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Inoculation of Sugarcane Plants with the Mosaic Virus Using the Airbrush

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INTRODUCTION AND REVIEW OF THE LITERATURE

Several mechanical methods are conventionally utilized to inoculate sugarcane plants with the causal agent of its common mosaic disease. According to Matz (5)² Kammerling, working in Java, claimed in 1903 that he had succeeded in transmitting the disease by injecting healthy sugarcane plants with sap expressed from affected ones. However, according to Matz, Kammerling's claim was invalidated by the fact that some uninoculated control plants also contracted the malady. In 1920 Brandes (1) transmitted the etiologic agent of the mosaic disease by injecting sap from infected plants into the growing points of healthy ones by means of a hypodermic syringe.

A somewhat different method was employed in Cuba by Bruner (2) who successfully transmitted the virus in 1922. This was achieved by superimposing affected upon healthy leaves and rapidly thrusting a fine hypodermic needle through the infected leaf into the healthy one. In 1930 Seín (6) devised a technique similar to the one employed by Bruner. Seín effected his inoculations with No. 0 or No. 2 insect pins and obtained high percentages of infection. Seín (7) also obtained infection by pricking in juices pressed out from mosaic tissues into the leaves of healthy plants. This was accomplished by smearing the leaves with inoculum and subsequently stabbing them repeatedly with an insect pin.

In 1933 Matz (5) published the results of his work on the artificial transmission of sugarcane mosaic and developed a new technique which was a refinement of the previous pin-thrust methods. In this latest technique a few drops of infective sap are placed in the wedge-shaped opening formed by the base of the youngest expanded leaf and the young rolled leaf. Re-

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² Italic numbers in parentheses refer to Literature Cited, p. 7.

peated thrusts are then practiced through the inoculum reservoir and into the young rolled leaf. Edgerton (3) mentioned a technique by means of which young sugarcane plants from true seed can readily be infected with the mosaic. The leaves of the young plants are merely clipped and then the seedbeds are sprayed with virus-containing juice from affected plants. The common method of inoculating plants by rubbing their leaves with virus-containing juices in the presence of carborundum is also employed to infect sugarcane plants with mosaic. This method is fairly efficient when dealing with seedling plants, but awkward, inefficient, and cumbersome for larger plants.

The Matz or pin-thrust method has been used regularly in Puerto Rico for a number of years because it results in higher rates of infection than those produced by the earlier manual techniques. Be that as it may, the Matz method is still cumbersome, and time-consuming, and also requires a certain skill for best results.

OBJECTIVES

Bearing in mind the inconveniences of the Matz method, it was decided to undertake work in an attempt to determine whether the airbrush method, devised by Lindner and Kirkpatrick in 1959 (4), could be successfully adapted to inoculate sugarcane plants with the common mosaic virus, and, if so, to evaluate an adaptation of the Lindner and Kirkpatrick method by comparing it in parallel inoculation tests with the standard Matz method.

MATERIALS AND METHODS

SPRAY EQUIPMENT

A Paasche "H 3-in-1" airbrush coupled to a 3-ounce glass bottle was used throughout the inoculation trials. The source of compressed air was a 1-hp. twin-piston industrial sprayer with a 12-gallon air storage tank. This unit was provided with a pressure switch to start the motor at 75 pounds per square inch, and to stop it at 100 pounds. The line pressure was maintained at 75 pounds with an air regulator which was connected to a filter unit capable of removing oil, water, and foreign matter. For inoculation, the flow-control tip on the Paasche airbrush was adjusted by revolving it clockwise $1\frac{1}{2}$ turns from the close-setting. This setting was used throughout all of the trials.

INOCULUM

Leaves from severely affected plants of different varieties were used as sources of inoculum. Upon collection the leaves were rinsed with tapwater, chopped with scissors, and ground with the aid of a precooled Universal

meat grinder No. 73. The juice from the ground leaves was then expressed using a Carver laboratory press. In all cases the macerated leaves were subjected to a pressure of 5,000 pounds per square inch for maximum juice yield. After extraction every batch of juice was agitated and immediately divided into two equal volumes. Carborundum (600-mesh) was added at the rate of 1 gm. per 100 cc. to the inoculum that was to be used for the airbrush method. No carborundum was added to the inoculum destined for the Matz method.

