AN INVESTIGATION OF TYPES OR STRAINS OF THE MOSAIC VIRUS OF SUGARCANE IN LOUISIANA

By

Eaton M. Summers

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Signature was redacted for privacy.

In charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College
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INTRODUCTION

This paper presents experimental data which establish the existence of strains of the virus responsible for the mosaic disease of sugarcane. This disease, the host range of which includes corn and many other related grasses both wild and cultivated, was first noticed by Dutch investigators in Java about 1890, before the existence of virus diseases as such had been definitely established. It was not, however, considered by them to be a transmissible disease because all efforts to transfer it by artificial means met with failure. It was known in Java as "gele strepenziekte" (yellow stripe) and has since been called by many names, mostly descriptive in nature, in the various countries where it has been reported. It seems to be present wherever sugarcane is grown but has not assumed such great economic importance in most places as it has in Louisiana. Here, apparently, the disease encountered the most adequate means for its rapid spread as well as conditions that often permitted it to very seriously affect varieties that are generally considered to be tolerant or resistant to mosaic. It has often been the deciding factor in abandoning varieties that show great commercial promise (see fig. 1) and is so severe on some varieties (see figs. 2 and 3) that they are eliminated even before they can be adequately tested from the agronomic standpoint.

Fig. 1. The disastrous effect of using mosaic, as compared to healthy, cuttings for planting in the otherwise very promising seeding, C. P. 29/291. There was, in this test, a reduction of 44 per cent in sugar per acre. The symptom pattern is quite mild on this variety.

Fig. 2. The dwarfing effect of using mosaic cuttings for planting on the seedling C.P. 28/8. Cane from diseased cuttings in front of stake and from healthy cuttings behind stake.

Fig. 3. The dwarfing effect of using mosaic cuttings for planting on the seedling C.P. 28-57. Cane from diseased cuttings growing in the foreground and from healthy in the background of the same row. The mosaic cane produced only a few shoots in the ratoon crop but the healthy cane was as good as in the plant cane crop shown here.

Very often varieties are encountered which show two types of mosaic, one which causes very mild symptoms and no stunting and another which causes severe chlorosis and stunting. A comparison of such symptoms is shown in figure 4.

The symptoms and general behavior of this disease soon identified it with that rapidly increasing group known as virus diseases. The causal virus has been treated by previous investigators as a single entity, although several of them have suggested the possibility of the existence of strains or variations of some nature but any adequate data in support thereof have been lacking. The results presented in this paper, therefore, represent the first systematic comparison and resultant identification of strains of the virus which produces the mosaic disease of sugarcane.

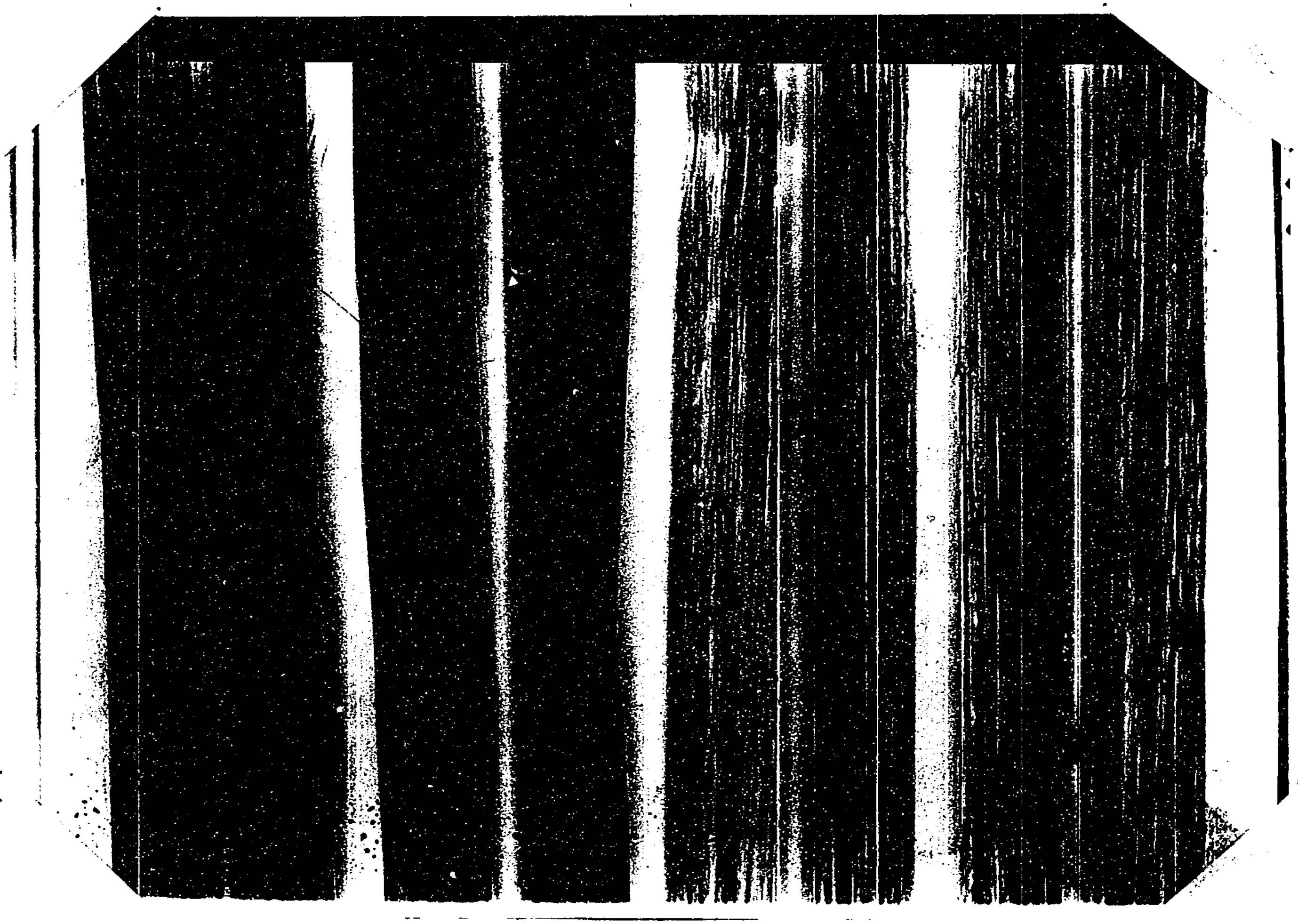

Fig. 4. Left (2 leaves): Mild or ordinary mosaic symptoms.
Right (2 leaves): Severe mosaic symptoms on leaves from adjacent stools, in the field, of the seedling C. P. 31/254.

EVIDENCE OF VIRUS STRAINS IN PLANTS OUTSIDE THE GRASS FAMILY

Indications that strains may occur in several plant viruses are to be found in the literature. Severin (23) reported aster yellows in California could be easily transferred to celery by its vector, Cicadula sexnotata Fall., but Kunkel (15) was not able to substantiate these results, working in New York and using a local source of the yellows virus. Having obtained a sample of the California material, however, he (16) was able to infect celery under New York conditions where the local virus failed and concludes, "whether the yellows from California is a strain of aster yellows or is a different disease is a question that cannot be answered at this time." Smith (25), commenting on this work, says:

> Here then is a case of a virus having 'mutated' or adapted itself to a new host plant in one district and after sojourn in this host has acquired the ability to infect it as easily as any other plant in its host range. Such a virus may be regarded merely as a slightly different strain of aster yellows or it may be regarded as a different entity and be referred to as "celery yellows." It is also possible that celery yellows is a stage in the evolution of an entirely new virus.

Séverin (24) later obtained yellows virus from asters or carrots from several widely separated sections of the United States and found celery to be highly resistant to all collections except those obtained in California. He obtained some infection with certain collections but the percentage never approached that obtained by the local virus.

Cooley (8), comparing what he calls "mild streak" and "severe streak" of black raspberries, suggests that he is dealing with two separate diseases of similar nature which have many diagnostic characters in common, suggesting a close relationship, but have certain others which are so widely divergent that the two must be differentiated. Their close relationships suggest the possibility that they are strains of the same virus rather than separate diseases. Bennett (2) classifies three types of red-raspberry mosaic based on severity of symptoms and suggests the possibility that they "may be produced by strains of the virus in different stages of virulence."

EVIDENCE OF VIRUS STRAINS IN SUGARCANE AND RELATED GRASSES

Storey and McLean (32) have shown that the "streak" disease of maize is definitely different from that of the sugarcane variety, Uba, in Natal. These are probably strains of the same virus, the latter having, presumably, evolved from the former since in Uganda, where "streak" is common on maize and the vector, Cicadulina (Balclutha) mbila Naude, is plentiful, the Uba cane has remained healthy. What is apparently a third strain of the "streak" virus is common on the wild grass, Digitaria horizontalis Willd., since it differs markedly from the two described above. What appears to be a similar disease on corn in Cuba is described by Stahl (26) as "stripe." Its symptomatology is almost identical with that described for "streak" by Storey (28) in 1925 but it has a different insect vector, Peregrinus maidis Ashm., which was unable to transmit "streak" in Natal. It is suggested by Storey (31) that this may be another strain of "streak" differing in its ability to come into association with P. maidis.

The first suggestion that there might be any variation in the virus causing the mosaic disease of sugarcane and related grasses was made by Brandes and Klaphaak (6) who stated "...it is possible that there is more than one mosaic disease affecting grasses, and there is some reason for believing this to be so, ..." They were working with the attempted transfer of one source of sugar cane mosaic to a long series of wild grasses and appended this remark as a possible qualification for any discrepancies that might appear in the future in the list of grasses that they reported as susceptible. The possibility that "a more virulent form of the disease" might have been responsible for a wave of mosaic spread in 1925 at Cairo, Georgia, which produced infection on a number of varieties that had previously remained mosaic-free and had been considered highly resistant, if not immune, was suggested by Yoder (39) in 1926. This hypothesis, however, was but one of several suggested as possible explanations of this occurrence.

It was shown by Storey (30) that a mosaic disease observed to occur on maize and Sorghum arundinaceum Stapf in Transvaal, which was indistinguishable from that produced by the sugarcane mosaic virus in other localities, did not produce infection on sugarcane varieties that were ordinarily susceptible to mosaic either in the field, although the vector (Aphis maidis Fitch) was abundant, or in cage experiments conducted with the same material in Natal. He concludes that "the Transvaal virus is not virulent to sugarcane and is therefore different from the common sugar cane mosaic virus." Tims and Edgerton (37) presented observations on the behavior of mosaic affecting P.O.J. 213 in Louisiana that suggest very strongly a difference or modification of the virus. They found that diseased cuttings from near

Baton Rouge, Louisiana, produced commonly a number of healthy plants, whereas similar diseased cuttings from Reserve, Louisiana, about 50 miles distant, showed no such germination recovery but produced only mosaic plants. They concluded, "This difference in the behavior of mosaic in the two different sections suggests that there may be two strains of the sugarcane mosaic virus present in Louisiana."

RECOVERY FROM MOSAIC

Prior to the discovery of strains of the sugarcane mosaic virus a large amount of data had been accumulated on what was apparently "recovery" from the disease by certain varieties. Certain of the P.O.J. varieties had been 100 per cent mosaic in 1925. During the years 1926 to 1930, a period of apparently low secondary spread in Louisiana, there occurred a widely noted and unexpected disappearance of mosaic symptoms which, in the case of P.O.J. 213, was practically complete throughout the state. Such marked ability to apparently recover from mosaic prompted the study of recovery as a factor in field control as well as in breeding for mosaic resistance. Evidence will be presented here to show (1) that actual and permanent recovery does in fact occur; (2) its incidence among the more important commercial varieties; (3) the variability of disease transmission by cuttings; and (4) the manner in which a natural diminution and even complete elimination of mosaic may be brought about over large areas by what may be conveniently called the "recovery process."

VARIATIONS IN SYMPTOM EXPRESSION OF VIRUS DISEASES OF PLANTS OTHER THAN GRASSES

It was found by Melhus (20), 1917, that potato plants, grown in Iowa from tubers produced by mosaic-diseased plants in Maine, showed no evidence of mottling. He also has shown that infected egg plant seedlings lose all mosaic symptoms when the seedling stage is passed. The latter finding was confirmed by Elmer (12), 1925, who also observed celery from which the mottling caused by celery mosaic had disappeared. However, the filiform leaves, characteristic of mosaic diseased celery, were present at all times. Allard (1), 1917, noted that the plants of <u>Nicotiana glauca</u>, showing typical tobacco mosaic, lost their symptoms of mottling shortly after having become infected. However, inoculation from these plants showed that the infective agent was still alive. A number of investigations have been made to determine whether the sudden disappearance of mosaic symptoms was due to recovery. Dickson (9) concluded that in the case of most of these, the mosaic virus within the plant was still virulent. Brierly (7), on the strength of the results of one negative inoculation trial, decided that he had observed a case of actual recovery by a tomato plant. Two plants of the cucumber variety, Chinese Long, were observed by Summers (33), 1928, to "outgrow" their symptoms of mosaic. This variety had been considered immune to mosaic previous to this time when nine plants, including the two mentioned above, had been infected by artificial inoculations. Porter (21), 1932, observed a number of parallel cases with the same cucumber variety. He also reported a new virus, "Cucumber virus 2", as being very virulent on a number of cucurbits. A number of watermelon plants infected with this virus were observed to outgrow the symptoms in the field.

VARIATIONS IN SYMPTOM EXPRESSION OF VIRUS DISEASES IN SUGARCANE AND RELATED GRASSES

Several virus diseases have been described for sugarcane. Among these is "streak disease", which is prevalent in South Africa. Storey (29), 1926, reported that occasional plants of Uba and P.O.J. 213 sugarcane varieties became infected with what he terms a sparse form of streak disease (when leaf hoppers from streak diseased maize were allowed to feed upon them.) Successful transfer of the disease to maize showed that the infective principle was present in the juices of the sugarcane, although observations over a period of several months thereafter revealed no signs of the disease. A later paper by Storey and McClean (32) indicates the existence of at least two strains of the streak virus, i.e., the maize strain and the sugarcane strain. They observed all infections of the sugarcane variety, Uba, from maize, as well as those from Eleusine indica Gaert. and Digitaria horizontalis Willd. to be transitory. Eleusine indica, which could be infected only by virus from diseased plants of the same species, resisted permanent infection, although a few cases of transitory infection were produced.

The mosaic disease of sugarcane has shown greater irregularity of behavior than most of the diseases mentioned previously. Brandes (4), 1929, reported numerous cases of apparent recovery in corn and also in crab grass (Syntherisma sanguinalis) and foxtail (Chaetochloa lutescens), all three hosts being affected by the virus of sugarcane mosaic. He also observed that occasional mosaic-diseased stools of both sugarcane and sorghum produced tillers with no signs of mosaic, and (5) that mosaic symptoms were not present in new leaves produced by mosaic-diseased stalks of the Badila variety of sugarcane. Lyon (17) reported Lahaina to exhibit the same phenomenon.

Grey (13), working in Cuba, claimed that sugarcane, if properly fertilized and cared for, would gradually throw off symptoms of mosaic. Earle (10) disputed the possibility of such an occurrence.

Kunkel (14), reporting in 1924 on his studies of sugarcane mosaic in Hawaii, says:

> It was observed that diseased stools of certain varieties frequently recover. Careful studies show that this may come about in either of two different ways. The diseased shoots of a stool may at times begin to produce healthy leaves. Later, the old diseased leaves die and fall off. Such stools may grow to maturity without showing any further signs of disease on the leaves. They show no evidence of having been diseased unless the joints from which the diseased leaves grow have markings characteristic of mosaic. These stools may become healthy through the recovery of the terminal buds of their diseased shoots. But the disease may also be overcome in quite a different way. Although none of the diseased shoots actually recover, the new shoots produced may be healthy. The diseased shoots remain small and are overgrown by the healthy ones. After a time, they die and the stool may remain healthy to maturity. In other instances, a stool may be diseased or partly diseased in the plant crop, or in one of the ratoon crops, but after this crop is harvested, it may produce only healthy shoots. Such stools may remain healthy through the next and subsequent crop periods. This is the manner in which many stools were observed to recover in experiments to test the effect of mosaic on yield...

Kunkel's observations resulted in detecting a number of cases of recovery. More detailed observations on Lahaina resulted in seven cases of the first type described above.

Stahl and Faris (27), in 1929, observed numerous cases of recovery from leaf symptoms in the varieties P.O.J. 2714 and P.O.J. 2725 in Cuba. These two varieties, as well as P.O.J. 2883, consistently produced healthy shoots from cuttings of mosaic plants as well as occasional healthy stalks in diseased stools.

Tims and Edgerton (36), in 1931, report great variations in severity of mosaic symptoms in the very susceptible D-74 and Louisiana Purple varieties, the greener and more vigorous variants apparently reproducing these characters through several vegetative generations.

Continuing vegetative selections among the newly introduced P.O.J. canes, they found that healthy plants were produced from diseased stalks. This was true to a much greater extent in P.O.J. 213 and P.O.J. 228, classed as resistant, than in P.O.J. 36 and P.O.J. 234, rated as susceptible. They found complete disappearance of foliage symptoms occurring in the same relative proportion as the several varieties had exhibited for apparent recovery upon germination.

East (11) has found, in Cuba, following up Grey's (13) observations, that sugarcane may recover from mosaic. He says, in addition, that the same plant may recover and be reinfected as many as three times. In his words,

...such phenomena are interpretable by either of two hypotheses, both of which are immunological in character. The host may kill the infective agent, throw off all symptoms of the disease, remain in a partially immune condition for a period, and then become reinfected. It is equally possible that the host merely reduces the virulence of the mosaic virus until the latter is unable to produce the usual mosaic symptoms, although the virus continues to live within its tissue. In this case, the visible symptoms of the disease could reappear without reinfection, if the resistance of the plant were lowered by the proper combination of external and internal conditions.

From the theoretical point of view, it is immaterial as to which of these hypotheses proves to be more acceptable. The important fact is that here, for the first time, there seems to be a critical evidence of the establishment of an acquired immunity to a definite infective agent in one of the flowering plants.

He concludes with,

...Slight as the evidence undoubtedly is, it points toward the truth of the second of our hypotheses; that is to say, it is perhaps more probable that sugarcane plants gain an apparent immunity by reducing the virulence of the mosaic virus than by throwing it off entirely.

Rands and Summers (22), in a preliminary paper in 1932, report observations of numerous instances of apparent recovery from mosaic in several varieties of sugarcane in Louisiana. Field observations on "Louisiana Striped" showed a reduction in mosaic percentage from 63 per cent on June 2nd to 14 per cent on July 21st during the summer of 1932. This was believed to be actual recovery from mosaic rather than a temporary suppression of symptoms even though a wave of secondary spread increased the mosaic to 86 per cent later in the season. Healthy plants of three other "noble" varieties, i.e., "Louisiana Purple", "L-511", and "D-95", were obtained from cuttings from similarly "foliage-recovered" plants. The variety "D-74" showed no such tendencies. The newly introduced P.O.J. varieties were 100 per cent mosaic in Louisiana in 1924. By 1930, there was almost no mosaic in P.O.J. 213 and it was greatly reduced in the others. Actual observations of individual plants showed 20.2 per cent foliage recovery in P.O.J. 36-M and 0.8 per cent in P.O.J. 234 in 1930. There were no mosaic P.O.J. 213 available for such observations. Incomplete transmission by diseased cuttings was very marked in P.O.J. 36-M and P.O.J. 234 but much less so in the other varieties mentioned above and incomplete transmission by diseased stubbles was much less common than by cuttings.

