SOME STRAINS OF POTATO VIRUS X AND THEIR SPONTANEOUS MUTATION

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Summary

One virulent and three avirulent potato virus X strains were characterized qualitatively and quantitatively by the reactions of *Gomphrena globosa* and *Capsicum frutescens*. Spontaneous virulent mutants arose from time to time in the avirulent strains cultured in *Datura stramonium*. Avirulent strains developing low virus concentrations in their host plant had higher mutation rates than one developing a higher concentration in the host. Mutation rate appeared to be influenced by environmental conditions. The mutants from the three avirulent strains were similar and did not appear to differ from the ordinary virulent control strain. The mutants, owing to their greater biochemical vigour, almost entirely suppressed their parent avirulent strains.

I. Introduction

Previous genetical work on the reactions to virus X of potato seedlings derived from parents tolerant to this virus resulted in the separation of strains differing in virulence (Hutton 1948). Three of the avirulent strains, which were symptomless in *Datura stramonium* L. and produced only a fleeting mottle in *Nicotiana tabacum* L., were cultured in *D. stramonium* for over two years. At the same time one of the virulent strains was cultured in *D. stramonium* as a control. Opportunity was thus afforded to study some of the characteristics of these strains. During the two-year period spontaneous mutations occurred from time to time in each of the avirulent strains and observations were made on the rate and type of mutation.

Matthews (1949) has presented evidence to show that mottle-type strains of potato virus X usually have a relatively high forward mutation rate compared with severe ones, and he indicated also the possibility of back mutation from severe to mild strains. The present paper furnishes additional data to that of Matthews (1949) on mutation rates in strains of virus X that were apparently milder than those used by him. Further, the strains described in this paper were characterized both qualitatively and quantitatively by means of globe amaranth (*Gomphrena globosa* L.) as described by Wilkinson and Blodgett (1948) and also by *Capsicum frutescens* L. so that an indication of the inherent qualities of the strains leading to differences in rates of mutation was obtained. Globe amaranth proved also of use in the characterization of the mutants that arose from the avirulent strains.

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The vital constituents of both viral and nuclear material are chemically similar and both viruses and genes are subject to spontaneous mutations. In addition, X-rays induce mutations in viruses (Gowen 1939) as was found for flowering plants by Stadler (1928). It is thus evident that studies on the mutation of plant viruses could assist in an understanding of gene mutation in plants.

II. Materials and Methods

The origin of the X strains used in this study has been mentioned already. The avirulent strains were designated 31-45, 50-57, and 50-58, and the virulent one 25-144. Over the two-year period of the experiments, each strain was transferred serially to a batch of six young *D. stramonium* plants at two- to three-weekly intervals. The inoculum for a transfer to a fresh batch of young plants was prepared by grinding composite leaf samples from symptomless plants of the previous batch with pestle and mortar at the constant proportion of 1 : 40 by weight with water. This was applied with a ground glass spatula after dusting the leaves with fine carborundum powder. After each transfer, the symptomless plants of the old batches carrying the avirulent strains were reinoculated with the virulent strain, and usually, protection was maintained in 50 per cent. or more of the plants of a batch thus proving the presence of the masked strains. The failure of complete protection by masked strains has been noted previously (Hutton 1948).

Wilkinson and Blodgett (1948) developed the use of globe amaranth for qualitative and quantitative work with potato virus X because of its opposite paired leaves, which are similar in appearance and sensitivity, and give well-defined local lesions without systemic infection. Preliminary tests with this plant showed it to be suitable for the study of some of the inherent characters of the virus X strains used in these experiments. To obtain optimum results with globe amaranth, the plants should have grown quickly and be used just prior to or at the commencement of flowering. Virus transfers to this species were made in the same way as those to *D. stramonium*.

Salaman (1938) showed that *Capsicum annuum* L. reacted to all the strains of potato virus X he isolated, including the masked one. It was thus of interest to compare the reactions of *Capsicum frutescens* L. to the four X strains with those of globe amaranth. Two varieties of *C. frutescens* were selected, World Beater No. 13 and an unnamed variety obtained from Professor C. M. Haenseler, Rutgers University, New Jersey, U.S.A.

