemed to us to be too coarse. Disregarding the extraction of the juices as troublesome and subject to too much variation, we sought to devise a new method by improving on the work of Earle and Bruner through the use of a fine insect pin. In the new method as originally conceived, we depended on the spindle of tightly rolled tender mosaic leaves as the traditional source of the inoculum. Later, however, we found that the expanded leaves were a much more convenient and equally effective source of inoculum.

Evolution of the New Method

The spindle-to-spindle method

Adhering to the traditional source of inoculum—the spindle or cylinder of tightly rolled tender leaves—our first attempts at devising a new method of artificial transmission consisted in holding a spindle pulled out of a mosaic cane plant tight against the exposed spindle of a healthy cane plant and running an insect pin through the mosaic into the healthy tissues. A No. 2 white Asta insect pin was used thrusting it rapidly in and out at several points on both spindles. The results were proportional to the care taken in performing the inoculations, the condition of the cane plants and possibly other factors. When vigorously growing cane plants were inoculated carefully and rapidly, this spindle-to-spindle method could be depended upon to produce about 70 per cent infection. The method, however, had several disadvantages. Only one spindle can be obtained from one mosaic shoot and it is troublesome to hold the spindles tightly together when performing the inoculation. We used it nevertheless, with satisfactory results during the years 1925–1928 on different sugar-cane varieties. During the year 1928, the method was simplified by the use of expanded mosaic cane leaves instead of the spindles of tightly rolled tender leaves as the source of the inoculum.

THE LEAF-SLIP METHOD

Contrary to the traditional belief, the expanded leaves of mosaic sugar-cane plants are as good a source of the inoculum as the more tender ones. Any leaf as soon as it has expanded can be used. The very young ones split easily and the older ones are not pliable. The medium leaves are therefore more satisfactory.

THE TOOL

Black number 0 and white No. 2 Asta insect pins have been used. It is possible that a finer pin than the black No. 0 but sufficiently stiff to enter the tissues without bending will produce higher percentages of infection and require a smaller number of pin pricks to be made at each inoculation, but for ordinary routine work those that have been used are very satisfactory. Ordinary pins have proved worthless since apparently they are too thick and short and allow the entrance of air into the wound.

TECHNIQUE

We ordinarily use one pin at a time to make the inoculations, but several can be tied together into a brush thus making many pricks at one time. When the plants to be inoculated are large and their spindles thick, the brush of pins is very convenient. Before using the pin we usually cleanse it by running it through cloth or the midrib of a cane leaf. No attempt has been made to sterilize the pin. No other disease than mosaic has developed in the inoculated plants as a result of the pin pricks and it is perhaps needless to add that mosaic does not result from pin pricks or from pricking in the juice of healthy cane leaves into healthy cane plants.

When sugar-cane plants from two feet in height and upwards are to be inoculated the procedure is as follows: The expanded leaves are bent down and the central spindle exposed. The outermost one of the leaves, the basal part of which is still tightly wrapped about the spindle, is stripped off exposing the whitish cylinder of tender leaves.

A slip about one inch wide and some eight inches long is stripped off a mosaic leaf, this is placed like a band around the base of the exposed spindle and held tightly with the thumb and forefinger of the left hand. The pin, held in the right, is thrust rapidly in and out repeatedly through different parts of the mosaic band into the healthy spindle. The pin is thrust in a slanting

position and is not made to go clear through the spindle. After the pin has been thrust in about twenty times, the mosaic leaf-band is moved upwards, held tightly again, and the pricking repeated at four or five points along the spindle using a fresh part of the mosaic leaf-band every time.

To inoculate very small cane plants whose stems are soft, the band of mosaic leaf is placed around the stem instead of the spindle. Corn and other grasses are inoculated in the same manner.

PERIOD OF INCUBATION

The period of incubation for sugar-cane mosaic is considered to be about fifteen days long. There is no difference in the length of the period between sugar-cane plants inoculated artificially by mechanical methods or through the agency of the insect vector, *Aphis maidis,* in nature.

The length of the period of incubation is measured by the appearance of the first symptoms of secondary infection. Since these symptoms appear in the tender leaves and not in those that were old or mature at the time of inoculation, it is obvious that the appearance of the symptoms depends on the rate at which the new leaves are growing and expanding.