IMPORTANCE OF RECOVERY

A detailed study of the recovery of sugarcane from mosaic offers many possibilities of being of great practical importance. A better understanding of this phenomenon in connection with aphid migrations and a detailed knowledge of the role of wild grass hosts (not considered in this report) would form a more intelligent basis for roguing, a common practice in many cane growing regions. Even without this and under conditions of variable secondary spread as in Louisiana, the disease was almost completely eliminated from P.O.J. 213 during the period 1925 to 1930. When a variety possesses this characteristic, roguing as a control measure (it is not now practiced in Louisiana) would be aided to the extent that recovery occurred. Unfortunately, many otherwise desirable varieties are often appreciably damaged by mosaic (as high as 50 per cent in 1932), so that such losses would be greatly lessened, particularly in ratoon crops during years of low secondary infection, if the variety grown had the ability to throw off the disease, or to produce new growth free of it. Any appreciable amount of recovery in a variety would naturally reduce the source of secondary infection, thus reducing the incidence of mosaic from two angles; i.e., the cane that recovered and the cane that would have been infected had the supply of the infective agent not been removed. Probably the most important practical phase of recovery is its possible utility in cane breeding where it is hoped that it will prove to be an heritable character and may be transmitted to desirable seedlings that are unfortunately not immune. It is entirely plausible to expect that this character might be greatly accentuated in an occasional seedling.

RECOVERY AMONG COMMERCIAL VARIETIES IN LOUISIANA

Numerous observations over a period of years in Louisiana have shown a strong tendency on the part of a number of commercial varieties to gradually reduce the incidence of mosaic in the field. This is evidently brought about in two different ways: (1) the production of healthy plants by the germination of eyes, either from planted cuttings or from stubbles in ratooning, both of which during the previous crop had supported continuously diseased foliage, and (2) the production of new foliage without mosaic symptoms by diseased plants which, by elongation of the stalk and natural death of the older leaves, common to all grasses, presently show no further signs of the disease.

RECOVERY MANIFESTED AT TIME OF GERMINATION

The sprouting of considerable numbers of healthy shoots from supposedly diseased cuttings and ratoons was noted among the newer introductions, P.O.J. 36-M, 213 and 234 as well as the long-cultivated L. 511 and Louisiana Purple, now discarded as commercial canes.

In order to definitely compare the varieties in this important characteristic, systematic observations have been made over a period of three years (1930-1932, inclusive) of thousands of plants grown under representative field conditions. Usually, the varieties were distributed in replicated single-row plots of 1/412 acre area and alternated with plots of healthy cane of the same variety to indicate the prevalence of secondary mosaic infections (i.e., spread by aphids). In conformity with field practice, all experiments were planted during the autumn preceding the year of observations. Mosaic readings were made as early in the following spring as it was possible to detect leaf symptoms.

The first experiment*, planted in the fall of 1929, was located about one-third of a mile from Bayou Black in a rear "black land" field of the United States Sugar Plant Field Station near Houma, Louisiana. The seed-cane had been selected from stools

*Planted by Dr. R. D. Rands.

inspected only at harvest time for the presence or absence of mosaic. In May 1930, evidence of recovery, shown by the sprouting of healthy shoots from the diseased seed, was noted as well as any mosaic sprouts from the healthy seed. Further readings were made in August and December. Then in the spring of 1931, when the stubbles from the previous crop sprouted, the observations were repeated on the ratoons and continued in 1932 on the second ratoons.

In the meantime, new plant-cane tests, including additional varieties, were put down each year and in most cases followed through at least the first ratoon crop. These were of necessity located on light soil near the bayou where unfortunately, as subsequent observations proved, heavy secondary mosaic spread by aphids has complicated interpretation of results on the ratoons. All records for this light soil area were on plants from cuttings or stubbles that had been observed regularly throughout at least one preceding growing season without any change in disease or disease-free status and thus called "pedigreed" in these tests. The healthy seed of D-74 and Louisiana Purple was selected in 1930 and grown during the next year before being used in the test. The Louisiana Purple seemed to be healthy when selected, but there was some question about the status of the D-74. The relative extent of recovery indicated by the early-spring observations for each of the three years is presented in table 1, and later seasonal readings of mosaic are shown in figure 5. Four plantings of mosaic pedigreed seed of P.O.J. 36-M over a

Table 1. Relative percentage of healthy and mosaic primary stalks in plant and successive ratoon crops of commercial varieties grown from pedigreed seed planted in localities subject to light and heavy secondary infection.

Variety	Year	Crop	Mosaic seed: Stalks observed	Mosaic seed: Stalks healthy	Mosaic seed: Stalks diseased	St[illegible] obs[illegible]
			Number	Per cent	Per ce	Nu[illegible]
"Black Land" test (slight secondary spread)						
P.O.J. 36-M Station seed	(1930	Plant-cane	210	59.4	40.6	[illegible]
	(1931	1st ratoon*	1379	39.0	61.0	1[illegible]
	(1932	2nd ratoon	1044	60.5	39.5	1[illegible]
P.O.J. 36-M Ashland seed	(1930	Plant-cane	198	50.9	49.1	
	(1931	1st ratoon*	1759	40.2	59.8	1[illegible]
P.O.J.	(1930	Plant-cane	242	24.8	75.2	
	(1931	1st ratoon*	1260	24.1	75.9	1[illegible]
	(1932	2nd ratoon	1062	49.5	50.5	1[illegible]
"Light Land" test near Bayou (heavy secondary spread)						
Co. 281	1932	Plant-cane	1004	0.9	99.1	1[illegible]
P.O.J. 36-M	(1931	Plant-cane	357	28.3	71.7	
	(1932	1st ratoon	1059	18.6	81.4	1[illegible]
P.O.J. 36-M	1932	Plant-cane	442	59.5	40.5	
P.O.J. 213	(1931	Plant-cane	140	0.7	99.3	
	(1932	1st ratoon	371	0.0	100.0	
P.O.J. 213	1932	Plant-cane	297	0.3	99.7	
P.O.J. 234	(1931	Plant-cane	232	24.2	75.8	
	(1932	1st ratoon	970	30.3	69.7	1[illegible]
D-74	1932	Plant-cane	114	1.8	98.2	
Louisiana Purple	1932	Plant-cane	47	4.3	95.7	

*Mosaic readings delayed until July; all others in April.

of healthy and mosaic primary stalks
ssive ratoon crops of commercial varie-
ligreed seed planted in localities sub-
leavy secondary infection.

Year	Crop	Mosaic seed: Stalks observed	Mosaic seed: Stalks healthy	Mosaic seed: Stalks diseased	Healthy seed: Stalks observed	Healthy seed: Stalks healthy	Healthy seed: Stalks diseased
		Number	Per cent	Per ce	Number	Per cent	Per cent
nd" test (slight secondary spread)							
30	Plant-cane	210	59.4	40.6	207	97.7	2.3
31	1st ratoon*	1379	39.0	61.0	1341	91.9	8.1
32	2nd ratoon	1044	60.5	39.5	1250	92.1	7.9
30	Plant-cane	198	50.9	49.1	202	97.3	2.7
31	1st ratoon*	1759	40.2	59.8	1947	84.4	15.6
30	Plant-cane	242	24.8	75.2	234	100.0	0.0
31	1st ratoon*	1260	24.1	75.9	1432	93.9	6.1
32	2nd ratoon	1062	49.5	50.5	1709	95.1	4.9
t near Bayou (heavy secondary spread)							
32	Plant-cane	1004	0.9	99.1	1127	98.3	1.7
31	Plant-cane	357	28.3	71.7	476	85.3	14.7
32	1st ratoon	1059	18.6	81.4	1351	62.4	37.6
32	Plant-cane	442	59.5	40.5	490	99.6	0.4
31	Plant-cane	140	0.7	99.3	100	99.0	1.0
32	1st ratoon	371	0.0	100.0	427	16.6	83.4
32	Plant-cane	297	0.3	99.7	459	99.8	0.2
31	Plant-cane	232	24.2	75.8	282	92.6	7.4
32	1st ratoon	970	30.3	69.7	1363	66.2	33.8
32	Plant-cane	114	1.8	98.2	119	22.7	77.3
32	Plant-cane	47	4.3	95.7	63	87.3	12.7

til July; all others in April.

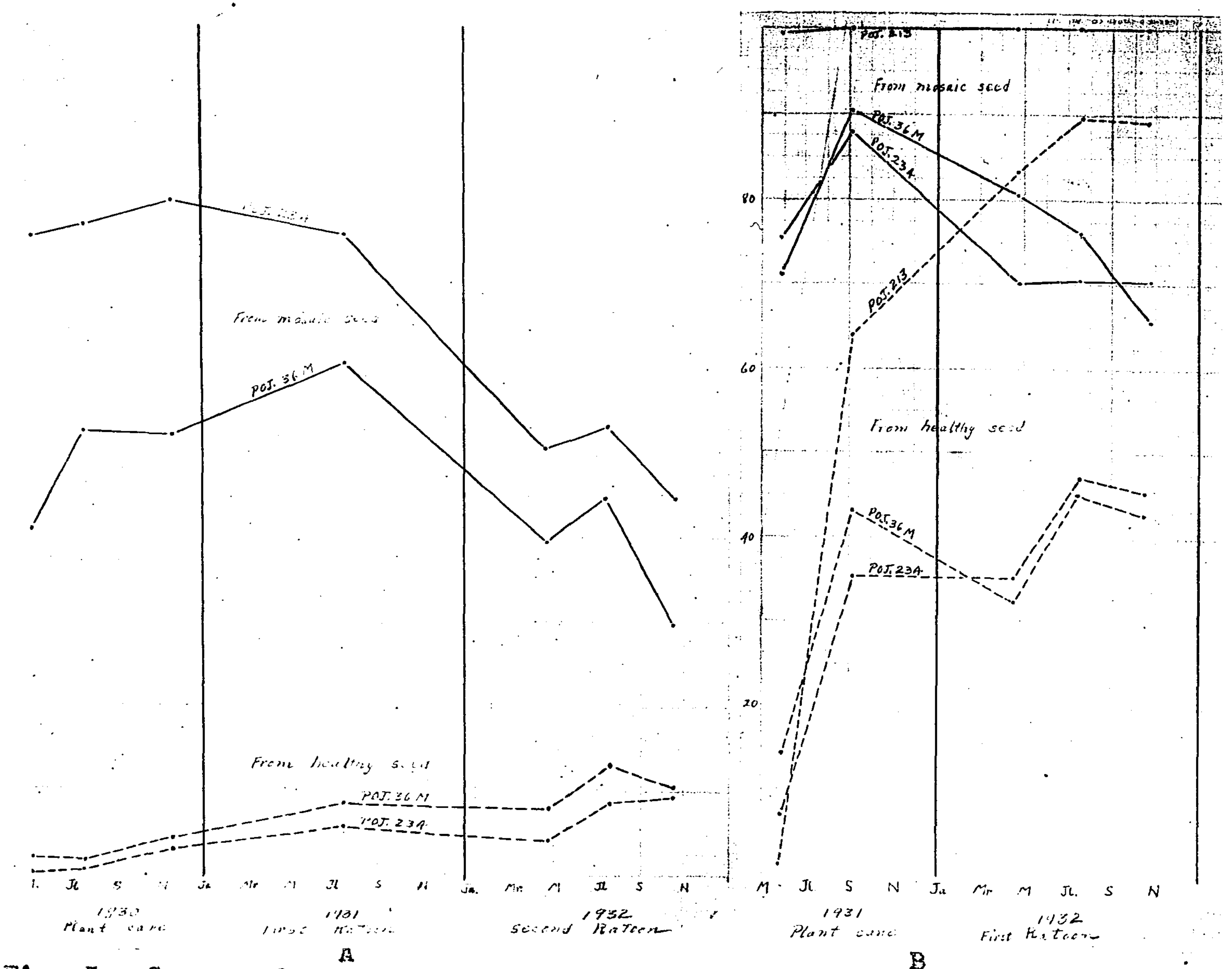

Fig. 5. Comparative mosaic incidence in plantings of healthy and mosaic seed of three varieties of sugarcane on areas subject to light (A) and heavy (B) secondary infection.

period of years produced from 28 per cent to 59 per cent of healthy shoots. The fact that more mosaic appeared in the first ratoon than in the plant-cane crop was obviously due to secondary spread during the last two growing seasons. However, in the single case where second ratoons were observed, there was a decided reduction in mosaic percentage over the previous year. A very slight reduction in the healthy plot also occurred, which is interpreted as being due to much less secondary spread in this particular field.

The behavior of P.O.J. 234 was similar to that of P.O.J. 36-M, except that a smaller percentage of healthy plants was produced. Two plantings on successive years gave almost identical results, slightly less than 25 per cent of healthy shoots. There was little change in the first ratoon crop, but the percentage of healthy plants was doubled in the one second ratoon. The four remaining varieties showed practically no recovery at the time of germination. Louisiana Purple, based on the smallest population observed, produced less than five per cent recovery, which was much higher than any of the others.

The data in table 1 include, in addition to notes on diseased seed, the percentage of mosaic from healthy seed. It will be noted that in only one case were all the germinating sprouts from this supposedly healthy seed free from mosaic at the first reading. This was in the variety P.O.J. 234. In the remaining varieties, excepting D-74, the percentage varied from 0.2 to 14.7 per cent. Three possible explanations might be offered to account for this: (1) The plants used for seed might have become infected the previous fall, but too late for symptoms to be manifested before harvesting; (2) natural spread in the spring before the cane was large enough for observing; (3) that some latent mosaic, possibly in an attenuated form, might be present in supposedly healthy cane derived from seed that was a few years previously 100 per cent mosaic-diseased. The first and last possibilities seem to be refuted by tests in an insect-proof greenhouse, later reported. This leaves secondary spread as the most plausible explanation although it is still difficult to account for as high as 14.7 per cent mosaic in the 1931 plots of healthy P.O.J. 36-M (fig. 5, B). However, they were located closer to the "bayou bank", where wild grass hosts of both mosaic and its vector (Aphis maidis Fitch) were plentiful.

The figure of 77.3 per cent mosaic for D-74 probably means that the seed was not mosaic-free. As indicated above, apparently mosaic-free stalks had been selected in 1930 from diseased stools. They were planted that fall and the resulting crop was very green and thrifty looking as compared to an adjacent row of obviously mosaic cane. This striking difference persisted throughout the season, but at the time when the seed was cut for the test, some faint suggestion of mottling made it questionable whether the plots could be called healthy. However, there was still a great difference between them and the cane grown from diseased seed, so the test was planted as originally planned, with the results indicated. The peculiar behavior of this variety agrees with the results reported for it by Tims and Edgerton (19) in 1931. They had observed it for a number of years and had selected seed each fall with the result that they were able to keep a selection of the variety that in gross appearance approximated a disease-free condition.

Influence of "Dormancy" or Unfavorable Winter Conditions on Extent of Recovery

The fact that so much recovery was manifested during germination (table 1) led to some conjecture as to whether it might be due to environmental factors. One question concerned the possible influence of the adversities encountered during the winter months in Louisiana, when the cuttings are subjected to periods of warm weather, which tend to initiate growth, followed at irregular intervals by combinations of wet and cold (often freezing) weather, which stop all growth and freeze back everything above the surface at least once every winter. In order to determine the effect of such factors, a planting of four varieties, Co. 281, P.O.J. 36-M, P.O.J. 213 and P.O.J. 234, was made on September 4, 1931, with mosaic seed selected in the field just prior to planting. The following fall and winter were unusually mild and four mosaic readings were made before March 9, 1932, on which date a killing freeze destroyed all cane, including the terminal buds, above the surface of the ground. This was the only killing frost during the winter and the cane came up again very quickly, representing in fact a ratoon growth since the terminal buds had been killed. Further readings were made in May and September.

The results (table 2) show that no recovery occurred in Co. 281. At the first reading, less than six weeks after planting, 31.5 per cent of the P.O.J. 36-M was healthy. The readings for the next two months were 50.4 per cent and 45.0 per cent healthy with practically no change in population. It jumped to 69.4 per cent on February 10 and retained this approximate percentage at both readings taken on the new shoots after the freeze. P.O.J. 213 showed no signs of recovery at the first reading on October 13, 1931; a few cases became apparent by November 16, but they were not evident on February 10, 1932. On May 11, two months after the frost, 5.0 per cent healthy cane was counted and this was increased to 7.3 per cent by September 9, due, presumably to a lesser natural mortality from crowding among the healthy stalks.

The cuttings of P.O.J. 234 produced 11.3 per cent healthy plants, which figure remained almost constant until the freeze. An additional 10 per cent recovered upon germination of the young stubbles after the freeze giving a percentage of 21.2 on May 11. This was practically the same in September.

It is of interest to note that there is about as much recovery in P.O.J. 36-M as in the average of the tests shown in table 1. This demonstrates that this variety has approximately the same ability to produce healthy plants under conditions of

Table 2. Percentage of healthy primary shoots produced by field-run mosaic seed cane with subsequent readings to show the combined effect of recovery in the field, tillering and natural mortality (due to crowding) on the subsequent proportion of healthy stalks. Planted September 4, 1931--all growth frozen back March 9, 1932.

Variety	October 13			November 16				De
	Stalks observed	Healthy		Stalks observed	Healthy		Stalks observed	
	Number	Number	Per cent	Number	Number	Per cent	Number	Num
P.O.J. 234	194	22	11.3	205	22	10.7	269	3
P.O.J. 36-M	124	39	31.5	133	67	50.4	127	5
P.O.J. 213	113	0	0.0	127	3	2.4	164	
Co. 281	99	0	0.0	100	0	0.0	127	

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			1932									
	December 11		February 10			May 11			September 9			
Stalks observed	Healthy		Stalks observed	Healthy		Stalks observed	Healthy		Stalks observed	Healthy		
Number	Number	Per cent	Number	Number	Per cent	Number	Number	Per cent	Number	Number	Per cent
269	33	12.3	277	32	11.6	728	154	21.2	272	60	22.
127	57	45.0	108	75	69.4	207	139	67.1	238	164	68.
164	6	3.7	239	0	0.0	526	31	5.9	355	26	7.
127	0	0.0	232	0	0.0	441	0	0.0	432	0	0.

quick germination as under ordinary conditions where it lies in the ground during the winter before much germination takes place. However, in this test, there is only about half as much recovery in P.O.J. 234 during germination as recorded in table 1. The percentage in P.O.J. 213 is too small in both experiments for reliable comparison.

RECOVERY SHOWN BY GREENHOUSE GERMINATIONS IN THE ABSENCE OF SECONDARY INFECTIONS

The obvious complicating effect of possible secondary infections in interpreting the results of field studies reported in tables 1 and 2 made it desirable to repeat the tests in an insect-proof greenhouse. Accordingly, during both 1932 and 1933, 20 to 100 pedigreed mosaic stools containing one to eight stalks each were selected at random from the plots that furnished the data for table 1, and every stalk cut into single-eye seed pieces and sprouted in the greenhouse. Preliminary indexing tests showed that reliable results could not be expected from very young plants, but that at least 8 to 10 weeks of growth were desirable before final judgment of their mosaic status.

The data presented in table 3 showed that pedigreed mosaic seed of Co. 281 and P.O.J. 213 has produced less than three per cent healthy offspring in greenhouse trials; P.O.J. 234 varied during the two years from about six per cent to 19 per cent, while comparable seed of P.O.J. 36-M produced approximately 59 per cent healthy cane. These figures from an aphid-free greenhouse are not significantly different from those obtained in the field and indicate that some of the variations in disease expression there observed were not necessarily due to secondary infection. In the greenhouse, there were also several cases among P.O.J. 36-M and P.O.J. 234 where individuals were healthy for a considerable part of the time they were under observation, but were tabulated as "mosaic" if they showed the disease distinctly at any time. Thus, the percentage of healthy plants shown in table 3 is less than the actual percentage at any stated time of observation.