III. Characteristics of the Four X Strains as Indicated by Globe Amaranth (Gomphrena globosa L.)

Six pairs of opposite leaves representing young, medium, and old stages of development were selected on each of four well-grown globe amaranth plants. A randomized experiment was then done so that each X strain was inoculated to 12 leaves which represented a leaf from the three growth stages on all four plants. To ensure accuracy, lesions were counted by artificial light eight days after inoculation. The results are tabulated in Table 1 and the differences between the X strains are depicted in Plate 1. It will be noticed in Plate 1 that the virulent strain, 25-144, tended to give better-defined lesions than the avirulent ones.

TABLE 1

MEAN NUMBER OF LESIONS ON LEAVES OF GLOBE AMARANTH FOLLOWING INOCULATION WITH FOUR STRAINS OF POTATO VIRUS X

	Avirulent			Virulent
Strain of X	31-45	50-57	50-58	25-144
Mean number of lesions per leaf	31.75	50.00	33.63	94.25

Minimum difference between treatment means for significance at 5 per cent. = 13.84; at 1 per cent. = 18.83.

The statistical analysis of the data presented in Table 1 was made on the results from leaves that were classified at the time of inoculation as being of old and intermediate age. The results from young leaves were too variable to be of value, so were not included. It will be observed in Table 1 that the virulent strain 25-144 produced from almost twice to three times the number of lesions of the avirulent strains on globe amaranth, the results being highly significant. There is no significant difference in mean number of lesions per leaf between the avirulent strains 31-45 and 50-58, and at the 5 per cent. level both of these strains produced significantly fewer lesions per leaf than 50-57. It is apparent from these results that the virulent strain existed in high concentration in *D. stramonium* and that significant inherent differences existed among the avirulent ones which attained a lower concentration in the host. The characteristics of the X strains determine their behaviour in host plants, and as will be indicated later, the differences between the avirulent ones affect their rate of mutation as well as their protective action against the ingress of a virulent strain.

IV. Reactions of Two Varieties of Sweet Pepper (Capsicum frutescens L.) to Four X Strains

The three avirulent strains and one virulent strain of virus X were inoculated separately to different plants of the two sweet pepper varieties. Each strain was inoculated to a large leaf midway on three plants of each variety, the plants being 10-12 in. high and growing vigorously. The reactions on the inoculated leaves were observed after eight days and the symptoms in the uninoculated portions of the plants after 22 days. The results are summarized in Table 2.

It is evident that there is a correlation between these and the results obtained previously with globe amaranth and that a difference in type of reaction to virus X exists between the two sweet pepper varieties. In the variety ex Haenseler none of the strains, including the virulent, produced necrosis, and growth, although mottled, approached normal, while abscission of the inoculated leaves occurred slowly. With World Beater No. 13, small necrotic spots in ill-defined rings appeared rapidly on the inoculated leaves, and the numbers and extent of the necrotic spots gave a virulence rating of the strains comparable to that obtained with globe amaranth. Abscission of the inoculated leaves in World Beater No. 13 was rapid, and the necrotic systemic symptoms were accompanied by marked growth depression, particularly with strains 25-144, 31-45, and 50-58.

It is apparent that certain sweet pepper varieties like World Beater No. 13 are useful in the characterization of X strains. Further, the necrotic reaction to virus X in sweet pepper appears to be genetically controlled.

TABLE 2

COMPARISON BETWEEN THE REACTIONS OF TWO SWEET PEPPER VARIETIES (*CAPSICUM FRUTESCENS* L.) TO ONE VIRULENT AND THREE AVIRULENT STRAINS OF VIRUS X

X Strain	Variety† ex Haenseler		World Beater No. 13	
	Inoculated leaf reaction after 8 days	Symptoms in leaves below growing point after 22 days	Inoculated leaf reaction after 8 days	Symptoms in leaves below growing point after 22 days
31-45 (avirulent)	None apparent	Severe mottling and distortion	25* Small necrotic spots in ill-defined rings	Severe necrosis, mottle, and distortion
50-57 (avirulent)	Slight interveinal yellowing	Light mottle and some distortion	96* Small necrotic spots in ill-defined rings	Some distortion and necrosis
50-58 (avirulent)	Slight interveinal yellowing	Severe mottling and distortion	19* Small necrotic spots in ill-defined rings	Severe necrosis, mottle, and distortion
25-144 (virulent)	Interveinal yellowing and vein banding	Very severe mottling and distortion	90* Spreading necrotic spots covering most of the leaves	Very severe necrosis, mottle, and distortion

* Mean from 3 leaves.