ACCOUNT OF EXPERIMENTAL WORK

Only one experiment will be reported since it is representative of many others that were conducted. For lack of green-house facilities, they were all carried out in the open and therefore subject to error by the almost unavoidable liability to infestation by *Aphis maidis.*

A field near the Entomological Laboratory at the Insular Station, Río Piedras, P. R., was prepared in the usual manner and SC–12(4) cane planted in 14 parallel rows of 200 seed pieces each. The seed pieces were cut 1 foot long, planted 1 foot apart and the rows were spaced 3 feet apart. The seed came from one of the Station fields free from mosaic. After germination all the plants were examined and found to be healthy.

On August 24, 1928, the plants were about 4 feet high. All were inspected again and none were found with mosaic. In row No. 7, in the center of the field, 100 shoots, each in a different plant, were inoculated by the leaf-slip method and tagged. Alternate plants in the row were selected for inoculating thus leaving a check

in between. As each seed piece had produced an average of five shoots there were some 13,000 shoots in the plot.

From September 8 to September 15, 1928, ninety-four of the one hundred inoculated cane shoots in row No. 7 developed mosaic. As soon as the symptoms of secondary infection were evident in the inoculated shoots, the entire plant to which it belonged was pulled out of the field. Up to September 15, 1928, when the experiment was closed no other case of secondary infection had appeared in the field except the 94 that had been inoculated.

On September 20, 1928, while looking over the field, two cases of secondary infection were found in cane plants that had not been inoculated artificially. One of the cases was near the outer borders of the plot, one out of a stool of four shoots; the other was in row No. 7, also one in a stool of five shoots.

Up to the time the experiment was closed no weeds had been allowed among the cane in the experimental plot. The finding of these two cases of secondary infection, evidently the result of natural transmission through the agency of *Aphis maidis,* is not surprising since the plot where the experiment was conducted was surrounded on two sides by fields of "malojillo" or Para grass, *Eriochloa subglabra,* a host plant of the insect, and on the other two sides by fields in which other host plants were also growing. A low percentage of natural transmission is to be expected in any such experiment conducted in the open.

DISCUSSION

Brandes (3) reached the following conclusion on the requirements for successful inoculation from his studies on the transmission of sugar-cane mosaic by *Aphis maidis:*

> "It has been found that a definite, measurable quantity of 'Virus' is necessary. This would eliminate from consideration as an explanation the carrying into the plant of the scanty amount of virulent material adhering to the minute mouth parts."

Brandes in the same publication shows by means of sections how the setae of *A. maidis* are inserted into the tissues of the corn leaf:

> "Typically, the setae of *A. maidis* pass through the sub-stomatal cavity, then through the mesophyll cells, either intercellularly or intracellularly, continuing between two cells of the starch sheath and finally into the phloem of the vascular bundle."

In devising our new method for accomplishing artificially what

A. maidis does in nature, we have started from a point opposite to that of Brandes in that we have assumed that possibly the scanty amount of "virus" adhering to the mouth parts might be sufficient to produce infection if it were conducted to the healthy tissues without undue exposure to the air. Whether the "virus" has to be carried to the phloem to produce infection or not, this is accomplished mechanically by thrusting the pin into the leaves a large number of times.

For lack of greenhouse facilities we have not attempted to determine the number of pin pricks necessary to produce infection. This would require a very large number of inoculations under conditions in which there would be no possibility for *A. maidis* gaining access to the plants. We have made some experiments in the open field in which lots of twenty cane plants each were pricked once, twice and three times, at the base, center and upper part of the spindles through mosaic cane slips thrusting the pin in and out as rapidly as possible. All such inoculations gave negative results and were not repeated since the very low percentage of infection to be expected would be questionable as there is always in the open field the possibility of infestation by *A. maidis*.

When a hundred or more pin pricks are made into each plant, the percentage of infection is so high and so constant that the danger of infection through *A. maidis* can be overlooked. In the first experiment reported above we selected the row of plants to be inoculated in the center of the field to minimize as far as possible the danger of infestation by *A. maidis* crawling in from outside or dropping down from the air. Lacking green-house facilities, this was the best that could be done. To further correct possible error, the only means we had was the repetition of the experiment a large number of times. The percentages of infection in all these repeated experiments conducted during the years 1925–1929 was as a rule over seventy with contrasting exceedingly low percentages of infection in the checks.

With green-house facilities soon to be available at the Insular Experiment Station, the experiment will be repeated under perfectly controlled conditions.