Table 3. Extent of recovery manifested at germination by single-eye cuttings from pedigreed mosaic stools sprouted in an aphid-free greenhouse.

Variety	Year	Cuttings germinating	Healthy plants	
		Number	Number	Per cent
Co. 281	(1932	411	8	1.9
	(1933	117	0	0.0
P.O.J. 36-M				
	(1932	1264	782	61.9
	(1933	104	56	55.8
P.O.J. 213	(1932	548	15	2.7
	(1933	100	0	0.0
P.O.J. 234	(1932	1947	372	19.1
	(1933	105	6	5.7

The fluctuating expression of symptoms on occasional young plants in the field could not be regarded as particularly significant because the situation was complicated by the possibility of new infections from aphids. However, its recurrence in an aphid-free greenhouse is significant in connection with an eventual explanation of the recovery process. The fluctuations here noted are not unlike the wavering host-parasite relationship in certain mycorrhiza and other better known parasitic diseases. The transient appearance and disappearance of mosaic symptoms on these greenhouse plants suggest that in certain cases at least recovery is not necessarily due to absence of the virus from those eyes producing healthy shoots, but is probably initially present and later dies, or is strained out during germination or early growth of the young plant. Whatever the explanation, the condition usually became stabilized in a few weeks, after which the plant remained either healthy or mosaic. This was demonstrated by growing the plants one season and re-indexing the new stalks. Thus, in 1932, 126 eyes from such recovered shoots were germinated in the greenhouse and gave 100 per cent healthy plants, whereas, 111 eyes from initially mosaic stalks gave 47.7 per cent healthy.

The sprouting of healthy plants from mosaic seed cane, as described in the foregoing sections is, according to Stahl and Faris (27), apparently not to be regarded as recovery. After some preliminary indexing of P.O.J. 2714 in Cuba, in which they obtained some healthy plants from mosaic seed, they conclude: "The main point of interest is the fact that the mosaic is not distributed throughout the whole stalk, as it is in susceptible varieties." Unequal distribution, as opposed to general distribution followed by recovery during or immediately after germination, appeals to the writer, in view of the above mentioned observations, as the less plausible explanation. When, throughout an entire growing season, the successive new leaves formed by the terminal bud in elongation of the stalk have continuously shown the symptoms, it is inconceivable that the lateral buds subtending those leaves should not also contain the virus.

Distribution of Recovery in Different Parts of the Plant as Shown by Greenhouse Germination

By indexing and germinating individual eyes of pedigreed mosaic planting material, the proportionate contribution of healthy offspring by different stools and by primary and secondary stalks of the same stool, as well as of the different eyes on the same stalk, may be appraised. In May 1931, a large number of mosaic primary shoots of four commercial varieties in the experiments reported in table 1 were tagged, as well as all subsequent secondary shoots or suckers, as they appeared, giving in October the following numbers of stools in which all stalks had shown the disease throughout their growth: 12 for Co. 281, 85 for P.O.J. 36-M, 69 for P.O.J. 213 and 145 for P.O.J. 234. During the winter of 1931-32, every stalk was divided into single-node cuttings, germinated in the greenhouse as in the preceding experiments, and the young plants grown until the presence or absence of mosaic symptoms could be definitely ascertained.

In table 4, the extent and distribution of healthy offspring

Table 4. Frequency distribution of healthy offspring from indexed pedigreed mosaic seed of four commercial varieties of sugarcane in Louisiana.

Variety	Frequency classes (per cent healthy) 0	1-20	21-30	31-40	41-	5
	Per cent	Per cent	Per cent	Per cent	Per	ce
1. DISTRIBUTION OF STOOLS ACCORDING TO THE PERCENTAGE OF WHOLE STALKS PER STOOL PRODUCING HEALTHY PLANTS						
P.O.J. 36-M (45 stools)	33.3	2.2	8.9	22.2		11.
P.O.J. 234 (58 stools)	87.9	6.9	1.7	3.4		.
2. DISTRIBUTION OF STOOLS ACCORDING TO THE PERCENTAGE OF TOTAL EYES PER STOOL PRODUCING HEALTHY PLANTS						
P.O.J. 36-M (45 stools)	11.1	6.7	6.7	6.7		4.
P.O.J. 234 (58 stools)	32.7	24.1	18.9	8.6		5.
3. DISTRIBUTION OF STALKS ACCORDING TO THE PERCENTAGE OF EYES FOR STALK PRODUCING HEALTHY PLANTS						
P.O.J. 36-M (257 stalks)	26.1	2.7	0.4	5.4		5.
P.O.J. 234 (359 stalks)	50.7	12.0	10.0	10.0		10.
P.O.J. 213 (166 stalks)	92.8	4.8	1.2	0.6		.
Co. 281 (59 stalks)	93.2	5.1	1.7	-		.

fspring from indexed
cial varieties of

classes (per cent healthy)								
:	21-30 :	31-40 :	41-50 :	51-60 :	61-70 :	71-80 :	81-99 :	100
nt	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
CENTAGE OF WHOLE STALKS								
	8.9	22.2	11.1	2.2	4.4	4.4	4.4	6.7
	1.7	3.4	-	-	-	-	-	-
CENTAGE OF TOTAL EYES								
	6.7	6.7	4.4	6.7	6.7	22.2	20.0	6.7
	18.9	8.6	5.2	6.9	1.7	1.7	-	-
CENTAGE OF EYES FOR								
	0.4	5.4	5.1	3.1	4.3	7.8	6.2	38.9
	10.0	10.0	10.0	3.6	3.8	1.8	0.9	3.3
	1.2	0.6	-	-	0.6	-	-	-
	1.7	-	-	-	-	-	-	-

from individual eyes representing the different varietal populations are classified on the basis of stool units and individual stalks. The data in the first two sections of the table (comparing only those stools of three or more stalks each) show that in only one-third of the stools of P.O.J. 36-M were there no stalks that produced only healthy offspring, although there were only about one out of five stools in which 50 per cent of the stalks gave no diseased plants. There is an even greater shift toward disease freedom when total eyes per stool irrespective of stalks (section 2) are considered. This is, of course, explained by the large number of stalks producing both healthy and diseased plants. In fact, but three (6.7 per cent) of the 45 stools gave entirely healthy offspring. P.O.J. 234, in conformity with earlier tests, showed much less recovery; less than 10 per cent of the stalks of most of the stools (87.9 per cent) gave exclusively healthy plants.

The classification of individual stalks, according to the status of their offspring (section 3, table 4, which includes the full population of primary and secondary stalks) shows the majority of the stalks of P.O.J. 36-M concentrated at the extreme of the frequency table. (See also figure 6). This tendency of individual stalks (65 per cent of the total) to produce either all diseased or all healthy shoots, which is not evident on the part of the entire stools (section 1), emphasizes the stalk rather than the stool as the more important physiological unit in reaction toward mosaic. However, this is strikingly evident only in P.O.J. 36-M, which most readily recovers from the disease, although ex-

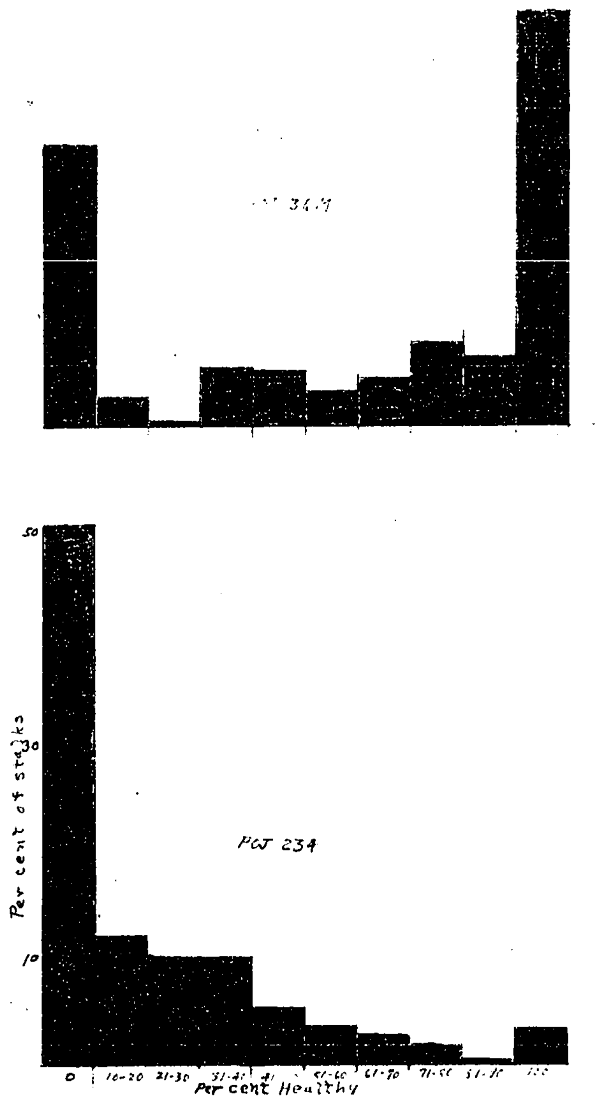


Fig. 6. Frequency distribution of percentage of stalks yielding different percentages of healthy shoots upon germination of pedigreed-mosaic seed of P.O.J. 36-M and P.O.J. 234.

amination of the detailed data on P.O.J. 234 reveals the same general tendency. (Figure 6). Co. 281 and P.O.J. 213 remained as in previous tests largely diseased.

The comparative extent of healthy offspring from primary or "mother" shoots and "suckers" or tillers revealed no significant difference, the figures for P.O.J. 36-M being 56.8 per cent contrasted with 56.5 per cent and for P.O.J. 234, 20.2 per cent compared with 18.2 per cent, respectively.

The data for P.O.J. 36-M and P.O.J. 234 on percentage of eyes of individual stalks producing healthy plants are classified in table 5 according to each quarter segment of the stalk from which they came. The stalks varied from eight to 20 joints each and the data on those not falling in any quarter were excluded. The basal quarter in each case yielded a lower percentage of healthy shoots than the upper three quarters of the stalks. However, the differences of about eight per cent and five per cent, respectively, are not statistically significant, although the agreement in trend of both varieties suggests that a repetition of the experiment on larger populations with more satisfactory germinations might establish beyond question less likelihood of recovery from the lower portion of the stalk.

In figure 7 the same data are shown graphically by individual nodes. An irregular but consistent rise in the percentage of healthy plants from the second to the fourth or fifth node (corresponding roughly to the bottom quarter of the stalk) is evident. Statistical comparison of suscessive nodes in this re-

Table 5. Percentage of healthy plants from individual eyes representing the different quarter segments of pedigreed mosaic stalks germinated in the greenhouse, 1931-32.

Variety	Stalks tested	First (basal) quarter			Second quarter			Third quarter			Fourth quarter		
		Eyes germinating	Healthy		Eyes germinating	Healthy		Eyes germinating	Healthy		Eyes germinating	Healthy	
	No.	No.	No.	Per cent	No.	No.	Per cent	No.	No.	Per cent	No.	No.	Per cent
P.O.J. 36-M	255	301	162	53.8	283	176	62.2	308	196	63.6	257	166	64.6
P.O.J. 234	363	433	65	15.0	466	96	20.6	497	110	22.1	408	79	19.4

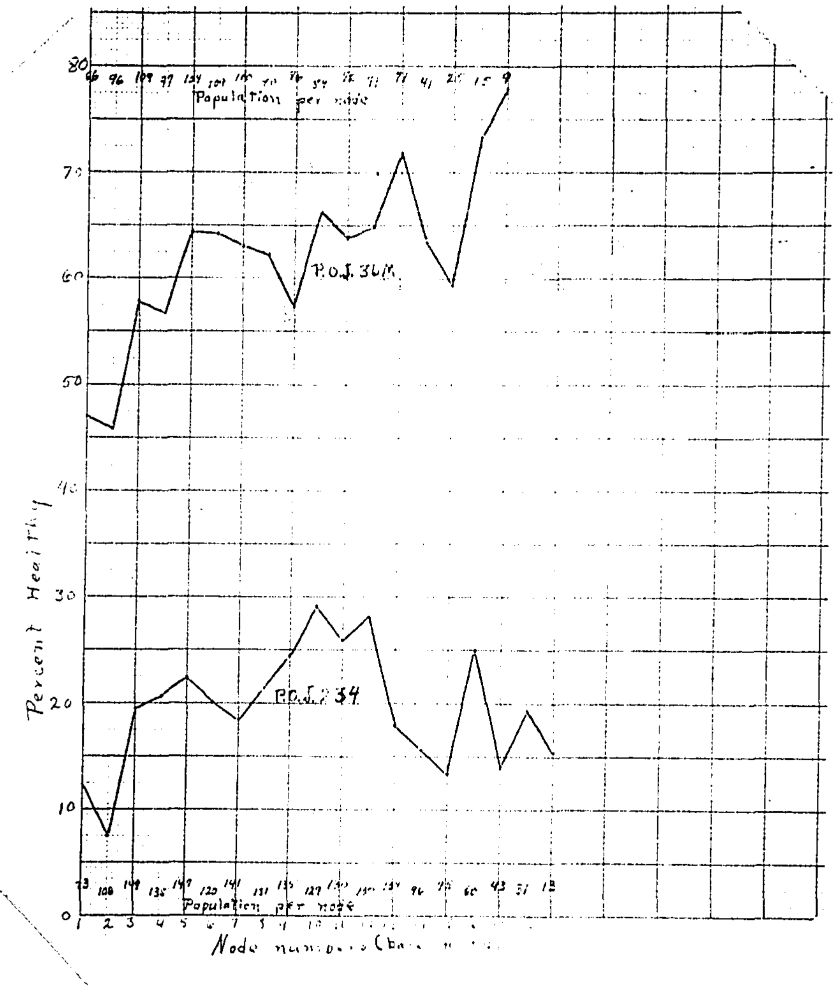


Fig. 7. Frequency distribution showing lower percentage of healthy shoots from lower nodes produced by germination of individual eyes of pedigreed-mosaic stalks of P.O.J. 36-M and P.O.J. 234.

gion indicates a reliable degree of probability against the differences being due to chance. For example, the curve for P.O.J. 36-M shows a gross difference of 12 per cent between the second and third joints. A fair comparison necessitates discarding all stalks in which one or other of these nodes failed to germinate, as well as all cases where both joints produced healthy or diseased plants. Two hundred and seventy-one stalks were indexed, of which joint 2 is represented by 96 shoots and joint 3 by 109 shoots (fig. 7). However, on 42 stalks the number 2 joint had no number 3 for comparison and on 55 stalks number 3 had no number 2 for comparison, the low percentage germination being due mainly to borer and handling injury and the higher incidence of red rot in such single-node cuttings. There are left 54 stalks on which both joints 2 and 3 produced plants, but on 36 of them both joints gave the same results, that is, either healthy or mosaic. Eighteen stalks remain for comparison, and on 15 of these stalks joint number 2 developed mosaic plants when joint number 3 was healthy, and conversely in only three cases did joint number 3 show mosaic when joint number 2 was healthy. The odds against such a difference in behavior between joints numbers 2 and 3 being due to chance are approximately 257: 1.

RECOVERY IN GROWING SUGARCANE

All of the data presented so far in this paper have had to do only with recovery manifested in the production of healthy offspring by the germination of cuttings from diseased stalks. It has been shown, for example, in repeated tests with P.O.J. 36-M that as high as 50 per cent of the stand derived from pedigreed-mosaic seed cane may start off in the spring free from the disease. Later during the growing season the total number of mosaic-free plants (barring excessive secondary spread) is further augmented by foliage recovery of many of those initially showing mosaic at the time of germination. As earlier indicated (p. 26) this is brought about by the production of new leaves without symptoms and the gradual dying and shedding of the earlier diseased leaves incident to the elongation of the stalk characteristic of all grasses.

An evaluation of this phenomenon as a varietal characteristic in relation to mosaic control necessarily involves the determination of: (1) Its comparative extent among commercial varieties, (2) variation in extent from year to year, (3) possible correlation between foliage recovery and germination recovery, (4) relative incidence among diseased plants from pedigreed mosaic and recovered lines of seed, and (5) proportion of healthy offspring shown by germination of recovered sugarcane. While additional information is desirable on most of these questions, it is believed that the data thus far obtained indicate sufficiently definite trends to warrant their publication at this time.

Systematic observations of mosaic plants for evidence of recovery by means of the individual stool and stalk-pedigree method, already described, were carried out during the growing seasons of 1930 to 1932, inclusive. The stools selected were always scattered through replicated plots of yield tests laid down to compare the effect on yield of planting mosaic seed. Therefore, the observations on different varieties are strictly comparable, and, furthermore, the amount of disease in the regularly distributed healthy plots gave some measure of secondary infections.

In 1930 only two varieties, P.O.J. 36-M and P.O.J. 234, were compared but in 1931 and 1932, as mosaic seed of other varieties became available, observations were extended to them. There also became available, during the latter two years, a considerable amount of sugarcane produced by germinating the individual eyes of the stalks of P.O.J. 36-M and P.O.J. 234 that recovered in 1930. These were indexed by nodes and germinated in the greenhouse and then transferred to the field where systematic observations were made on them in comparison with stools from pedigreed mosaic seed. A number of these individual eye-cuttings of recovered stalks produced mosaic plants in the greenhouse and many others initially healthy, later became diseased in the field, due presumably to secondary infection. These two categories furnished an appreciable supply of diseased stalks, all of which were observed for recovery.

Unfortunately, the results for 1931 and 1932 are complicated by a large amount of secondary spread, as shown by mosaic incidence in the healthy plots, so that they are unreliable from an absolute standpoint, but, due to the very large populations observed, they are nevertheless significant from a relative standpoint and are indicative at least of results to be expected under such conditions. The data for 1930, however, were obtained under conditions of approximate freedom from secondary spread and, therefore, should be fairly representative of the natural tendencies of the two varieties compared that year. These data are presented in table 6.

Varietal Differences in Foliage Recovery

During the one year (1930) when recovery was not appreciably influenced by secondary spread of mosaic, P.O.J. 36-M with 14 per cent is outstanding compared with only 0.9 per cent for P.O.J. 234.

During 1931 five varieties were observed for recovery but none occurred in P.O.J. 213, Co. 281, or L-511, and but 1.9 per cent (19 cases) in P.O.J. 36-M and 0.16 per cent in P.O.J. 234. Similar results were obtained in 1932 when the percentages of the latter were reduced to 0.4 per cent and zero, respectively.

Table 6. Summary of three years' observations on occurrence of foliage recovery in commercial varieties, including comparisons of pedigreed mosaic and recovered lines, and the proportion of healthy offspring produced on germination of recovered stalks.