TEST PLANTS

The sugarcane plant varieties B. 34104, P.O.J. 2878, P.R. 980, H. 328560, and B.H. 10(12) were used in the inoculation trials. The varieties B. 34104, and B.H. 10(12) are known to be very susceptible to mosaic under natural conditions in Puerto Rico. They have also been shown to be very susceptible when inoculated by artificial means. In Puerto Rico the varieties P.R. 980 and H. 328560 have been found less susceptible than the former under natural conditions and rather hard to infect by artificial means. As far as is known there have been no observations in Puerto Rico of an infected cane plant of the variety P.O.J. 2878 in the field. A few instances of infection of plants of this variety via the Matz method have been recorded by Adsuar in the Island. Apparently, the variety is refractory to infection when inoculated by pin-thrust methods.

The test plants were grown from one-eyed seedpieces. The canes from which the seedpieces originated were in all cases obtained from mosaic-free plants in the Experiment Station's collection. Immediately after the canes were reduced to one-eyed-seedpiece size they were separately treated with phenyl mercuric acetate (500 cc. per gallon of water) and then planted in flats containing a mixture of clay loam, sand, and filter-press cake. All plants germinated normally except those belonging to the variety B.H. 10(12). On pulling and observing some of these plants no roots were detected at the root bands of the seedpieces. These plants recovered when roots began to spring from their bases. Inoculations using the two means under evaluation were effected 22 days after the seedpieces were planted. In general, at the time of inoculation, the plants had attained a height which varied from 7 to 14 inches.

INOCULATION PROCEDURE

A group of plants was inoculated via the Matz method as described previously. A set of plants of each variety was left undisturbed as an uninoculated control. The following procedure was effected in inoculating plants via the airbrush method: The two youngest leaves of the plant to be inoculated were held as depicted in figure 1, and the aircap of the atomizer was



FIG. 1.—Manner in which the airbrush was used on the youngest leaves of sugarcane plants for inoculation purposes.

brought perpendicularly to rest on the unfurled leaf's upper surface at a height of about $1\frac{1}{2}$ inches from the spindle. Spraying began at this site. While still pressing the release lever the aircap was quickly drawn downwards, but in contact with the unfurled leaf until the tender base tissues of the selected leaves were reached, and then was speedily brought upwards to the starting site by the same route. This procedure was repeated until about six passes were completed.

This technique was found to be the least awkward and produced excellent results. Attempts to set distances from the nozzle to the leaf surfaces by various small mechanical devices complicated the procedure and were postponed for later studies. By applying the aircap to the leaf surfaces as indicated a distance of about 0.5 cm. was found at most times to separate the surface of the youngest expanded leaf from the tip of the aircap; possibly the distance was shorter during most of the operation. The entire procedure of inoculating a plant via the airbrush took at the most 4 seconds by one man as against 10 seconds employed by two men using the Matz method. Consumption of inoculum was about the same in both cases. Inoculations via the Matz and airbrush methods were started simultaneously in the two first tests herein reported.

EXPERIMENTAL RESULTS

As a rule, symptoms began developing 10 days after the plants were inoculated via the airbrush. This was not true for plants inoculated by the Matz method, in which symptoms first became noticeable about 12 days after inoculation. The final results of the tests reported in table 1 were recorded 1 month after inoculation. The airbrush-inoculated plants of the P.O.J. 2878 variety are of particular interest since such high incidence of disease was not expected to occur. To study the behavior of affected P.O.J. 2878 plants, 46 of them were marked and observed further. Forty-four days after inoculation 20 of these plants still manifested severe symptoms of disease in all their leaves. Of these 20 plants, 3 were almost totally necrotic and about to die. The course of the symptoms was dissimilar in the remaining 26 plants; the top three leaves of these were completely devoid of symptoms 44 days after inoculation. The plants that appeared to recover are still being observed and studied.