The plants were considered mosaic as soon as the symptoms of secondary infection became well marked. The symptoms as described by Stevenson (**18**), Brandes (**2**), Lyon (**12**) and others are unmistakable. The incubation period of about fifteen days is the normal for the disease. During the period of incubation, the soil was moist and the plants in the experiment were growing vigorously.

In using a pin for performing the inoculation, the limiting factor seems to be the exposure to the air of the "virus" that is carried on the pin. Success therefore depends on how rapidly the pin is thrust in and out. When the cane plants are growing vigorously the tissues are turgid and the sap is flowing freely. This probably favors infection because the wound made by the pin seems to close up on it thus excluding the air and possibly because the "virus" is rapidly distributed through the plant. Thrusting the pin in a slanting position would also favor the exclusion of air from the wound.

When resistant or immune varieties of sugar cane are inoculated, other factors are concerned of which as yet we do not know much about. Some of the sugar-cane plants inoculated artificially by us in our experiments and some of the checks that developed mosaic through the agency of *Aphis maidis* were transplanted and kept under observation for several years.

There is no difference between sugar-cane plants inoculated artificially with sugar-cane mosaic and those inoculated in nature by the insect vector of the disease, the corn aphid, *Aphis maidis Fitch*. The period of inoculation is the same, (varying naturally with the rate of growth of the plants), the symptoms and the manner in which they appear are identical and the course of the disease is the same in either case.

SUMMARY

1. A new mechanical method for artificially transmitting sugar cane mosaic has been developed and used at the Insular Experiment Station of Porto Rico during the years 1925–1929.

2. The method is very simple and easy to operate and produces constant high percentage of infection.

3. Former investigators had sought to transmit sugar-cane mosaic artificially by extracting the virulent juices and injecting them into the healthy plant by means of a hypodermic needle syringe or by pricking them in after smearing the leaves. The extraction and injection of the virulent juices is always troblesome and the "virus" is quickly rendered non-infectious possibly by exposure to the air. Such methods besides being laborious and troublesome do not produce constantly high percentages of infection.

4. Bruner obtained a few infections by pricking in the virulent juices directly from the mosaic into the healthy tissues without previous extraction. The percentages of infection obtained were quite low. This was possibly due to the use of too coarse a needle.

5. The spindle of tightly rolled tender leaves has been the traditional source of the inoculum in artificial transmission experiments. Our new method (spindle-to-spindle) originally consisted in pricking in the inoculum from a mosaic into a healthy spindle held tightly together. This arrangement, though producing a constant high percentage of infection was somewhat cumbersome.

6. In its final form the leaf-slip method consists in pricking in the inoculum from a slip of mosaic cane leaf held tightly as a band around the exposed spindle of a healthy cane plant. To inoculate small cane plants, corn and other grasses, the band is held around the stem.

7. Black No. 0 and white No. 2 Asta insect pins have been used with equally good results. Ordinary pins do not produce the desired results.

8. The number of pin pricks necessary to produce infection has not been determined. Experiments in which groups of cane plants were pricked one, two and three times each, failed to develop mosaic. Ordinarily, about a hundred pin pricks are sufficient to insure infection.

9. The limiting factor seems to be exposure of the "virus" to the air. By thrusting the pin in and out rapidly in a slanting position a large number of times if there are any other requirements for infection, they will be fulfilled mechanically. The larger the number of pricks made, the larger the number of chances for infection.

10. Using a bunch of pins tied up into a brush makes the inoculation work more rapid, especially when inoculating plants with a thick spindle. Equally good results have been obtained with a bunch as with only one pin at a time.

11. Success depends on the care taken to shorten the exposure to the air, of the "virus" that is carried on the pin and on the rate at which the inoculated plant is growing. In resistant or immune cane varieties, other factors are involved, of which we as yet do not know much about.

12. When the inoculated cane plant is growing vigorously, the tissues are turgid and the sap is flowing freely. This seems to favor infection because (1) the wound made by the pin closes up on it thus excluding the air and (2) possibly because the "virus" is carried and distributed rapidly through the plant.

13. Inoculated cane plants of susceptible varieties, when growing rapidly show the first symptoms of secondary infection usually fifteen days after the inoculation. The symptoms appear and are

in every respect identical with those shown by plants that have been inoculated through the agency of the insect vector of the disease, the corn aphid, *Aphis maidis* Fitch. The course of the disease is the same in sugar-cane plants inoculated artificially as it is in plants inoculated in nature through the agency of the insect vector.