Variety and source of seed	Year	Crop	Soil type	Stools	Mosaic stalks	[illegible]
				Number	Number	[illegible]
P.O.J. 36-M:	(1930	Plant-cane	Sandy	10	48	[illegible]
	(1930	"	Clay	15	95	[illegible]
	(1931	First ratoon	"	165	537	[illegible]
Pedigreed mosaic	(1932	Second ratoon	"	50	146	[illegible]
	(1931	Plant-cane	Sandy	94	448	[illegible]
	(1932	First ratoon	"	50	299	[illegible]
	(1932	Plant-cane	"	50	303	[illegible]
	(1931	Plant-cane	Clay	54	392	[illegible]
Recovered line	(1932	First ratoon	"	114	546	[illegible]
	(1932	Plant-cane	Sandy	9	72	[illegible]
P.O.J. 234						
	(1930	Plant-cane	Clay	34	219	[illegible]
Pedigreed mosaic	(1931	First ratoon	"	86	368	[illegible]
	(1932	Second ratoon	"	50	150	[illegible]
	(1931	Plant-cane	Sandy	174	870	[illegible]
	(1932	First ratoon	"	50	162	[illegible]
	(1932	Plant-cane	"	50	331	[illegible]
	(1931	Plant-cane	Clay	3	20	[illegible]
Recovered line	(1932	First ratoon	"	7	35	[illegible]
	(1932	Plant-cane	Sandy	89	641	[illegible]
P.O.J. 213:						
	(1931	Plant-cane	Sandy	131	824	[illegible]
Pedigreed mosaic	(1932	First ratoon	"	50	385	[illegible]
	(1932	Plant-cane	"	50	317	[illegible]
Co. 281:						
	(1931	Plant-cane	"	215	1125	[illegible]
Pedigreed mosaic	(1932	First ratoon	"	50	370	[illegible]
	(1932	Plant-cane	"	50	301	[illegible]
L-511:						
Pedigreed mosaic	1931	Plant-cane	"	27	64	[illegible]

ns on occurrence of foliage
including comparisons of
es, and the proportion of
ination of recovered stalks.

Soil type	Stools	Mosaic stalks	Recovered stalks		Recovered stalks indexed			
					Number	Eyes germinating	Eyes producing healthy shoot	
	Number	Number	umber	Per cent		Number	Number	Per cent
Sandy	10	48	7	14.6	7	43	29	67.4
Clay	15	95	13	13.7	13	104	60	57.7
"	165	537	6	1.1	6	38	38	100.0
"	50	146	2	1.4	2	10	10	100.0
Sandy	94	448	13	2.9	13	83	82	98.8
"	50	299	1	0.3	1	8	8	100.0
"	50	303	0	0.0	0	0	0	-
Clay	54	392	46	11.7	9	98	97	99.0
"	114	546	24	4.4	21	154	153	99.4
Sandy	9	72	4	5.6	3	33	31	93.9
Clay	34	219	2	0.9	1	6	2	33.3
"	86	368	0	0.0	0	0	0	-
"	50	150	0	0.0	0	0	0	-
Sandy	174	870	2	0.2	2	10	10	100.0
"	50	162	0	0.0	0	0	0	-
"	50	331	0	0.0	0	0	0	-
Clay	3	20	0	0.0	0	0	0	-
"	7	35	2	5.7	2	20	20	100.0
Sandy	89	641	2	0.3	0	0	0	-
Sandy	131	824	0	0.0	-	-	-	-
"	50	385	0	0.0	-	-	-	-
"	50	317	0	0.0	-	-	-	-
"	215	1125	0	0.0	-	-	-	-
"	50	370	0	0.0	-	-	-	-
"	50	301	0	0.0	-	-	-	-
"	27	64	0	0.0	-	-	-	-

L-511 was not observed in 1932. The discrepancies in the percentages for 1931 and 1932 as compared with 1930 can be explained by the great waves of secondary spread of mosaic during the last two years. This spread, entailing more or less constant introduction of new sources of infection by aphids, apparently thwarted most cases of recovery before they could be observed. In a few at least the plants put out healthy new leaves but later again showed the symptoms which persisted until harvest.

Probably, the most surprising disclosure of these tests is the failure of P.O.J. 213 to show any foliage recovery. It was expected, in view of its behavior during the period 1926 to 1930 when mosaic became practically non-existent in this variety in Louisiana after it was approximately 100 per cent diseased in 1925, that it would exhibit more recovery than P.O.J. 36-M. A possible explanation of this is presented in the discussion.

Correlation of Foliage and Germination Recovery

Comparison of the percentage of foliage recovery, as shown in table 6, with those for germination (tables 1, 2 and 3) indicate sufficient agreement in trend to suggest a positive correlation between the two "types" of recovery. The comparative figures for the four commercial varieties, P.O.J. 36-M, P.O.J. 234, P.O.J. 213 and Co. 281, reveal the latter two showing no foliage recovery and practically no germination recovery, while P.O.J. 234 shows a small amount of foliage recovery and a very considerable amount (up to 25 per cent) at the time of germination. P.O.J. 36-M showed the greatest recovery of foliage and likewise the greatest from germination.

These preliminary observations suggest that should recovery at such widely separated stages of growth have the same physiological basis, the responsible factor or factors operating during the early stages in both varieties later apparently regains a considerable intensity only in the P.O.J. 36-M. Such a hypothesis naturally presumes recovery at germination and not simply unequal distribution of the virus in the seed piece.

Recovery by Pedigreed-Mosaic and Previously Recovered Lines

The observations in 1931 and 1932 on foliage recovery among plants grown from pedigreed mosaic seed (table 6) included more or less comparably situated plants of P.O.J. 36-M and P.O.J. 234 which had been transplanted in the spring from the greenhouse. These were the mosaic plants obtained by indexing and germinating individual stalks showing foliage recovery the preceding year (1930) and, therefore, represent a recovered line.

Due to the possible influence of transplanting, uncertainty of secondary infection, differences in recovery between plant and ratoons and the fact that the plots were neither located nor arranged to compare these two sources of seed, definite conclusions regarding the relative tendencies toward recovery of pedigreed and recovered lines can not be made. However, it is of interest to note that in all possible comparisons, regardless of year, location, or crop involved, the percentage of recovery among the previously recovered lines in spite of secondary spread was never less than three times, and more often 10 to 12 times, as great as any comparable percentage of the pedigreed mosaic selections.

The counts to determine mosaic spread in healthy sugarcane showed no appreciable variation within either soil area, so that it is permissable to compare the two objects on the same soil type. Therefore, in 1932, so far as secondary spread is concerned, the two ratoon crops on clay soil and the two plant-cane crops on sandy soil are comparable and show such difference in favor of the recovered line as to suggest a definitely greater tendency toward recovery.

Production of healthy suckers from initially mosaic mother shoots of a recovered line of seed.

The preceding studies showed that mosaic plants from certain joints of recovered stalks gave an appreciably larger amount of foliage recovery when grown to maturity in the field than similar pedigreed mosaic planting material. Throughout this work it has been noted that in the growth and tillering of mosaic primary shoots from such previously recovered stalks, occasional suckers appeared from the beginning free of symptoms. No such development has ever been observed from pedigreed mosaic cuttings, the observations covering hundreds of stools over a period of three years. Invariably, the suckers from such pedigreed mosaic plants have come up diseased.

In order to determine the frequency of production of healthy suckers from mosaic mother shoots derived from recovered seed, the data have been examined, and out of a total of 30 stools in which the primary shoot was initially diseased, five stools were followed in which part or all of the suckers came out and remained healthy. A typical case observed in 1931 is shown diagrammatically in table 7. The proportion of unquestionable cases might have been higher were it not for the masking effect of secondary mosaic spread.

Proportion of healthy shoots from foliage-recovered cane.

The last column in table 6 shows the results of germinating in the greenhouse indexed single joint cuttings of most of the stalks that showed foliage recovery. A glance at the figures shows a much higher production of healthy shoots than obtained from mosaic stalks from the same plots, as shown in tables 1 and 3. There the average for P.O.J. 36-M was approximately 50 per cent and here varies from 57 per cent to 100 per cent. Therefore, the totals in table 6 must represent a summation of the effect of foliage recovery as well as any germination recovery that may be operative in sprouting of eyes that otherwise might

Table 7. Production of healthy suckers from a continuously diseased mother shoot derived from a recovered stalk.

Stalk recovery in field September 1930. Indexed and germinated in greenhouse, November 1930. Node number (base to top)	Condition of plants	Mosaic primary shoot from node no. 4 transplanted to field, June 20, 1931. Successive observations.	July 25	July 31	August 13	August 29	September 11	September 21	September 28	Dec. 4
11	Healthy									
10	"									
9	Mosaic									
8	"									
7	Failed	(Mother shoot	M[1]	M	M	M	M	M	M	M
6	Healthy	(Suckers 1	-	H[2]	H	H	H	H	H	H
5	"	2	-	H	H	H	H	H	H	H
4	Mosaic	3	-	H	M	M	M	M	M	M
3	"	4	-	H	H	H	H	H	H	H
2	Failed	5	-	H	H	H	H	H	H	H
1	Healthy	6	-	H	(3)	(3)	(3)	(3)	(3)	(3)

1 M = mosaic
2 H = Healthy
3 = Died

have manifested the disease. An evaluation of the relative influence of these two factors has not been attempted.

It may be noted that in 1930 in the absence of appreciable secondary spread a high percentage of foliage recovery resulted in a much lower percentage of healthy progeny than was obtained in 1931 and 1932 when secondary infections were abundant and foliage recovery low. This suggests a selective process whereby, under conditions of abundant secondary infection, only the most persistent cases of recovery were manifested.

The distribution of the nodes on recovered stalks giving rise to diseased plants shows no grouping towards the base of the stalk, as illustrated in figure 7, for pedigreed-mosaic seed. Healthy plants obtained both from such foliage-recovered stalks as well as from diseased stalks have been grown for three successive vegetative generations and produced only healthy sugarcane. This is believed to be sufficient proof that they no longer contained the virus and had, in fact, completely recovered from the disease.

RESUME OF RECOVERY

The "recovery" presented here demonstrates a widespread disappearance of symptoms and actual recovery from mosaic by certain "tolerant" varieties of sugarcane that occurred in Louisiana over a period of years. It was shown that, in the case of P.O.J. 36-M, "germination recovery" to the extent of about 50 per cent could be expected when pedigreed mosaic cuttings were used and that, under conditions of light secondary spread at least, an appreciable amount of "foliage recovery" would occur. P.O.J. 234 gave similar results but to a much less degree. Very little recovery of either type was observed in Co. 281 and P.O.J. 213 in these studies, although the latter had, during an earlier period of low secondary spread, practically elminated the disease in sections where it had previously been 100 per cent mosaic. The fact that this variety was unable to recover after new infections in 1930 and subsequently, and that differential rates of recovery were demonstrated for two "lines" of P.O.J. 36-M suggests very strongly that an adequate explanation for the recovery phenomenon is to be found in the assumption that different "strains" of the virus were concerned. This theory replaces the earlier one that attenuation of the virus was responsible for recovery also because negative results were obtained in inoculation tests designed to demonstrate attenuation in the "line" that showed greater recovery.

VARIATIONS IN SYMPTOM TYPES OR PATTERNS ENCOUNTERED IN THE FIELD

Slight but consistent differences in mosaic patterns have been characteristic of the different commercial varieties in Louisiana, as elsewhere. Widely divergent types of symptoms have long been noted in the seedling nurseries, due presumably to greater genetic variability and consequent greater range of host susceptibility. In the extremely susceptible class have been many seedlings whose growth was greatly retarded by the disease and many of which were either killed during the first season they showed the disease or failed to germinate the following spring, while, on the other hand, adjacent seedlings of the same cross have often shown but mild symptoms of mosaic and no visible growth retardation or other apparent deleterious effect. No striking difference, or unexplainable variation, has, however, been observed between individual plants of the same seedling. However, in the fall of 1932, while mosaic notes were being made in a group of the Canal Point 1928 series, one seedling, C.P. 28/60, was encountered which showed two radically different types of mosaic, viz., on one stool a severe pattern, typified by extreme chlorosis and some necrosis, causing marked stunting of growth and on an adjacent stool a mild pattern that was barely discernible and had caused little, if any, growth retardation.

Plantings were made, both in the greenhouse and in the field, with cuttings from each of these as well as from healthy cane of the same variety. With the exception of a few that recovered in the greenhouse, each symptom type was reproduced in the resultant shoots and maintained through successive vegetative generations. In the field, the plants produced by the cuttings from stools showing the "severe" symptoms were rather scattered, due to poor germination, and made a very poor growth the first year, and the first ratoon crop was even worse, while the other planting produced a good stand of plants with very "mild" symptoms that made a good growth comparing favorably with the healthy cane at all stages. During the following year several additional (unnumbered) seedlings of the C.P. 31-series were discovered that showed two similarly divergent types of mosaic.

At about this same time several stools, all apparently being the result of shoots from a single stalk of cane, of Co. 281, which is at present the most widely planted commercial variety in Louisiana, were discovered that showed very severe mosaic symptoms characterized by extreme chlorosis, severe necrosis, and pronounced stunting. These stools were in a commercial field that showed a fairly high percentage of ordinary mosaic. Cuttings from these stools with severe symptoms, as well as from adjacent stools showing ordinary symptoms, were planted in the greenhouse and the types have persisted through several vegetative generations except for some variation in the amount of necrosis on the plants with the severe symptoms due, presumably, to differences in growing conditions.

INOCULATION TRIALS

Materials and Methods

The inoculation technique that has proven most suitable for greenhouse work is essentially that described by Matz (19) in which the exposed "spindle", or tightly rolled young leaves, is pricked a number of times through a small quantity of juice, freshly expressed from a mosaic cane plant, placed in the axis of the youngest open leaf in such a way that it will adhere to and completely encircle the spindle mentioned above. The pricking is done with a very small needle or small insect pin, equal success having been achieved with several different types. In expressing the juice two types of grinders or juice presses have been employed successfully. The larger grinder is more desirable if a large quantity of juice is required or there is ample material available as a source of inoculum. The smaller grinder is more economical of limited supplies of inoculum and lends itself more readily to sterilization where accommodations are limited. In a majority of the tests here reported, however, both types were employed.

The grinders used for obtaining the juice used in these tests were sterilized by boiling, usually being placed in water that was already boiling and being left for some time after it had again come to a boil, as were also the containers used for catching the juice, the pipettes, needles, vials, etc. This treatment is considered an ample precaution particularly in view of the low thermal death point of the entities here treated which point will be discussed later.

The portion of the cane plants used as a source of juice was always the leaves. In earlier tests only the blades were used but eventually, it was discovered that the more succulent roll of young leaves and sheath was just as desirable so long as none of the very tender, rapidly proliferating, growing point was included. If this region was used there appeared to be a more rapid deterioration of the juice which is, of course, undesirable if it has to be kept for any length of time. The expressed juice is strained through an ordinary 16-mesh screen, to make it more amenable to handling with pipettes, into a small glass vial and, if it is not to be used within a very short time, is stored in an icebox. Whenever practical, all inoculations are repeated on successive days or with one intervening day in order to secure as high a percentage of takes as possible. All inoculation experiments were conducted in an aphid-proof greenhouse which was fumigated frequently as an additional precaution.

Results of Inoculations

It seemed probable, considering the field observations on the five varieties and the greenhouse experience in propagating C.P.

28/60, that such definite symptom variation indicated the existence of at least two causal entities, the magnitude of whose variation could be determined only by further detailed observations and carefully conducted inoculation trials. In the fall of 1933, therefore, a series of tests was instituted to establish the status of a number of the varied collections mentioned above with relation to one another.

Since C.P. 28/60 was the first variety to show two distinct types of symptoms it was selected as a possible differential host. Parallel inoculations were made into Louisiana Purple, a very susceptible variety, for comparative purposes. The first test was started prior to the discovery of the "severe" mosaic on Co. 281 and so it included, as virus sources, only C.P. 28/60, three of the unnumbered seedlings mentioned above, each of which exhibited two types of symptoms, and the three commercial varieties, P.O.J. 213, P.O.J. 36-M, and Co. 281 (two collections).

A summary of these inoculations is shown in Table 8. It will be seen that some successful inoculations were secured in all but two series, one on each C.P. 28/60 and Louisiana Purple. The symptoms produced on the latter were, in all cases, typical of mosaic previously observed on this variety, there being no greater variation between different series than within the individual series. The results of the inoculations on C.P. 28/60 were, however, of quite a different nature. Mild symptoms were produced by all inoculations with juice from individual plants

Table 8. Experiment 4. Preliminary mosaic inoculations to compare symptomatology on two host varieties produced by virus from four seedlings, each of which exhibited two distinct types of symptoms in the field, and from three commercial varieties.

Source of virus		Plants mosaic		
		C.P. 28/60 (10 plants inoculated)		La. Purple (5 plants inoc.
Variety	Type of symptom	Number	Type of symptom	Number
C.P. 28/60	Mild	9	Mild	4
C.P. 28/60	Severe	9	Severe	4
No. 196	Mild	8	Mild	5
No. 196	Severe	1	Severe	2
No. 335	Mild	8	Mild	5
No. 335	Severe	4	Severe	0
No. 393	Mild	5	Mild	4
No. 393	Severe	4	Severe	3
P.O.J. 213	Ordinary	1	Severe[1]	5
P.O.J. 36-M	Ordinary	3	Severe	5
Co. 281	Ordinary	9	Severe	5
Co. 281	Severe[2]	0	-	4

1 Not the same as secured from other juice sources, but produced a different type of chlorosis and death of growing point.

2 Characterized by more severe chlorosis than usual and no necrosis.

of the four seedlings that had shown only mild symptoms, and severe symptoms by the four corresponding lots of juice from plants showing severe symptoms as well as from P.O.J. 36-M and two lots of Co. 281, one of which had been called "severe" because it had exhibited a noticeably more severe chlorosis in the field than is usually encountered in this variety. Since no symptoms of any kind were discernible for some time on the C. P. 28/60 plants inoculated with juice from P.O.J. 213 and since 10 of the other 11 virus sources had apparently divided themselves into two natural groups, the logical conclusion seemed to be that there were two strains of the sugarcane mosaic virus present in Louisiana. Of these two it appeared that the "severe" strain was probably the "mosaic" that was common in the cane fields while the "mild" strain was a much less common one, possibly being harbored on some wild grass, that found congeniality only in occasional seedlings. Since no variety had been observed which exhibited more than two types of symptoms this hypothesis seemed to be tenable until one of the plants inoculated with P.O.J. 213 juice began to show some symptoms of a rather unusual character for C.P. 28/60. They consisted of a few elongated, whitish lesions on the leaf, the plant became stunted, and the growing point was blighted and eventually died. As a result of this several suckers appeared, most of which showed similar but even more severe lesions and similar blighting of the growing point, which sometimes resulted in the death of the shoot and sometimes in recovery and consequent resumption of growth. Since juice from this same source had produced ordinary symptoms of mosaic on Louisiana Purple it seemed likely that this was a third type of symptom on C.P. 28/60 and also a third strain of the sugarcane mosaic virus. It will be noted that these are differentiated wholly by their symptoms on one variety and that all of them produce indistinguishable symptoms on another variety.

Another inoculation test was instituted for the purpose of confirming or disproving the results obtained above. Juice was again obtained from the original stock of "mild" and "severe" mosaic plants of C.P. 28/60 as well as from Louisiana Purple plants infected from each in the previous experiments but showing identical symptoms. Since the original collection of P.O. J. 213 was from field-run material another sample, with a known mosaic history, was secured for this second test. Juice from each of the two collections of Co. 281 that were characterized by two widely different symptom patterns, as described in a previous section of this paper, was also included. The results of these inoculations are shown in table 9 and it will be noted that similar results were obtained with inoculations direct from C.P. 28/60 and from Louisiana Purple previously infected from the same source. Inoculations with P.O.J. 213 juice gave the same results as in the previous test, producing a symptom pattern on C.P. 28/60 readily distinguishable from that produced by either of the original collections from that variety.