† Variety from Professor C. M. Haenseler, Rutgers University, New Jersey, U.S.A.

V. Spontaneous Mutation of Avirulent Strains of Virus X to Virulent in *Datura stramonium* L.

Following their separation from the virus X complex in the Brownell potato through the use of seedling potatoes (Hutton 1948), the X strains described in this paper were cultured continuously in *D. stramonium* for over two years. During the first year of this period the distinctive properties of the virulent and three avirulent strains were confirmed a number of times with globe amaranth. However, it became obvious that, in contrast to the virulent strain, the avirulent strains were unstable and mutated to a severe form from time to time in one or two, and sometimes three, of the six replicates of *D. stramonium* used at a serial transfer of each strain. It was thought at first that the appearance of the severe strains in the cultures of the avirulent strains represented an initial admixture of a severe type. The fact that they occurred occasionally and discontinuously during relatively long periods of serial transfer, and then only in one or two replicates of *D. stramonium* at a transfer, disproved this and was strong evidence in favour of mutation.

The mode of development of the severe types in the avirulent cultures was interesting and was additional evidence for their occurrence by mutation. When the instability of the avirulent strains was suspected, close observation of the recently inoculated *D. stramonium* plants after a serial transfer of these strains often revealed one or two small yellow flecks close together in otherwise symptomless leaves of certain plants. When these yellow flecks were excised and inoculated to *D. stramonium* a severe yellow mottle resulted, whereas tissue taken from near the flecks did not produce symptoms in *D. stramonium*, cross-protection tests subsequently proving the avirulent strain to be present. It is apparent from these results that the virulent mutant arises spontaneously in one sector of a leaf, possibly from a single virus particle or aggregate, and owing to its greater biochemical activity is able to compete successfully with the parent avirulent strain so that a small but visible zone of multiplication results. There is no evidence to suggest that virulent mutants arise simultaneously at several separated loci on the same leaf.

It is certain that during the first year of serial transfer of the avirulent strains the number of mutants observed was in excess of the actual number developing, owing to the failure to recognize the first occurrence of a mutant in a leaf. Where a composite leaf sample carrying the avirulent strain contained one leaf with a small unrecognized mutant zone and was used in a serial transfer, the number of apparent mutants developing subsequently would be increased out of proportion. It has been found, using single *D. stramonium* plants, that it usually requires three serial transfers from a leaf carrying a single small yellow fleck to establish finally the virulent mutant in high concentration and suppress the avirulent parent strain. This is illustrated in Plate 2. In Plate 2, Figure 1, on the leaf carrying the avirulent strain 50-58, the light yellow fleck containing the mutant cannot be distinguished. Plate 2, Figure 2, illustrates the results after two serial transfers from the leaf in Figure 1, and Figure 3 illustrates the result of the transfer from Figure 2. When the virulent mutant had become established to the extent shown in Plate 2, Figure 2, it was difficult, although not impossible, to excise tissue giving only the avirulent strain.

During the first year of serial transfers of the avirulent strains the fact that spontaneous mutation occurred was established and the mode of development of the mutants observed. It was evident that little notice could be taken of the rates of mutation of the different strains in this period. In the next 12-

months period a more accurate assessment of the mutation rate of the strains was made by means of serial transfers from a number of separate *D. stramonium* lines of each strain.

The *D. stramonium* lines for this experiment were obtained by inoculating each avirulent strain to batches of 24 young plants and then reinoculating these with a severe strain 21 days later. In both 31-45 and 50-58, eight of the lines failed in this cross-protection test, 50-57, the strain previously shown to give the highest virus concentration in *D. stramonium* with globe amaranth, maintained protection in all 24 plants. Serial transfers were then made from the surviving lines at fortnightly intervals to young duplicate *D. stramonium* plants in the cotyledon stage. Although close observation was made of each line for the occurrence of mutations, a line was not regarded as having produced a spontaneous mutation until yellow flecking was clearly evident. The lines in which mutations developed were discarded. In this way a reasonably accurate comparison of the rates of mutation among the three avirulent strains could be made. The results are summarized in the graph by plotting the number of lines of each avirulent X strain that had not mutated at consecutive monthly periods over the year from March 1949 to March 1950. Thus in the graph the first six months represent the autumn-winter period and the last six months the spring-summer period.