More successful inoculations by both the Matz and the airbrush method were expected in the case of the variety B.H. 10(12). Although planted at the same time as the rest of the varieties in test No. 2, its germination was very poor and for a time its growth was very stunted. About 2 weeks after germination, and prior to inoculation, more than 80 percent of these plants became wilted and developed terminal burning of the youngest leaf. At the time of inoculation the plants of this particular variety were still

visibly affected. Possibly higher rates of infection by both methods of inoculation might have resulted had the B.H. 10(12) plants been healthier.

DISCUSSION

The high incidence of mosaic in plants of the variety P.O.J. 2878 when inoculated via the airbrush was unexpected because this cane had been relatively resistant to mosaic when inoculated by the older methods. As far as is known this variety has not been found infected in the fields of the Island. As mentioned previously, the variety P.O.J. 2878 is apparently

TABLE 1.—*Number and percentage of successful inoculations of healthy sugarcane plants of several varieties in tests designed to compare the efficiency of the standard Matz method with that of the airbrush method*

Test No.	Varieties	Number of plants infected by the Matz and the airbrush methods over plants treated by each method, and percentage infection ¹			
		Matz method		Airbrush method	
		Infected/ treated	Percentage infection	Infected/ treated	Percentage infection
1	P.O.J. 2878	3/162	1.8	56/137	40.8
	H. 328560	3/127	2.4	19/348	5.5
2	P.O.J. 2878	0/156	0	52/134	38.8
	H. 328560	0/140	0	26/150	17.3
	P.R. 980	0/148	0	9/133	6.8
	B. 34104	45/142	31.6	67/118	56.8
	B.H. 10(12)	21/105	20.0	30/97	30.9

¹ For each treatment a set of 25 uninoculated plants of each variety was set aside as a control. None of these plants became affected.

capable of “recovering” from the disease. However, much more work is needed on this before a definite conclusion can be reached. At any rate the “recovery” phenomenon could, in part, explain the complete absence of mosaic on the variety P.O.J. 2878 under our conditions. It is possible that other factors such as preferences of the aphid vectors might also be responsible for the absence of mosaic in Puerto Rican fields planted to the variety P.O.J. 2878.

To some it may seem that the airbrush method of inoculation is undesirable because, by means of it, the so-called “immune” variety P.O.J. 2878 has been consistently and easily infected. As mentioned earlier, even before the introduction of the airbrush method several instances of successful pin-thrust inoculation of plants of P.O.J. 2878 had been recorded by Adsuar in Puerto Rico. During the late stages of these studies claims were made by

some that the Uba variety, commonly known to be immune, would, like the variety P.O.J. 2878, contract mosaic if and when inoculated by the airbrush.

To clarify these questions 100 Uba (immune to mosaic) and 100 M. 336 (susceptible to mosaic) plants were inoculated by the airbrush with infective sap obtained from mosaic plants. Twenty-five plants of each variety were set apart as uninoculated controls. All inoculated plants of the variety M. 336 developed symptoms of mosaic; not one of the similarly treated plants of the Uba variety did so. The control plants remained healthy. It is believed that these results clearly demonstrate that the airbrush method is simply a more efficient method of inoculation, but not one capable of infecting immune plants.

The airbrush method is not affected by several of the variables which usually produce inconsistencies in the results of inoculation by the older methods. In this method the impelling force is air, the pressure of which can be satisfactorily regulated. The flow of inoculum and abrasive can also be so regulated that comparatively little variation should result.

SUMMARY

The results of the studies reported herein show that the airbrush method is more easily used and more efficient in infecting sugarcane plants with the common mosaic virus than the standard Matz pin-thrust method.

RESUMEN

Los resultados de estos trabajos demuestran que el método, en el cual se utiliza un pincel de aire, es más eficiente que el método de Matz para inocular plantas de caña de azúcar con el mosaico común de esta cosecha.

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