The Co. 281 sample, that exhibited only ordinary symptoms in the field proved to be harboring the same kind of mosaic as was the P.O.J. 213 while in the previous test the two collections tested gave the same reaction as the "severe" mosaic from C.P. 28/60. The other sample of Co. 281, that had exhibited such severe symptoms in the field, produced the same identical reaction on C.P. 28/60 but, on Louisiana Purple, produced a very "severe" pattern, characterized, as was the Co. 281 in the field, by severe chlorosis and necrosis. This was the first time that symptoms of this sort had been encountered or produced on this variety and so the logical conclusion was that a fourth strain of the sugarcane mosaic virus had appeared.

Table 9. Experiment 6. Mosaic inoculations to compare symptomatology on two host varieties produced by virus from selected sources.

Source of virus		Plants mosaic			
		C. P. 28/60 (8 plants inoculated)		La. Purple (5 plants inoculated)	
Variety	Symptom history	No.	Type symptom	No.	Types symptom
C.P. 28/60	Mild	2	Mild	4	Ordinary
La. Purple	28/60 Mild [1]	7	Mild	5	Ordinary
C.P. 28/60	Severe	5	Severe	5	Ordinary
La. Purple	28/60 (Sev.)[1]	7	Severe	5	Ordinary
P.O.J. 213	Ordinary	2	Severe[3]	5	Ordinary
Co. 281	Ordinary	2	Severe[3]	5	Ordinary
Co. 281	Severe[2]	5	Severe[3]	3	Severe[3]

1 From previous test.
2 Severe chlorosis, necrosis, and stunting.
3 Similar to symptoms produced with virus from P.O.J. 213 in previous test.

Another inoculation test was planned to check the results obtained in the two previous tests but, due to a shortage of inoculable plants, only the more pertinent virus sources were included. An examination of Table 10 will show that the results obtained were entirely consistent with those previously discussed. In the last column of this table the virus sources which have fallen into specific groups based on symptomatology on C.P. 28/60 and Louisiana Purple are designated provisionally by number. The numbers chosen for the groups were selected for convenience in differentiation rather than with any idea of indicating relative importance of virulence.

Table 10. Experiment 9. Mosaic inoculations to substantiate previous results of symptomatology on two host varieties with a classification of the cultures into four strains on this basis.

Source of virus		Plants mosaic				Strain of virus
Variety	Symptom history	C.P. 28/60 No.	C.P. 28/60 Symptoms	La. Purple No.	La. Purple Symptoms	
Co. 281	Severe	2	SR[1]	4	SR	3
Co. 281	Ordinary	6	SR	6	Ord.	4
La. Purple	Severe (281 Severe)	4	SR	6	SR	3
La. Purple	Ordinary (281 ordinary)	6	SR	6	Ord.	4
La. Purple	Ordinary (28/60 severe)	6	S	6	Ord.	2
La. Purple	Ordinary (28/60 mild)	6	M	6	Ord.	1

[1] A designation used to denote the type of severe symptoms first observed on Co. 281 in the field.

A very good idea of the difference in effect of strains 1 and 2 on C.P. 28/60 can be obtained from figure 8 which shows how badly this variety is stunted by the latter. In figure 9 is shown a section of a row of the same variety which had been filled in with healthy stools and was subjected to secondary spread under field conditions. The picture shows typical effects of infection by strains 1, 2 and 4 with a healthy stool for comparison. A definite differentiation of the strains based on a careful comparison of symptoms will be included later in this paper.

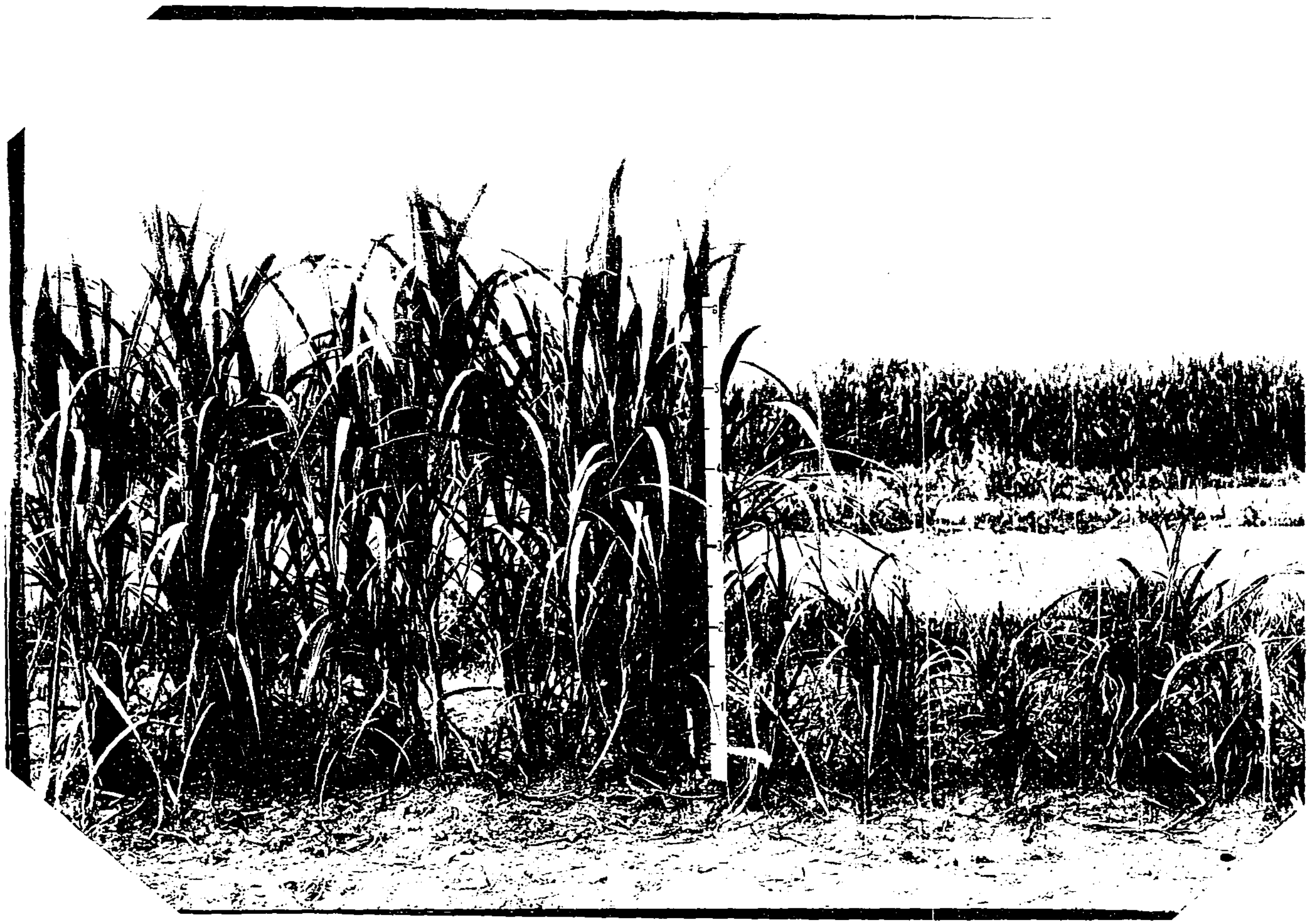


Fig. 8. Inoculation with strain 1 (left) and strain 2 (right) on C.P. 28/60 showing the retardation in growth caused by the latter. Inoculations made in greenhouse and plants transferred to field.

Fig. 9. C.P. 28/60, healthy plants from the greenhouse after five and one-half months exposure to secondary spread. Stunted stools at left infected by strain 2, large stool in center by strain 1, and the next three irregular stools by strain 4 which kills back some shoots and permits others to make a fair growth. Stool at right is still apparently healthy.

Since at this time a number of mosaic collections, particularly on Co. 281 and P.O.J. 213, together with a supply of healthy plants of six varieties of suitable size, were available, it was determined to inoculate these varieties with as many juice sources as possible with a view to obtaining further information on strain differentiation and distribution. It will be seen from table 11 the collections of Co. 281 and P.O.J. 213 represent a rather widely scattered number of localities in Louisiana sugar district.

All collections of P.O.J. 213 yielded but one strain of mosaic, i.e. strain 4, while the Co. 281 produced both strains 2 and 4. Probably the most significant development in this test was that the severe mosaic collected on Co. 281, that had caused such severe symptoms on C.P. 28/60 and Louisiana Purple, here again produced similar symptoms on Co. 281 and the three P.O.J. nos. 36-M, 213 and 234. These results indicated that there was very little of this kind of mosaic present in the state because such symptoms had not previously been recorded for any of these varieties which had been growing here for 10 or 12 years under conditions of heavy secondary spread.

The data secured from these four inoculation tests seemed sufficiently conclusive to warrant the publication of a preliminary note (34) in 1934 announcing the existence of four types of sugarcane mosaic in Louisiana. Tims (35), in 1935, reported the occurrence of two types of mosaic on C.P. 28/70, a yellow or severe type causing a reduction in tonnage of 32 per cent and in sucrose of 20 to 30 per cent and a green or mild type which apparently causes little or no reduction in growth and only a slight reduction in sucrose. His yellow type virus produced the ordinary green type when transmitted to other cane varieties. A later paper by Tims, Mills and Edgerton (38) reported essentially the same results.

Table 11. Experiment 10. Preliminary strain survey with a selected group of collections on P.O.J. 213 and Co. 281 using six host varieties.

Variety	Source of virus: Symptoms	Source of virus: Locality or source	Type of mosaic symptoms on: La. Purple	C.P. 28/60	Co. 281	POJ 234	POJ 213	POJ 36-M	Strain of virus
P.O.J. 213	Ordinary	Station(PM)	Ordinary	SR	Ord.	-[1]	-	-	4
P.O.J. 213	"	Station	"	"	"	-	-	-	4
P.O.J. 213	"	Sterling	"	"	"	-	-	-	4
P.O.J. 213	"	Haas	"	"	"	-	-	-	4
P.O.J. 213	"	Washington, La.	"	"	"	-	-	-	4
P.O.J. 213	"	Rosewood	"	"	"	-	-	-	4
Co. 281	"	Kamperdown	"	"	"	-	-	-	4
Co. 281	"	Station(PM)	"	S	"	-	-	-	2
Co. 281	"	Station	"	"	"	-	-	-	2
La. Purple	"	Rosewood(281)	"	SR	"	Ord.	Ord.	Ord.	4
La. Purple	Severe	" (281 sev.)	SR	"	SR	SR	SR	SR	3
C.P. 28/60	"	Station	-	-	Ord.	Ord.	Ord.	-	2
C.P. 28/60	Mild	"	-	-	"	"	"	-	1

[1] No plants available for inoculation.

FURTHER CORROBORATIVE EVIDENCE ON INDIVIDUALITY OF STRAINS

Following the discovery of the "severe" mosaic on Co. 281 in 1933 and the demonstration of its effect on other varieties it became apparent what great havoc might be wrought by its general distribution in Louisiana. A visit in 1934 to the plantation where it was originally found revealed the existence of 25 stools of this variety affected with the same symptoms. Most of these stools were in plant cane and the evidence indicated that they were probably the result of using seed affected by the "severe" mosaic although there was some doubt about the source of the seed, the overseer seeming to feel that it had come from a field where there was no visual evidence of this type of mosaic. It is very probable, however, that it came from the field where the first stools were discovered, in which an additional stool was found at the time of this second visit. This would not, however, explain the appearance of the original stools. These 25 stools were all removed and destroyed at the time of this later visit.

Individual stools of Co. 281 from four additional localities in the State that appeared to be affected by the same severe type of mosaic were found during 1934. These have not all been identified but at least two of them are similar to, if not identical with, the original collection and certainly different from anything previously observed.

Another visit, in the spring of 1935, to the plantation where the severe mosaic was originally found on Co. 281 revealed some rather startling information. Several additional stools of this variety were discovered showing these same symptoms, some being in the same approximate locations and others in plant cane, the seed for which had been procured in a field where no severe mosaic had previously been found but in which, subsequently, a few stools were located. At the same time a large number of stools, showing approximately the same symptoms but much less growth retardation, were found in P.O.J. 36 and P.O.J. 36-M, not only in plant cane but also in stubble fields, where the pattern of occurrence was such as to indicate that most of it had been planted there rather than having been introduced by secondary spread. This, in turn, was very good evidence that the disease had been present on the plantation and in these varieties for at least four or five years. This conclusion is easily reached because of the planting practice in vogue in Louisiana cane fields where it is customary for three to five men to follow a wagonload of seed cane, each planting a row. Thus the chances are very good that all the stalks from a certain stool will be planted within a very small area on these few rows and hence the logical conclusion that each such area is directly traceable to a certain stool in the field from which the planting material was obtained. Previous observations of a similar nature, particularly where varietal mixtures are concerned, lend credence to this hypothesis.

Preliminary inoculations, from both of these varieties onto differential hosts, indicated that the same virus was concerned here as in Co. 281. Further and more comprehensive comparisons will be necessary for final proof.

As a result of these findings it was decided by the owner of the plantation to make an honest effort to eliminate all cane showing these symptoms from his place. A trained man was employed to make a systematic search for this type of mosaic. He was supplied with a crew to dig and remove all such stools and the final results showed that over 100 of them had been located in these three varieties and quite a large number of what appeared to be the same thing in P.O.J. 234. Inoculations to confirm this latter have not been performed as yet but the symptoms agree with those produced by inoculations to this variety with the original virus from Co. 281. The location of each of these stools has been recorded and will be revisited from time to time to check on possible recurrence of the disease either from accidentally left fragments of the original stools or from possible cases of secondary infection that had not as yet evidenced themselves when the fields were being rogued.

A large number of the plants used in the greenhouse inoculation trials, excepting those inoculated with the "severe" mosaic from Co. 281, were transplanted to the field and observed during the 1934 growing season. The three types of symptoms were maintained very well on C.P. 28/60 in the field, only a few cases appearing that indicated the presence of two types on the same plant. All three had apparently been transmitted by natural spread to adjacent stools of healthy C.P. 28/60. These field observations substantiate the greenhouse experiments and offer further evidence that separate entities are involved that may be considered separate strains of the mosaic virus.

Preliminary results from a mosaic survey of Louisiana, involving about 300 collections, indicate that the three strains identified, and which produce only ordinary mosaic on commercial varieties, are all rather widely distributed although there seems to be a tendency for some localities to have but one strain and also for certain varieties to be more favorable to some strains, e.g., all collections of P.O.J. 213, to date, have yielded only one strain, but Co. 281 has yielded all three, as well as the additional "severe" strain.

Evidence of the ability of strain 3 to spread under field conditions is shown by the appearance of three stools showing type 3 symptoms in a planting of healthy cane of two varieties near a few stools of Co. 281 infected with strain 3. Two of these transfers were to P.O.J. 36-M (see figures 10 and 11) and the other to Co. 281 (figure 12).

Fig. 10. Symptoms produced by strain 3 on P.O.J. 36-M in the field (left) by secondary spread. Compare with adjacent stool showing ordinary mosaic symptoms generally found on this variety.

Fig. 11. Type 3 symptoms on young shoots of P.O.J. 36-M in the field, produced by secondary spread. The early symptoms are well shown in the younger shoot with a few long, white, chlorotic areas. Many of these have coalesced in the older shoot but the linear or striping tendency is still evident as well as a number of necrotic areas. (Compare with C.P. 28/60 in Fig. 17).

Fig. 12. Symptoms produced by strain 3 on Co. 281 in the field (right) by secondary spread. Compare with adjacent stool of healthy cane.

OCCURRENCE OR DISTRIBUTION OF STRAINS

During the period of two or three years, immediately preceding the determination of the existence of strains of the sugarcane mosaic virus, a number of collections of mosaic cane had been made and were being carried in the greenhouse. A number of these were tested for strain identity as a matter of general interest and the results reported in tables 3 and 4. These early tests indicated that there were decided tendencies for certain strains to be somewhat localized in distribution and for certain varieties to harbor certain strains, i.e., P.O.J. 213 yielded only strain 4 in these tests while Co. 281 seemed to prefer strain 2 although strains 3 and 4 were secured from it.

In 1934 a comprehensive survey of the state was conducted to check more closely on these points and possibly get some leads that would open up the problem for further investigation possibly from some different angle. It seemed to be of more than passing interest to ascertain, if possible, the geographical distribution of each strain of the virus, particularly strain 3 which threatened so great destruction and strain 1 which seemed so mild on many of the seedlings. The survey was held up by a scarcity of plants of C.P. 28/60 and so most of the collections were inoculated onto Louisiana Purple to be held there until seed material of the former variety should be available. Of a total of over 300 collections, strain determinations have now been completed on 127, including those mentioned above. Figure 13 shows a view of the greenhouse in which these inoculations were made. Many collections were lost because the original inoculations failed to produce infection, this being particularly true of a very valuable series of collections from isolated regions of the state which were secured late in the fall of 1934 and met with such unfavorable growing conditions that all were lost. It was hoped that this series would yield much of historical significance because most of the plantings were of the old "noble" varieties, Louisiana Purple and D-74, and no new mosaic had been introduced into these localities for many years.

The results of this survey, so far as it has been completed, are summarized in tables 12 and 13, the former from the variety standpoint and the latter from the locality standpoint. It will be noted that the collections are predominantly from the two varieties, Co. 281 and P.O.J. 213. This is partly due to the fact that several collections of each were available prior to the initiation of the main survey but chiefly because these two varieties seemed to hold the keynote to the strain situation. The other collections listed are fairly indicative of the variety picture in 1934 at the places included in this survey, with the exception of the collections on Louisiana Purple which were made from areas just beyond the main cane-growing area of the state and represented established plantings of long standing with

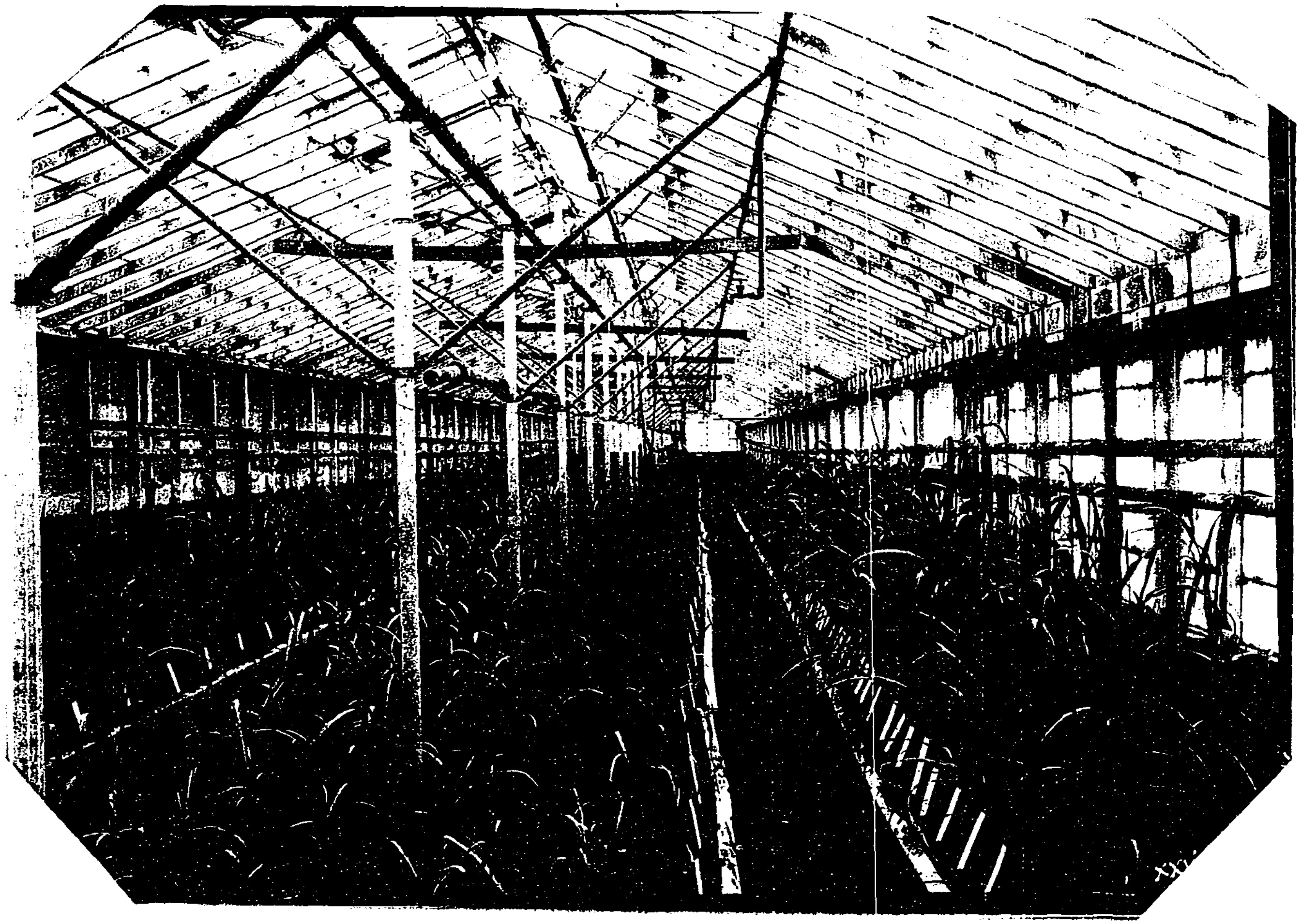


Fig. 13. Interior view of greenhouse in which inoculations were made with collections from the strain survey. C.P. 28/60 in center bed and Louisiana Purple in side beds.