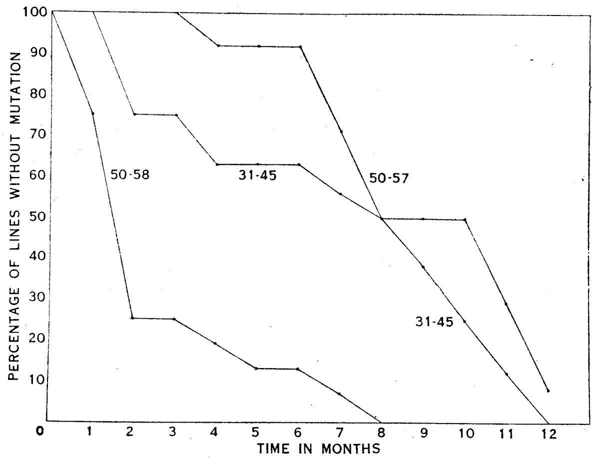

Fig. 1.—Rate of mutation in three avirulent strains of virus X. Strain 50-58 commenced mutating at the beginning of the experiment while 31-45 and 50-57 commenced mutating after one and three months respectively.

In Figure 1 the different mutation rates of the three avirulent X strains can be seen clearly. Strain 50-58 was the least stable, all of its lines being discarded because of mutation in eight months. Strain 50-57, which had been shown previously to develop the highest virus concentration of the avirulent types in *D. stramonium,* had the lowest mutation rate with two of its lines remaining stable after 12 months. Strain 31-45 had a mutation rate intermediate between 50-58 and 50-57, all lines being finally discarded in 12 months. It is of interest to note that strains 31-45 and 50-58, which developed the lowest concentration in *D. stramonium,* as shown by globe amaranth and *Capsicum frutescens,* had higher mutation rates than 50-57. This suggests that low virus concentrations tend to favour a high mutation rate. Another point of interest in the graph is the suggestion that the increasing photoperiod and rising temperatures of spring and early summer have influenced the mutation rates of the more stable 31-45 and 50-57 strains.

Four times during the course of these experiments *D. stramonium* plants in which mutants from each avirulent strain had developed to the stage shown in Plate 2, Figure 3, were inoculated in properly designed experiments to globe amaranth in comparison with the virulent strain 25-144. No significant differences in virus concentration between the mutants and the virulent strain 25-144 were observed. In addition, there were no real differences between their symptom pictures in *D. stramonium.* It is apparent that when a virulent mutant arises in a culture of an avirulent strain, the mutant, owing to its greater biochemical vigour, eventually suppresses the parent strain to such an extent that it has little or no effect on the virus concentration of the mutant. In addition the mutants observed are indistinguishable from ordinary virulent strains, the course of mutation following the same pattern irrespective of the parent avirulent strain.

VI. Discussion

The mutability of plant viruses is now accepted generally (Bawden 1950) although critical data on the process is rather scanty. This is due in part to the difficulties associated with obtaining strain cultures of many of the plant viruses in a pure condition. Some of the most conclusive early work on the occurrence of mutations in plant viruses was that of McKinney (1935) with tobacco mosaic, while more recently Matthews (1949) demonstrated the mutation of mottle strains of X. From a biochemical standpoint Stanley (1943) considered that the mutation of plant viruses involved changes inducing differences in the proportions of amino acids in the molecules. Bawden (1950) considers that a quantitative change in amino acids is not involved but rather a rearrangement in the sequence of the amino acids on a peptide chain. This is more in line with the type of results obtained by Wyss *et al.* (1948) for induced mutations in bacteria. It will be of considerable interest if it can be shown that the mutations induced in bacteria, fungi, and higher plants are similar in character to those observed in viruses. The work of Gowen (1939) with X-ray-induced mutations in tobacco mosaic, if substantiated by other workers, could be an important link in establishing these relationships.

The work described in this paper gives some indication of the factors influencing spontaneous mutation in avirulent strains of potato virus X. Cultures of these strains maintained in *D. stramonium* without contamination from virulent mutants did not change their relationship to each other as shown by frequent quantitative experiments on globe amaranth during a two-year period. Thus there was no evidence of a back mutation as suggested by Matthews (1949) for his strains. All the evidence presented in this paper indicates that for the avirulent strains of X described, the mutational process is in the direction of increasing virulence as judged by virus concentration and symptomatology in *D. stramonium*. It would be of interest to know whether this type of mutation is usual for mild or avirulent strains of plant viruses, and also whether back mutations occur or could be induced in virulent strains. It is possible that in evolution avirulent strains precede virulent and that avirulent strains could thus remain unnoticed in a host until transferred to a plant favourable to their mutation.