LITERATURE CITED

1. **Brandes, E. W.** The Mosaic Disease of Sugar Cane and Other Grasses. U. S. Dept. Agr. Bul. **829.** (1919.)
2. _____ Artificial and Insect Transmission of Sugar Cane Mosaic. Jour. Agr. Research **19**: 131–138. 1920.
3. _____ Mechanics of Inoculation with Sugar-Cane Mosaic by Insect Vectors. Jour. Agr. Research **23**: 279–285. (1923.)
4. **Bruner, S. C.** Sobre la Transmisión de la Enfermedad del "Mosaico" o "Rayas Amarillas" en la Caña de Azúcar. Rev. Agr. Com. & Tra. Cuba **5**: 11–22. (1922.)
5. **Bonazzi, Augusto.** Study on Sugar Cane Mosaic. Science, pp. 529–530. (Nov. 26, 1926).
6. **Earle, F. S.** The Year's Experience with Sugar-Cane Mosaic or Yellow-Stripe Disease. Jour. Dept Agric. Porto Rico **3**: 3–33. (1919.)
7. **Fawcett, G. L.** Apuntes sobre el Mosaico de la Caña de Azúcar. Rev. Ind. Agric. Tucumán **18**: 205–209. (1928.)
8. **Hadden, F. C.** Sugar-Cane Mosaic and Insects. Hawaiian Planters' Record **32**, No. 1. (Reprinted In Facts About Sugar, August 11, 1928, pp. 758–762.) (1928.)
9. **Kamerling, Z.** De Gele-Strepenziekte der Bladeren. Verslag 1902 Proefs. Suik. Oost Java "Kakok" to Pekalongan, pp. 76–81. (1903.)
10. **Kobus, J. D.** Vergelijkende Proeven Omtrent Gele-Strepenziekte. Arch. Java-Suikerindus., jaarg. **16,** pp. 350–374, (Reprinted as Meded. Proefs. Javasuikerindus., No. 12, 319–342). (1908.)
11. **Kunkel, L. O.** Studies on the Mosaic of Sugar Cane. Bul. Exp. Sta. H. S. P. A. Bot. Ser. **3**: 115–167. (1924.)
12. **Lyon, H. L.** Three Major Cane Diseases: Mosaic, Sereh, and Fiji Diseases. Bul. Exp. Sta. H. S. P. A. Bot. Ser. **3**: 1–43. (1921.)
13. **Matz, J.** Infection and Nature of the Yellow-Stripe Disease of Cane (Mosaic, Mottling, etc.). Jour. Dept. Agric. Porto Rico **3**: 65–82. (1919.)
14. **McRae, W.** Mosaic Disease of Sugar Cane in India. Agric. Jour. India **21**: 198–202. (1926.)
15. **McRae, W.** and **L. S. Subramanian.** A Further Note on the Mosaic Disease of Sugar Cane. Jour. Agric. India **23**: 239–255. (1928.)
16. **Menéndez Ramos, R.** Estudios sobre el mosaico de la caña. Revista de Agricultura de Puerto Rico. **13**: 219–220. (1924.)

17. **Smyth, E. G.** Annual Report, Division of Entomology. Ann. Rpt. Insular Expt. Sta. Porto Rico, **1919–20,** pp. 83–89. (1920.)

18. **Stevenson, John A.** The Mottling or Yellow-Stripe Disease of Sugar-Cane. Jour. Dept. Agric. Porto Rico **4** (i. e. **3**) : 3–76. (1919.)

19. **Stok, J. E. van der.** Verschijnselen van Tusschenrasvariabiliteit bij het Suikerriet. (Proeve eener verklaring der Gele-Strepenziekte en der Serehziekte.) Arch. Java Suikerindus., jaarg. **15,** pp. 581–601. (Reprinted as Meded. Proefs. Oost-Java S. 4, No. 36, pp. 457–477.) (1907.)

20. **Tower, W. V.** Mottling Disease of Sugar Cane. Rpt. Porto Rico Agr. Exp. Sta. **1919**, pp. 21–25. (1919.)

21. **Wilbrink G.,** and **Ledeboer, F.** Bijdrage tot de Kennis der Gele-Strepenziekte. Arch. Suikerindus. Nederl. Indie, jaarg. **18,** pp. 465–518. (Reprinted as Meded. Proefs. Java-Suikerindus., deel 2. No. 39, pp. 443–495.) (1910.)