Table 12. Summary of survey for strains of the sugarcane mosaic virus showing number of individual collections of each strain identified from each variety.

Variety	Number of each strain identified				
	Strain 1	Strain 2	Strain 3	Strain 4	Total
Co. 281	3	16	4	15	38
P.O.J. 213	1	6	0	15	22
P.O.J. 234	2	4	0	6	12
P.O.J. 36-M	0	1	1	3	5
P.O.J. 36	0	1	1	1	3
Co. 290	1	5	0	4	10
La. Purple	0	0	0	9	9
Others	10	14	0	4	28
Total	17	47	6	57	127

Table 13. Summary of survey for strains of the sugarcane mosaic virus showing number of individual collections of each strain identified from each locality in Louisiana.

Place	Number of each strain identified				
	Strain 1	Strain 2	Strain 3	Strain 4	Total
Albania		2			2
Alma	1	1		3	5
Ashland				1	1
Baton Rouge		1		6	7
Broussard		1	1	1	3
Bunkie		1		5	6
Cinclare		2			2
Cheneyville				3	3
Crowley				1	1
Eunice				2	2
Ellendale			1		1
Georgia		7			7
Greenwood		1			1
Haas				1	1
Hessmer				2	2
Houma	10	13		4	27
Jeanerette		3			3
Kamperdown				1	1
Mansura				1	1
Meeker	6	3		3	12
Poplarville, Miss.				1	1
Raceland		2			2
Rosewood		1	3	15	19
Sterling				2	2
Stonewall		5		2	7
Washington				1	1
Waterproof		1	1		2
West Baton Rouge		2		1	3
White Castle		1		1	2
Totals	17	47	6	57	127

little or no introduction of new stock. It will be noted, incidentally, that all of these collections proved to be strain 4. The group listed as "others" is composed largely of C.P. seedlings which exhibited two or more symptom patterns in the field.

The predominance of strain 4 in these collections is probably explainable on the basis that the survey was more concentrated in the northern part of the district. It is expected that the completion of determination of the collections now on hand will demonstrate the prevalence of strain 2 in the main section of the sugar district. With one exception, strain 1 was collected only on C.P. seedlings at the Houma station and on Meeker Plantation where mosaic has always been considered of minor importance. Whether this is explainable on the basis of what appears to be the prevalence of this "mild" form of mosaic or not it seems to be very significant.

Strain 3 was collected in three locations on Co. 281 in addition to the original location on Rosewood Plantation. Recently this strain was collected on P.O.J. 36-M and 36 and possibly also on P.O.J. 234, the latter not having been determined as yet. Since all original distributions of Co. 281 in the state were 100 percent healthy, its occurrence in even four locations indicates that this strain was not introduced with the variety but is coming from some local source.

RESISTANCE OF STRAINS TO HEAT, AGING IN VITRO, AND DILUTION

Recent attempts to clasify virus diseases of plants have stressed, among other things, their physical properties, particularly their thermal death points. Consistent variations of any extent are considered important factors in differentiating between the viruses. Experiments were planned, therefore, to determine the thermal death point of the four strains of the sugarcane mosaic virus. Preliminary tests also were run on resistance to dilution and aging in vitro.

Thermal Death Point Studies

Materials and methods.

Juice was extracted for these tests in the same manner and with the same precautions as described earlier in this paper. It was then strained through cheese cloth and 2 cc. transferred to each of a series of vials for the thermal death point tests and placed in an icebox until needed. The remainder was placed in a flask and stored for use in the other tests. The vials used were of glass, long and thin-walled, so designed as to eliminate, so far as possible, the unavoidable lag in heat penetration. Cork stoppers were used so that the vials could be completely submerged in the water-bath. A wire basket had been so designed as to hold four vials, one with each strain, in comparable positions. Exposure was for 10 minutes, after which the basket was removed and held under running water until well cooled.

The constant temperature bath was equipped with a 500-watt heating element, controlled by a mercury thermostat which was operated through a relay, and guaranteed to be accurate to within one-tenth of a degree Centigrade. A good thermometer was inserted into the bath and the control was so efficient that it was impossible to detect any fluctuation in it after the desired temperature had been reached. The bath itself was home-made from available material and discarded equipment. A four-gallon garbage can of corrugated iron was placed in a wooden box and insulated with sawdust, at least two inches thick, below and all around. It was covered with a cap, constructed of two-inch lumber, varnished to prevent warping, through which the heating element, thermostat, thermometer, and stirrer were inserted. A hinged door was provided in this cap to provide access to the bath for the virus samples. A very efficient stirrer was provided, from discarded equipment, and was operated by a belt from an electric fan motor, which had been equipped with a pulley after the blades had been removed.

In order to set the thermostat for each temperature level another piece of apparatus was devised. This was merely a small granite pail with about two-thirds of the top covered by a wooden cap to which a small, hand-operated stirrer was attached and a hole for inserting a thermometer was provided. The thermostat was set for each higher temperature level by forcing a small amount of mercury from the column where the contact was made. The bucket was filled with water and heated as slowly as possible with constant agitation of the water. The entire bulb of the thermostat was immersed until the desired temperature was obtained when the excess mercury was removed from the tip of the column by a few sharp raps and the thermostat removed. It was necessary, only a few times, to repeat the procedure because of the sensitiveness of the instrument. In the first test a glycerine solution was used for setting at the higher temperatures because the thermostat had a setting factor of 18.5 degrees Centigrade and so temperatures in excess of 100 degrees were necessary for setting it where the bath was to be controlled at 85 degrees or higher.

Procedure.

Martin (18) had stated that the thermal death point of the sugarcane mosaic virus was probably between 53 and 54 degrees Centigrade but his technique had consisted of boiling the mosaic leaves and then using them as a source of inoculum. Many viruses are resistant to much higher temperatures, however, so the first test was planned to use a range from 50 to 90 at intervals of five degrees. This required nine samples of each virus strain in addition to the untreated check which was used at once. Ten plants of the variety, Louisiana Purple, were inoculated with each of these 40 samples of juice and held in the greenhouse for approximately two months to observe the appearance of mosaic. This was considered to be a sufficiently long period of observation because, under optimum conditions, symptoms usually begin to appear on this variety in seven to eight days and very few ever show up after three weeks.

The results of this test are shown in table 14. Infection was obtained with each strain on either one or two plants out of 10 inoculated at 50 degrees but on none at 55, whereas 100 percent infection was obtained in all the checks except the one inoculated with strain 3 where only seven of the 10 plants became infected. The small number of plants infected indicate that exposure at 50 degrees Centigrade for 10 minutes has the effect of greatly reducing the infectivity of the virus.

A second experiment was planned in which the temperature levels were placed at two degree intervals from 48 to 56 degrees Centigrade. In view of the previous results it was assumed that, since the number of plants available for inoculation was limited, this was probably the best range of temperatures to use. The results presented in table 15 again show 100 percent

Table 14. Experiment No. 19. Results of inoculation trials to determine the thermal death points of the four strains of the sugarcane mosaic virus. Juice samples subjected to temperature indicated for 10 minutes and then inoculated into 10 plants of the variety Louisiana Purple.

Temperature, in degrees Centigrade, at which juice was exposed	Number of plants infected by			
	Strain 1	Strain 2	Strain 3	Strain 4
Check, not heated	10	10	7	10
50	2	1	1	2
55	0	0	0	0
60	0	0	0	0
65	0	0	0	0
----	0	0	0	0
90	0	0	0	0

Table 15. Experiment No. 22. Results of second inoculation trials to determine thermal death point of the four strains of the sugarcane mosaic virus. Juice sample subjected to temperature indicated for 10 minutes and then inoculated into five plants of each Louisiana Purple and P.O.J. 234.

Temperature, in degrees Centigrade, at which Juice was exposed	Number of plants infected by			
	Strain 1	Strain 2	Strain 3	Strain 4
Check, not heated	10	10	9	9[1]
48	7[1]	3	5	4
50	1	0	0	2
52	1	0	1	0
54	0	0	0	0
56	0	0	0	0

[1]One of 10 plants died before symptoms could appear.

infection for all the check plants except one that remained healthy in the strain 3 series. Two varieties, Louisiana Purple and P.O.J. 234, were used in this test but, since they are apparently entirely comparable for routine inoculation tests, the results are grouped together. A fairly high percentage of "takes" were obtained at 48 degrees which would indicate that all strains were much less affected than at 50 degrees in the previous test. No infection was obtained with strains 2 and 3 at 50 but strains 1 and 4 produced respectively one and two infected plants. At 52 degrees one infected plant appeared among those inoculated with each of the strains 1 and 3 but none where the other strains were used nor with any of the strains at 54 and 56 degrees.

The results of these two tests point out rather definitely the approximate thermal death point of the virus of sugarcane mosaic. Probably no sample would retain any infective properties after the 10 minute treatment, as described above, at 54 or 55 degrees Centigrade or any higher temperature. Whether there are really any significant differences between the individual strains would require repetition of these tests with probably some change in the temperature range and possibly an increase in the number of plants inoculated with each sample. From the standpoint of the temperature range it seems desirable to start at a sufficiently low temperature that no effect on the virus is apparent. By increasing the temperature levels gradually from this point and using a sufficiently large population it seems likely that some significant difference might be shown between certain strains. There is, for instance, some indication that strain 1 might have slightly greater tolerance of heat than strain 2 but the data presented here are not sufficient for definite conclusions.

Resistance to Aging in Vitro

This test was run simultaneously with the first thermal death point test and the same set of checks was used for both tests. This preliminary test was planned merely to get some indication of the length of time the virus strains would remain infective and so the periods of exposure were set at three, nine, 27, and 81 hours. Two cc. of juice were placed in a stoppered vial, one for each virus strain for each period of exposure, placed in a covered box, and stored in an incubator in which the temperature was controlled at 35 degrees Centigrade. One vial of each strain was removed at each designated time and inoculations made to 10 plants of Louisiana Purple. The results, given in table 16, show some infection with all strains, an average of about half that obtained in the checks, at the end of three hours but only with strain 3 at the end of nine hours. Since two plants were infected in the latter group, there may be some indication of greater resistance to aging by this strain than obtains in the others. This test will be repeated with shorter time intervals and storgge at probably a slightly lower

Table 16. Experiment No. 20. Results of inoculation trials to determine resistance to "aging in vitro" of the four strains of the sugarcane mosaic virus. Samples were stored in stoppered glass vials in a dark incubator at 35 degrees Centigrade. Inoculations made to 10 plants of Louisiana Purple.

Time of aging in hours	Number of plants infected by Strain 1	Strain 2	Strain 3	Strain 4
0 - Check	10	10	7	10
3	6	4	3	4
9	0	0	2	0
27	0	0	0	0
81	0	0	0	0

temperature, i.e., about 28 to 30 degrees Centigrade.

Resistance to Dilution

This test was run concurrently with the ones described above. The dilutions were made as follows: one cc. of undiluted juice was added to nine cc. of water to give one part of virus in 10 parts of the mixture. A similar portion of the latter was again diluted in the same way and the procedure repeated with the resultant mixture, so that dilutions of one part in 100 and one part in 1000 were available for the inoculations. These were made with as little delay as possible after grinding on 10 plants of Louisiana Purple and observed as before. The results are reported in table 17, and show some infection with all strains at the first dilution, with strains 1 and 3 at 1-100 and all but strain 2 at 1-1000. There is again a definite indication that this strain is less resistant than the other three.

Conclusions

The two "thermal death point" tests and one each to determine resistance to "aging in vitro" and "dilution" indicate that there is probably very little, if any, difference in the four strains of the sugarcane mosaic virus from these standpoints. There seems to be a slight tendency for strain 2 to show the least resistance and for strains 1 and 3 to exhibit

Table 17. Experiment No. 21. Results of inoculation trials to determine resistance to "dilution" of the four strains of the sugarcane mosaic virus. Inoculations made to eight plants of Louisiana Purple. (10 plants in check)

Extracted juice diluted to one part in-	Number of plants infected by			
	Strain 1	Strain 2	Strain 3	Strain 4
1 (check)	10	10	7	10
10	7	3	2	3
100	1	0	2	0
1000	1	0	1	1

somewhat more than strain 4. All of the tests need to be repeated, giving due weight to the results here reported in planning the new tests, and probably should then be followed up by final confirmatory tests because, at best, there seems little likelihood of there being any differences sufficiently great to be obvious after just one more experiment.

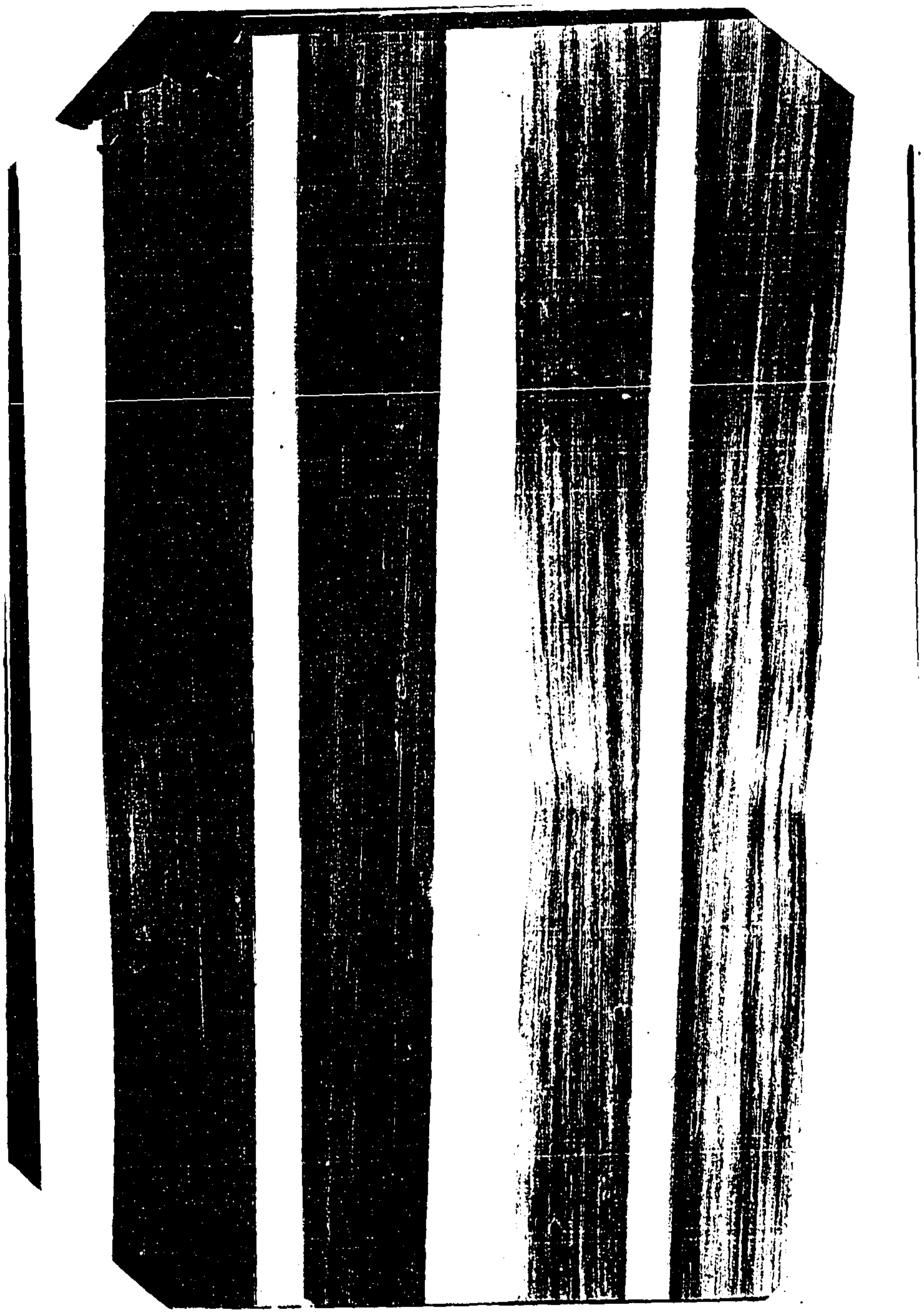
DESCRIPTION OF FOUR STRAINS BASED ON SYMPTOMATOLOGY

In view of the evidence offered above, four strains of the sugarcane mosaic virus are hereby differentiated by symptom expression on one- to four-month-old plants of C.P. 28/60 and Louisiana Purple, as follows:

Strain 1 is distinguished by a slight mottling with very little chlorosis and no noticeable stunting of C.P. 28/60 (Fig. 14, B) and by the production of ordinary (typical for the variety) symptoms on Louisiana Purple and several other varieties (Fig. 15, B).

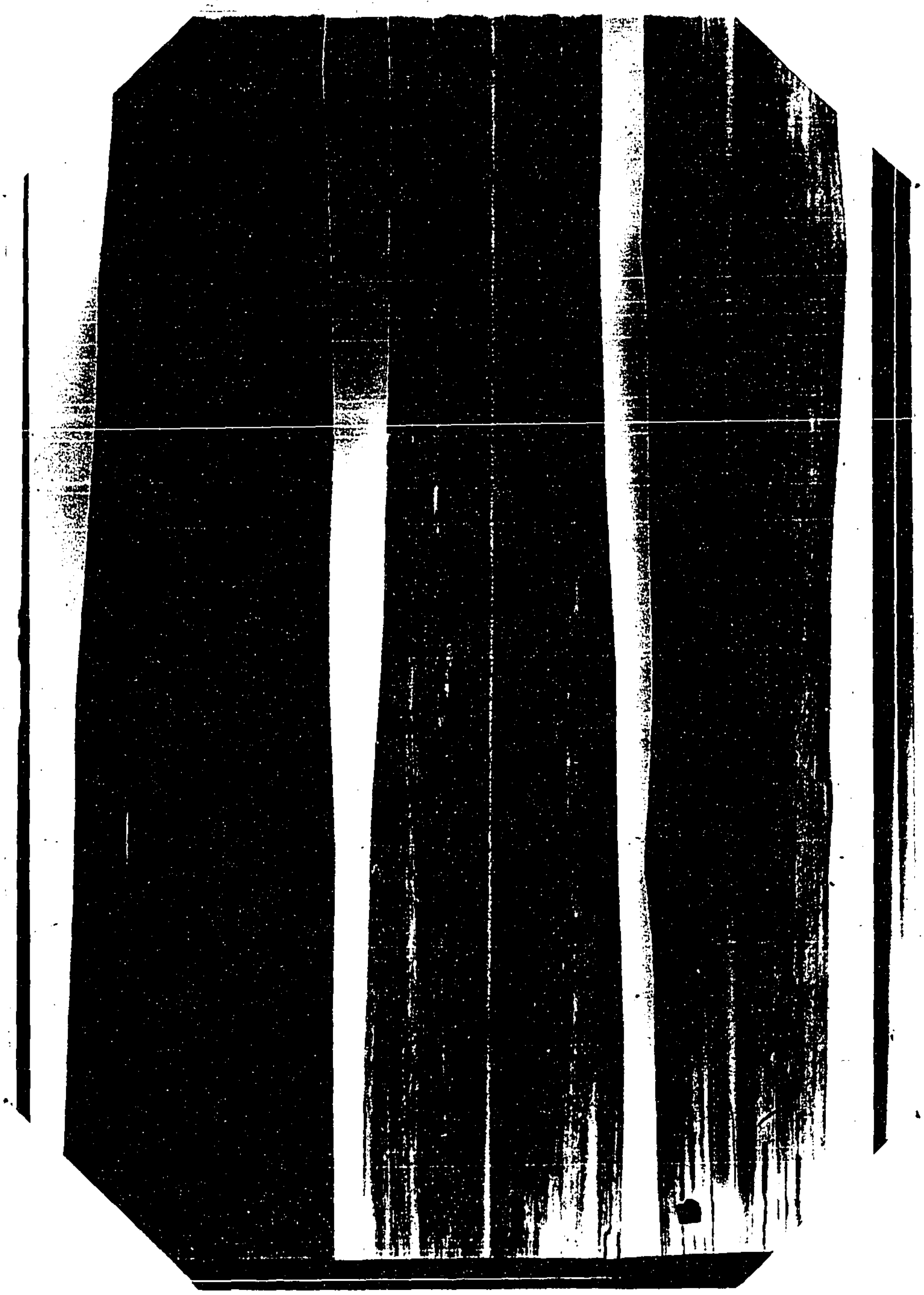
Strain 2 causes a severe mottling with large chlorotic areas, a varying extent of necrosis, and marked stunting of C.P. 28/60 (Fig. 16, A) and only ordinary mosaic symptoms as for strain 1 on Louisiana Purple and certain other varieties.

Strain 3 is first indicated in C.P. 28/60 by the development of elongated, almost white blotches or islands, some of which later coalesce into long, yellowish-white streaks or ribbons, often running the full length of the older leaves (Figs. 16B and 17). The streaks may appear only on the back of the midrib, and are frequently accompanied by necrosis, sometimes so severe as to produce temporary blighting or even death of the growing point, causing either a temporary or permanent



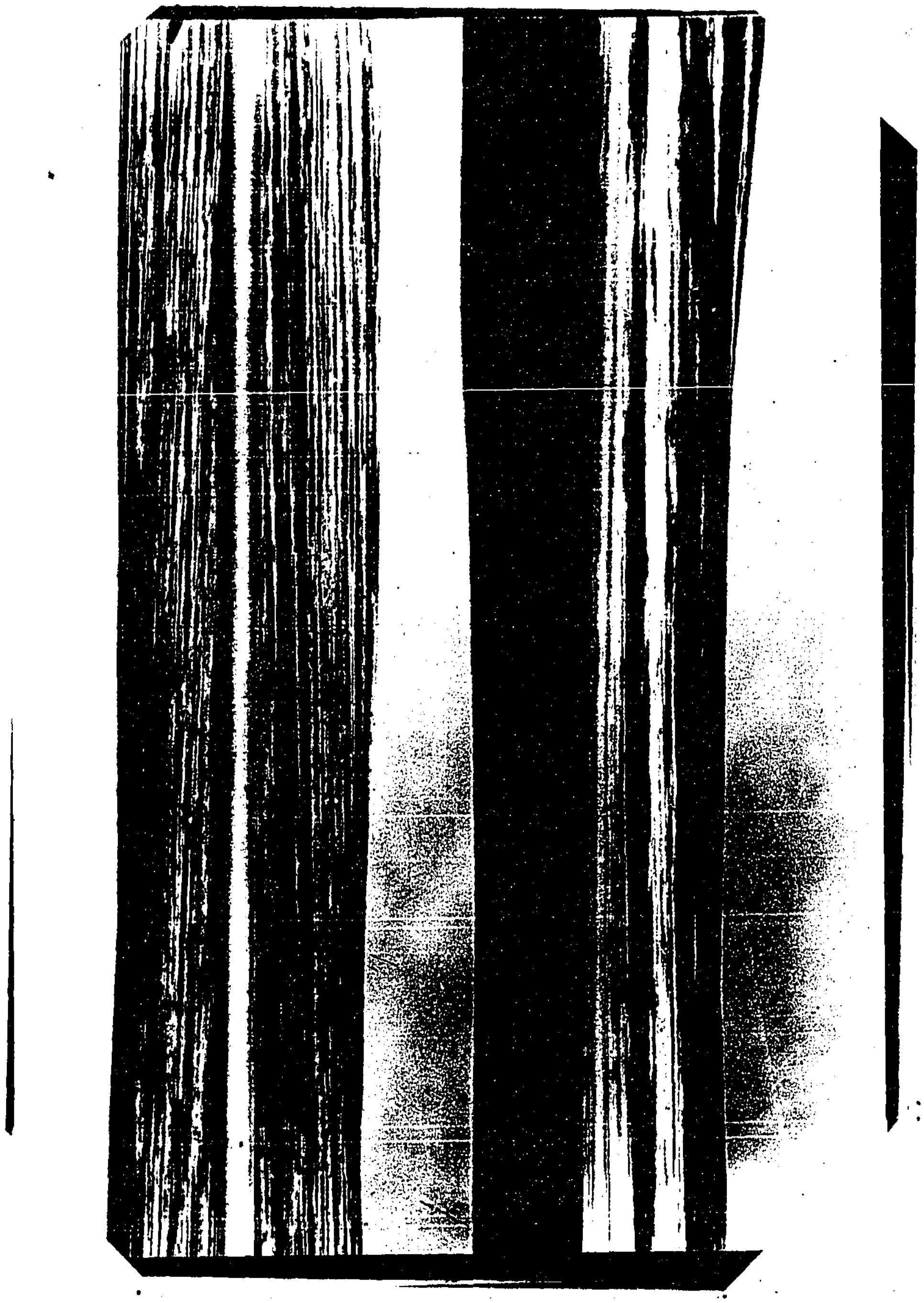
A B

Fig. 14. C.P. 28/60:
A. Healthy.
B. Type 1 symptoms of mosaic.



A B C

Fig. 15. Louisiana Purple:
A. Healthy.
B. Ordinary symptoms of mosaic.
C. Severe symptoms of mosaic.



A B

Fig.16. C.P. 28/60:
A. Type 2 symptoms of mosaic.
B. Type 3 symptoms of mosaic.

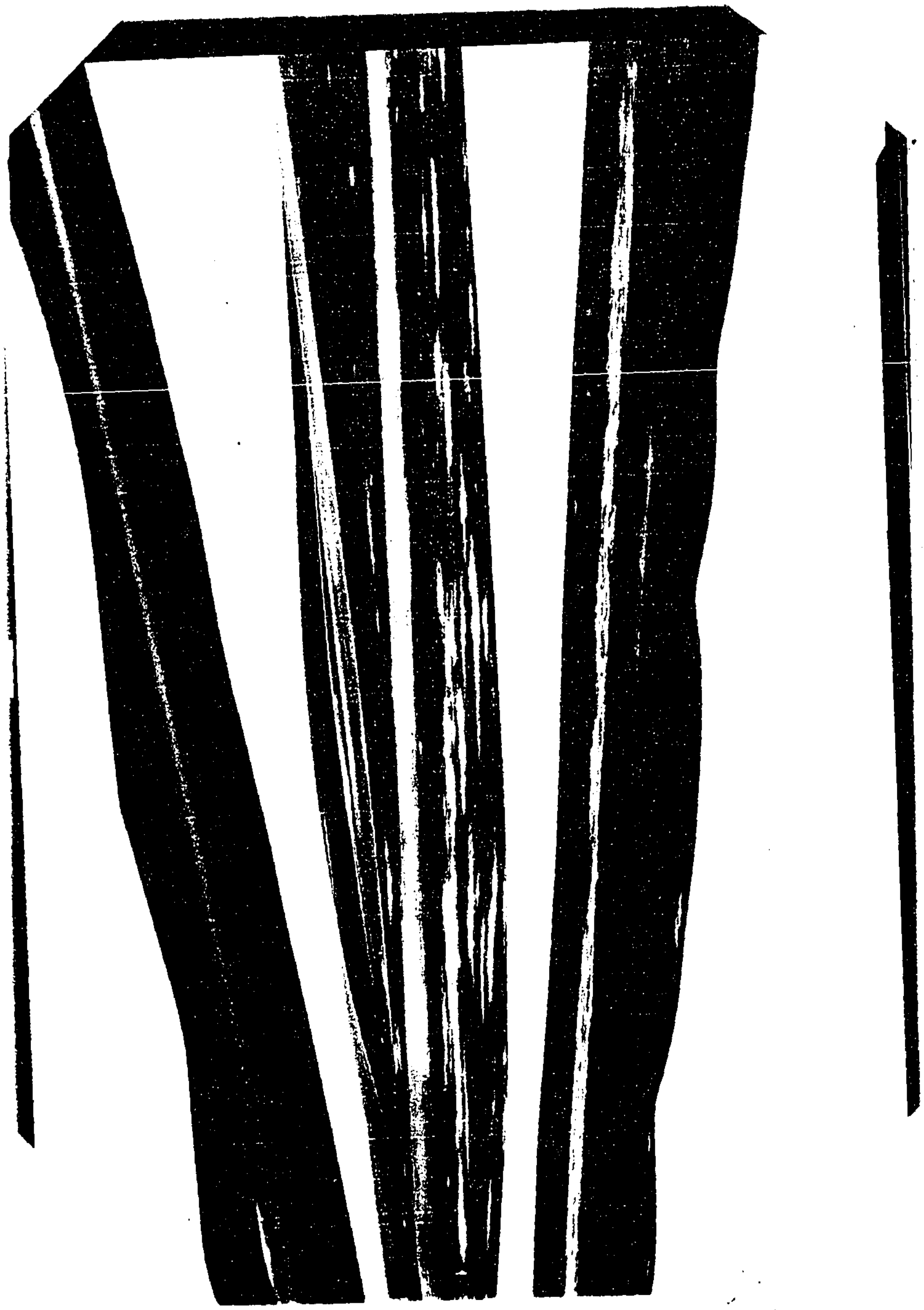


Fig. 17. Early symptoms of strain 3 on plant of C.P. 28/60 inoculated in greenhouse. Note long, white, chlorotic areas, many of which show necrosis also. Compare with P.O.J. 36-M in Fig. 11.

cessation of growth in the affected shoot. Severe symptoms similar to the above appear also on Louisiana Purple and all other varieties infected (Fig. 15, C).

Strain 4 on C.P. 28/60 produces symptoms identical in appearance and effect with strain 3 but, in common with strains 1 and 2, manifests only ordinary mosaic symptoms on Louisiana Purple.

In all tests where they have been used, four other commercial varieties, i.e., Co. 281, P.O.J. 36-M, P.O.J. 213, and P.O.J. 234, have reacted exactly as Louisiana Purple to these four strains and any one of them could well replace it as a differential host. A graphic representation of the symptom relations on the two differential hosts, upon which the differentiation of the four strains is based, is shown in figure 18.

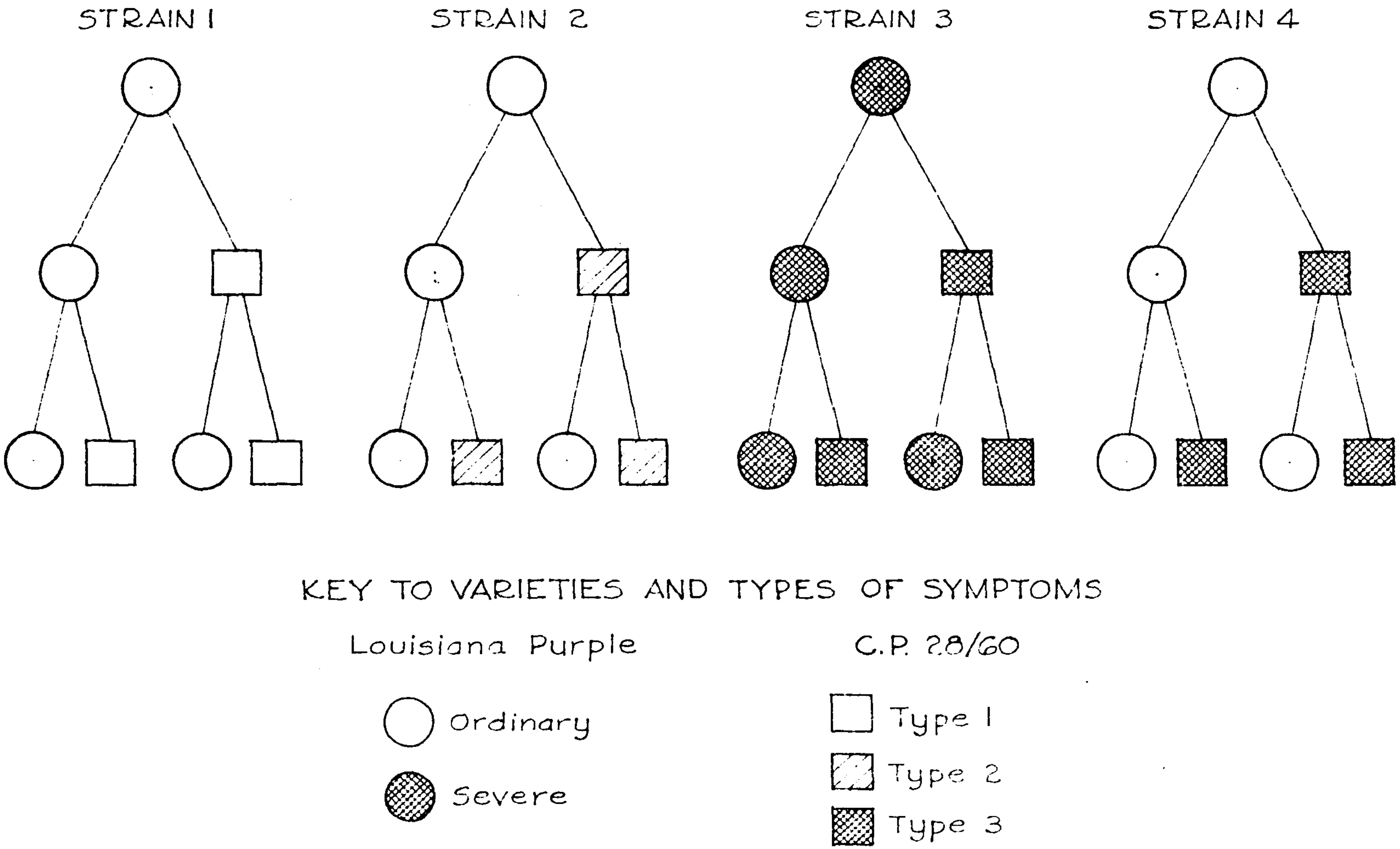


Fig. 18. Graphic representation of strain differentiation based on symptomatology.

THE SEARCH FOR FURTHER DIFFERENTIAL HOSTS

Since the four strains described above were so readily obtained and differentiated it seemed likely that further strains might be present in Louisiana or that these four strains might be further resolved by the use of other varieties as differential hosts. A survey of the possibilities indicated that such would most likely be forthcoming from the multitude of unselected C.P. seedlings of family groups available at the station, many of which were affected by a severe type of mosaic and quite a number also by a mild type or, at least, a distinct variation in symptoms. In one or two cases there were suggested three distinct types of mosaic patterns on adjacent stools of the same seedling in the field. However, there may be summarized here only the separate reactions of certain of these seedlings to known strains of the virus. Accordingly, healthy seed of a number of them was obtained and the plants compared in an inoculation test with C.P. 28/60. Five plants of each were selected for inoculation in the greenhouse with virus from each of the four known types of mosaic described above.

The results of these inoculations showed quite conclusively that at least three of the seedlings could readily replace C.P. 28/60 as differential hosts, i.e., they gave three distinct pattern reactions to the four strains of mosaic virus, and all three were easier to inoculate than C.P. 28/60 making it possible to depend on fewer inoculations for the determination of the strain of any mosaic sample. The three new seedlings are: C.P. 31/294. Seedling No. 31 from the 1931 progeny of Co. 281 x P.O.J. 2878, and Seedling No. 280, from the 1931 progeny of Co. 281 x U.S. 1694.

The other development of any great interest was that two varieties showed no infection at all with the strain 3 virus. Whether this was merely a coincidence or an indication of selective action by the hosts in question will require further investigation. There was not, however, any definite indication that any of these hosts would separate any of the current strains into two or more parts.

EVIDENCE ON THE EXISTENCE OF FURTHER STRAINS

A number of observations have been made during the course of this work that indicates a very strong probability that there are several additional strains of the sugarcane mosaic virus. Final or definite determinations of these must wait until sufficient seed of proposed new differential hosts is available for inoculations or, in some cases, even for the discovery of new differential hosts. It would seem, from the evidence about to be presented, that further strains will be more difficult to demonstrate because they are probably closely related to one of the four strains already described as witnessed by the fact that their symptom history often coincides, at least at certain stages, with that of one of the others.

During the early part of the 1933 growing season occasional mosaic stools of Co. 281 were observed that seemed to be more severely chlorotic than the general run of mosaic in this variety. This was especially true on one plantation and a few stalks were procured for testing. A small planting was made in the field and some of the juice was used for inoculating into C.P. 28/60 and Louisiana Purple. Resultant symptoms classed it as strain 2 and it was so considered for nearly two years. Shortly after the above inoculations a transfer was made from the diseased Louisiana Purple back to a few plants of healthy

Co. 281. These were later transferred to the field and seed from them was used for planting in an additional location. Observations, in 193., in both plantings revealed what appeared to be a new symptom pattern for Co. 281. The first three or four leaves of each plant appear to be harboring only ordinary mosaic but, as new leaves unfold and these become older they take on a very marked, necrotic appearance which closely resembles "stipple" in occasional susceptible seedlings but which is undoubtedly a stage in mosaic development since no analogy to this condition could be fund in extensive observations. Only strain 3 of the virus had previously produced necrosis on Co. 281 but there was not even a vague resemblance between the strain 3 symptoms and those just described. It seems likely that this potential new strain can be differentiated on Co. 281 but that the inoculated plants will have to be kept under observation for several weeks before final determination.

Another instance of variation appeared when a quantity of Co. 281 inoculated with a source of mosaic that had been classified as strain 4 exhibited about 40 per cent germination recovery and later some foliage recovery. This was the first time that authentic germination recovery had been observed in this variety, although occasional healthy stalks have appeared in mosaic rows, and it seems that such a sharp differentiation must surely predicate the existence of another strain of the virus.

A third observation that might be construed as indicative of a difference in the causal virus was the discovery that the variety C.P. 807, which had previously been considered extremely resistant if not actually immune to mosaic, had, on a certain plantation, suddenly exhibited a large number of mosaic stools which seemed to be much more common on one end of the field. There was very little mosaic in this variety on the remainder of the plantation and only one infected stool had ever been discovered previously although thousands of acres are now growing in commercial fields. It seems highly probable that a definite difference in the virus concerned here is indicated.

A few stools of C.P. 28/19 have been encountered that appear to have a very unusual mosaic pattern. These stools are also greatly stunted and generally unthrifty in appearance. It is, of course, possible that these symptoms may be the result of infection by one of the known strains but the unusual symptoms certainly warrant careful investigation before the identity of the strain is decided upon.

Several other instances suggesting variation have been encountered but the evidence, in most cases at least, is of a negative nature. In this category are a number of virus collections that seem to be rather specific in their host relationships. Several instances have come to notice where a certain virus source seemed unable to infect a certain cane variety when other sources, apparently identical in other respects, infected the same variety quite readily. This condition seemed to be particularly likely to occur when the source of virus was a certain variety from a rather limited portion of the State. As stated above, however, such evidence is of such a nature as to preclude its being given much weight at this stage of the investigation.

It is believed that the observations reported here offer very good evidence that additional strains of the sugarcane mosaic virus are extant in Louisiana. That some of these, at least, are probably closely related to one or another of the four strains described in a previous section seems to be indicated by the fact that, at first, they were classed with them but that later developments suggested minor, but nevertheless significant, differences. Final proof will, of course, depend upon the results of further inoculation trials.

DISCUSSION

The observation of two distinct types of mosaic on the seedling variety C. P. 28/60, in 1932, followed by similar observations on several other varieties the following year, led to a series of inoculation tests that established the existence of four strains of the sugarcane mosaic virus based upon the symptom patterns produced upon two differential hosts, C.P. 28/60 and Louisiana Purple. These four strains were differentiated by the production of three types of symptoms on the former variety and two on the latter. In all cases where cuttings were planted, both from the original collections and inoculated material, the mosaic patterns persisted in the new shoots. Further evidence on symptomatology, differential germination recovery, and possible host (varietal) specificity indicate a strong probability of the existence of a number of additional strains. Determination of the status of each individual "suspect" apparently will demand additional refinement of technique from the standpoints of differential hosts, virus preparation, and so on.

The search for differential hosts has been centered on seedling varieties that show two or more symptom types, several of which offer much promise, but may have to be extended to include some of the older commercial varieties with which the disease has been associated in the field. Virus preparation is a matter of major importance because of the difficulty experienced in obtaining infection with juice expressed from certain varieties that are very resistant to the disease but occasionally become infected. The variety C. P. 807, for instance, has long been considered immune to mosaic but it is now showing quite an infestation on one plantation. Attempted juice transfers have, to date, always failed. A solution of this difficulty would be of great benefit in strain studies.

Strains 2 and 4 are widely distributed in Southern Louisiana, as shown in a strain survey which is still in progress, although there is a marked tendency for localization in certain sections. Strain 1, which produces very mild symptoms on all varieties, has been found chiefly on Canal Point seedlings at the Houma Station but has also been collected on Meeker Plantation at the northern edge of the "sugar-bowl" where mosaic has always been considered of little importance. Strain 3 causes very severe chlorosis and necrosis on all varieties which have been infected with it. It has been collected mainly on only one plantation although three isolated stools from other sections apparently represent the same strain. All collections of this strain have been from Co. 281 until 1935 when it was found also on P.O.J. 36, P.O.J. 36-M and probably P.O.J. 234 in considerable amounts. The severity of strain 3 in all varieties makes it a very great potential hazard to the sugar industry of the state. A roguing program has been initiated in the hope of eliminating it before it becomes generally distributed. The pattern of occurrence on Rosewood Plantation, where it was first encountered, suggests that this strain has been present there at least four or five years.

Preliminary experiments to test resistance to heat, aging in vitro, anddilution indicate the possibility of minor differences between certain strains but probably none of very great magnitude. The thermal death point, for all strains, is probably below 54 degrees Centigrade for a 10-minute exposure.

Evidence that the entities here reported on are sufficiently different to be considered strains seems incontrovertible while, on the other hand, these strains seem to have sufficient characteristics in common to preclude any great possibility of their being considered separate diseases. On the basis of symptoms alone they are shown to be similar in that strains 1, 2 and 4 produce indistinguishable symptoms on Louisiana Purple while the same is true of strains 3 and 4 on C.P. 28/60. The four strains are further alike in that there is apparently little, if any, variation in their respective thermal death points. The same insect, *Aphis maidis*, is the vector of all the strains. There is but little evidence that there is any difference in host range, although some varieties of sugarcane seem to yield certain strains rather consistently even when other strains are known to be present on other varieties and some varieties show a tendency to resist infection by some strains although readily infected by others.

Brandes (3), in announcing the discovery of sugarcane mosaic in the United States in 1919, showed that it had probably been introduced prior to 1913 since previously there had been no quarantine laws regulating the importation of sugarcane from other countries. Large numbers of introductions had been made from all over the world and mosaic, of course, may have been introduced from a number of sources. Whether some of these sources may have furnished separate strains of the virus that have persisted, without producing any visible symptom differences, on the varieties available in Louisiana until the advent of suitable differential hosts, is a question that would involve the determination of the mosaic strains extant in all sugarcane-producing countries where the disease is known. That this would offer a satisfactory explanation for the origin of strains is highly improbable because of the promiscuous interchange of varieties between various countries prior to general recognition of mosaic. This practice would have tended to make negligible the probability of one strain existing alone in one country and still others in other countries. Strain 3, which ostensibly has arisen simultaneously in several sections of Louisiana but in such limited amounts as to preclude any great possibility that it has been present for such a long period, would most certainly have been recognized had it been long present because of its very severe effect on all varieties.

Another possibility, which has analogies in many other fields of virus research, is that we have been dealing with a complex virus which is being gradually fractionated by vectors or by its host plants, which include, in addition to sugarcane and a number of other cultivated grass plants, a large number of known, and probably many unknown, wild grasses. The possible selective action of some of these hosts has much to recommend it as a theoretical explanation of the appearance of so many strains. There have been observed, also, in the symptomatology of these strains, certain indications that some of them have much in common which would tend to establish their common origin or something common in their make-up. However, preliminary attempts to synthesize strain 3 by combining the other three strains in various ways have met with failure.

A third possible explanation for the sudden appearance of new types is infection of cane by a virus, not highly specialized as to host range, such as, for example, celery mosaic virus No. 1. Attempts to transmit strain 3, the most divergent of the new cane strains to Commelina nudiflora, a common wild host of the celery mosaic virus, have thus far met with failure. This plant, a common weed in Louisiana cane fields, has never been observed there showing symptoms of mosaic.

The possibility that these strains have arisen as variants, or mutants, of what had previously been a single, constant entity must not be overlooked. Strains of other plant viruses and some animal viruses have long been known and new ones are being increasingly described with often considerable evidence of spontaneous origin, as for example, by heat treatment or incubation in a different host.

The virus that causes sugarcane mosaic is readily transferred to many other graminaceous hosts and is often found naturally occurring on them, but there has not been reported any authentic occurrence of grass mosaic except in or near areas where sugarcane is grown, although both corn and sorghum are, under favorable conditions, readily infected with the disease. The absence of a source of infection or suitable vector is not believed to be an adequate explanation of this condition because a number of perennial grasses are known to be susceptible to mosaic and to carry it over from year to year and Aphis maidis is common in areas far removed from any sugarcane. Mosaic, therefore, is essentially a disease of sugarcane and its occurrence on other hosts is dependent on the proximity of that plant.

The existence of four strains, and the probable existence of others, of the sugarcane mosaic virus in Louisiana has offered a plausible explanation for many conflicting data accumulated on recovery from mosaic. The most logical interpretation, at the end of about three years work, seemed to be that recovery was the result of a reduction in concentration or even a qualitative attenuation of the virus. These conclusions were, however, based on purely observational data and, for this reason, open to criticism.

The extensive germination and foliage recovery demonstrated in these studies, particularly for P.O.J. 36-M, emphasizes its importance as a varietal character in reducing field losses and in the selection of promising new seedlings that may not be immune to the disease. That recovery, as here implied, is such in fact, and not mere masking or temporary disappearance of symptoms so often recorded in the literature for other plants, has been proven by continued vegetative propagation under various conditions in both greenhouse and field. Pedigreed-mosaic seed of P.O.J. 36-M has usually produced about 50 per cent healthy plants. Unpublished results of replicated yield comparisons made during the same period show from 12 per cent to 20 per cent reduction in tonnage of plots, showing about this proportion of healthy plants, compared with about 50 per cent when the planting was done in hills with sufficient seed to permit subsequent removal of all healthy plants from mosaic plots and thinning to comparable numbers in the healthy plots.

In the first type of yield experiments, additional recovery during sprouting and growth of the successive ratoon crops has further minimized the effect of the disease. At the rate of recovery shown in these tests and in the absence of new infections, this variety should nearly eliminate the disease over a period of a few years as actually occurred over large areas during the period of 1926 to 1930 in the case of P.O.J. 213.

An understnading of the physiological or structural basis of recovery from mosaic must await further knowledge of the multiplication and spread of the virus in different parts of the plant and its relation to the processes of germination and growth. No evidence on these questions other than the mere expressions of symptoms has been obtained in these studies nor found in the literature. Therefore, the germination of a healthy sprout from a diseased stalk does not necessarily prove the absence of the virus from that particular bud, as suggested by Stahl and Faris (27); it may represent merely recovery during germination of a diseased bud. This point might be determined by population comparisons in which one representative lot of diseased stalks is germinated as usual and the expressed sap from the individual buds of the other tested by artificial inoculation. Unfortunately, facilities have not been available for such comprehensive comparisons. However, the early fluctuation and disappearance of symptoms noted in some of the germination tests shown in tables 1 to 3 suggest strongly the initial presence of the virus in all the buds and that its subsequent irregularity of transmission must be due in part to physiological variation among the different buds during germination.

The results at this point suggested a reduction in concentration and in some cases a qualitative change or attenuation of the virus brought about by continued association with an uncongenial host. Eventually, through possible weakening or reduced multiplication of the virus, the balance between host and parasite might conceivably become so delicate as to permit failure of disease transmission.

A quantitative or qualitative change in the virus might explain the occurrence of foliage recovery, which experiments show, does not necessarily mean elimination of the virus from the stalk. The delicate balance might be presumed to persist for some time, even until the stalk is germinated, whereupon occasional eyes may give rise to diseased plants. Such diseased plants, however, as shown in table 6, are apparently much more likely to exhibit foliage recovery than diseased plants from seed that has not previously recovered.

Reduced concentration and even qualitative attenuation of the virus is suggested by the relative behavior of the two varieties, P.O.J. 36-M and P.O.J. 213, during 1931 and 1932. The latter variety, until 1931, had been considered to be the most resistant of the four P.O.J. varieties grown in Louisiana. It has further been conceded to exhibit far more recovery than P.O.J. 36-M both in the field and at the time of germination. The superiority of P.O.J. 213 in these respects was entirely supported by the facts as they appeared in 1930 when it was nearly impossible to find a field of this variety containing any considerable amount of mosaic. Therefore, the planning of a yield test in the fall of 1930 made it imperative to locate a supply of diseased seed. This was finally found in a narrow strip of cane along a bayou bank entirely surrounded by wild grasses in an area notorious for heavy mosaic infestations. The seed planted here the previous year had come from a field that was mosaic free, so that the infected plants had presumably received their inoculum from wild grasses. This newly infected cane furnished the "seed" for all the P.O.J. 213 tests listed in this paper with the exception of the one reported in table 2, which, incidentally, showed more recovery than any of the others.

The failure of this newly infected seed of P.O.J. 213 to show any foliage recovery or any appreciable germination recovery suggests very strongly that it had become infected with a different strain of the virus. On the other hand, the P.O.J. 36-M obtained from the center of a field of several hundred acres where direct transfer from grasses would for several years have been negligible continued to show quite an appreciable amount of recovery even in the face of heavy secondary spread, presumably largely from cane to cane, in 1931 and 1932. If attenuation were the correct explanation of the above paradoxical behavior of these two varieties, P.O.J. 213, after prolonged association with the virus, should again exhibit active recovery. This has not, however, proven to be the case. In a preliminary test in 1932 four plants of this variety derived from mosaic-free seed from the Washington quarantine house were artificially infected in a screened enclosure with extfacted juice from mosaic plants of a recovered line of P.O.J. 36-M. Two of the four subsequently showed foliage recovery and the stalks gave only healthy plants after indexing in the greenhouse. Therefore, the behavior of the variety in this limited test is in striking contrast with the recent field experience.

Practically no recovery had been recorded in four years' observations on Co. 281 until the spring of 1935 when germination readings on several lots of this variety, inoculated with mosaic juice from a number of sources, showed 40 per cent healthy shoots in one lot. The virus source used here had been determined to be strain 4 as had several others in comparable lots of seed planted in the same test none of which showed a single healthy plant.

East (11) suggested that recovery of sugarcane from mosaic might be due to acquired immunity in the host. This hypothesis is refuted by artificial inoculation trials with shoots from recently recovered plants and they have proven to be at least as readily infected as plants of the same variety that had never had mosaic previously.

Although there seems to be little similarity in the background of the phenomena observed in these three varieties, it is readily discernible that, in all three instances, the assumption of the participation of more than one virus strain would offer a satisfactory explanation for what took place. In the case of P.O.J. 36-M, for instance, it seems very probable that the original material, from which the lines used in this study were selected, contained individual stools infected with at least two different strains of mosaic. That one of these strains would find its relationship with the host variety much less compatible than the other is an assumption that certainly does not lack for precedent. Such a situation would naturally presuppose more recovery where such conditions were present. Whether a third strain or a mixture of two or more strains may have further complicated the situation is, at present, a matter for conjecture. In seeking an explanation for the behavior of P.O.J. 213 it is only necessary to suppose that its early experience with mosaic involved only a strain that, as in P.O.J. 36-M, did not find this variety a sufficiently congenial host to warrant a permanent domicile without constant reinforcements which were supplied by abundant secondary spread throughout the few years immediately preceding 1925. As soon as this wave of secondary spread subsided the host began to get the upper hand and was soon able to entirely eliminate the invading virus. During these lean years, however, a different strain of the virus was slowly but surely becoming established. Possibly it had not even been in existence during the period just described. So when a new wave of secondary spread got under way in 1931 it was a different strain that took possession of the variety and found greater compatibility than its predecessor had enjoyed.

With Co. 281, there is a more clearcut case. Out of a half dozen lots of the variety, each inoculated with separate collections of virus all of which had been determined as strain 4, there was not even a single case of germination recovery except in the case of the lot receiving virus collected in an isolated community where the cane fields are small and infrequent. Of some 65 or 70 plants appearing in plots planted with this seed, approximately 40 per cent were healthy and have remained so. The logical conclusion is that this represents a different strain of the virus, possibly closely related to strain 4, that cannot as yet be identified by symptomatology on the present differential hosts.

The desirability of a series of experiments for the observation of possible differential recovery rates in a variety, aliquots of which have been infected with known sources or strains of the mosaic virus, is acknowledged. That the results of such tests would lend support to the theory that certain varieties are able to recover more readily from one strain of the virus than from another seems quite likely. Such differential response to strains would, of course, entirely displace the theory of "attenuation" as the explanation for recovery.

SUMMARY

Two distinct strains of the sugarcane mosaic virus have been differentiated from several seedlings showing two types of symptoms in the nursery plantings of the United States Department of Agriculture at Houma, Louisiana. Two additional strains were identified from fields of Co. 281, the leading commercial variety of the state.

Through repeated vegetative propagation of the original and various sub-inoculated hosts, each of these four strains has maintained its separate identity, as readily shown by the distinctive symptoms reproduced when inoculated into certain differential varieties.

The seedling variety C.P. 28/60 differentiates strains 1 and 2 by the respective mild and severe patterns produced. It exhibits still a third type of symptom with strains 3 and 4. These two strains can, however, be separated by parallel inoculations on Louisiana Purple and several commercial varieties. On these, strain 3 maintains its severe C.P. 28/60-pattern, while strain 4, in common with strains 1 and 2, produces only the ordinary mosaic.

Strain 3, which causes very severe symptoms on every variety so far infected, seems to be as yet quite limited in distribution. Strains 1, 2, and 4 all seem to be widely dis-

tributed in Louisiana although separately localized to some extent in certain areas.

Possible explanations for the origin of strains are discussed.

Preliminary comparison of resistance of the strains to heat, aging in vitro, and dilution indicate the possibility of no more than minor differences.

The probability is suggested that the demonstration of strains of the sugarcane mosaic virus offers a suitable explanation of the widespread disappearance of symptoms and actual recovery from mosaic by certain "tolerant" variaties of sugarcane in Louisiana. The results emphasize the importance of this disappearance of symptoms for reducing mosaic losses and as a varietal character to be incorporated in the breeding of improved varieties that may not be immune to the disease.

Recovery results mainly by the sprouting of healthy shoots from diseased stalks used for "seed", although during years of minimum secondary spread by aphids, visible recovery of the foliage during the latter part of the growing season is an important contributing factor. Recovery in P.O.J. 234 is largely limited to germination, while in P.O.J. 36-M both types are pronounced. P.O.J. 213 and Co. 281 show, in these studies, no recovery of either type.

Statistical analysis of germination data on pedigreed-mosaic lines of P.O.J. 36-M reveals significantly lower production of healthy plants from nodes representing the basal quarter of the stalk. Similar studies of foliage-recovered stalks showed no such correlation in the 1930 experiments, while nearly 100 per-cent of healthy plants were produced in the 1931 and 1932 tests.

Preliminary comparisons of pedigreed-mosaic and recovered lines (i.e., mosaic plants produced by some of the lateral buds on germination of foliage-recovered stalks) with respect to extent of foliage recovery revealed a very much greater tendency on the part of the latter to throw off the symptoms. The accentuating effect of this is believed to have contributed materially toward the practical elimination of the disease during a three-year period of low secondary spread in the variety P.O.J. 213. However, as noted above, P.O.J. 213 has shown almost no germination or foliage recovery in the writer's experiments. The seed source of this variety had been exposed to infection from wild grasses, whereas that of P.O.J. 36-M and P.O.J. 234 represented older infections in large fields less subject to such natural spread.

The paradoxical behavior of P.O.J. 213, in conjunction with greater recovery among recovered lines of P.O.J. 36-M; the lessened tendency of the lower eyes of the stalk to produce healthy plants, and other facts brought out in this investigation seem best explained by the assumption that different strains of the virus are concerned and that the host varieties are able to combat and overcome one, or another single strain, but cannot throw off infection by others.

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VITA

Father: William Edgar Summers

Mother: Ethel Grace Pearce

Born: January 11, 1901,
Macedonia, Iowa

Schools attended:
Dist. 40, Merrick Co., Nebraska
Silver Creek High School, Silver Creek, Nebr.
University of Nebraska, B. Sc., 1922
Iowa State College, M. Sc., 1928

Fields specialized in:
Plant Pathology
Mycology

Major Professors:
Dr. F.D. Keim, University of Nebraska
Dr. J.C. Gilman, Iowa State College
Dr. I.E. Melhus, Iowa State College