VII. Acknowledgments

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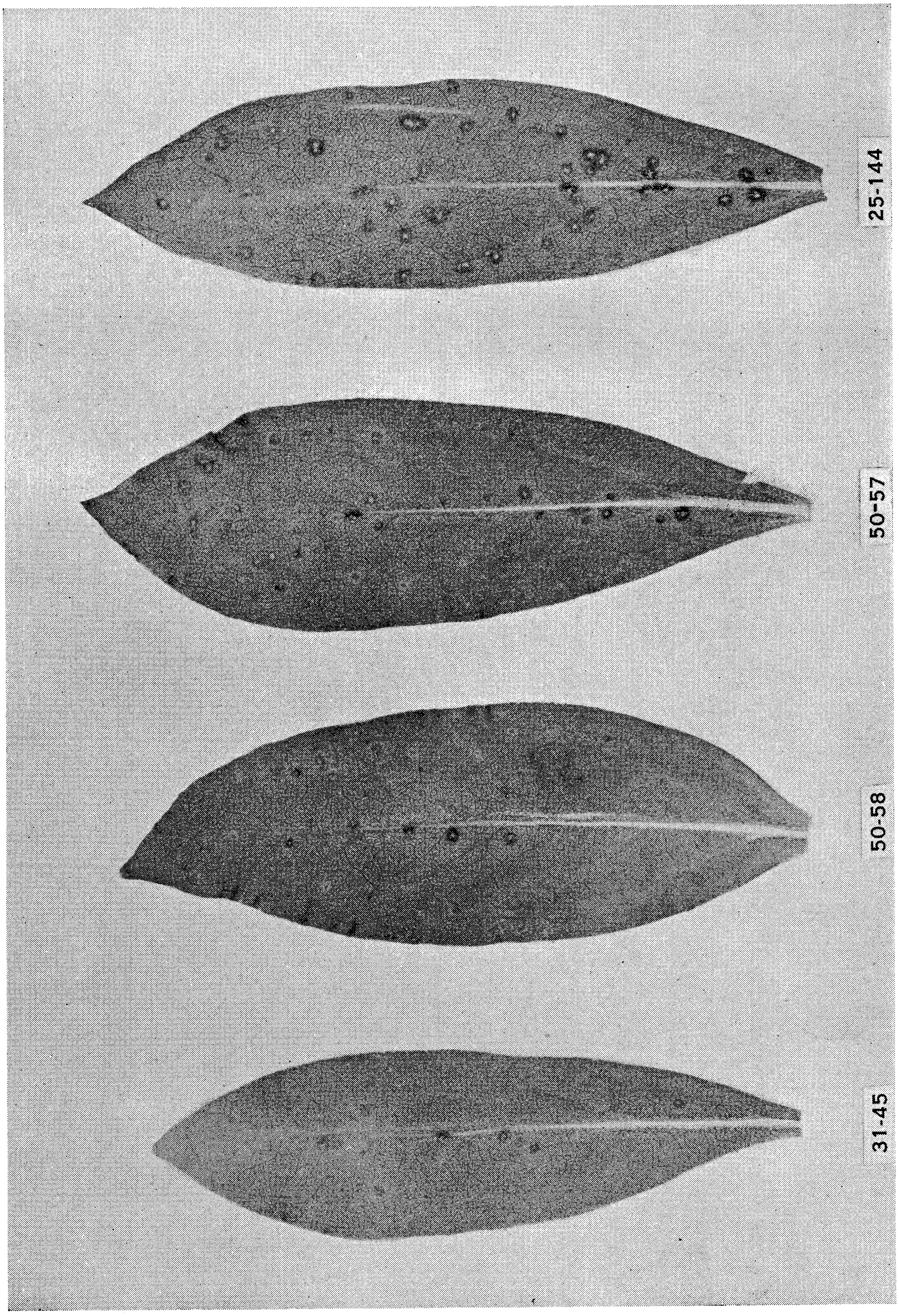
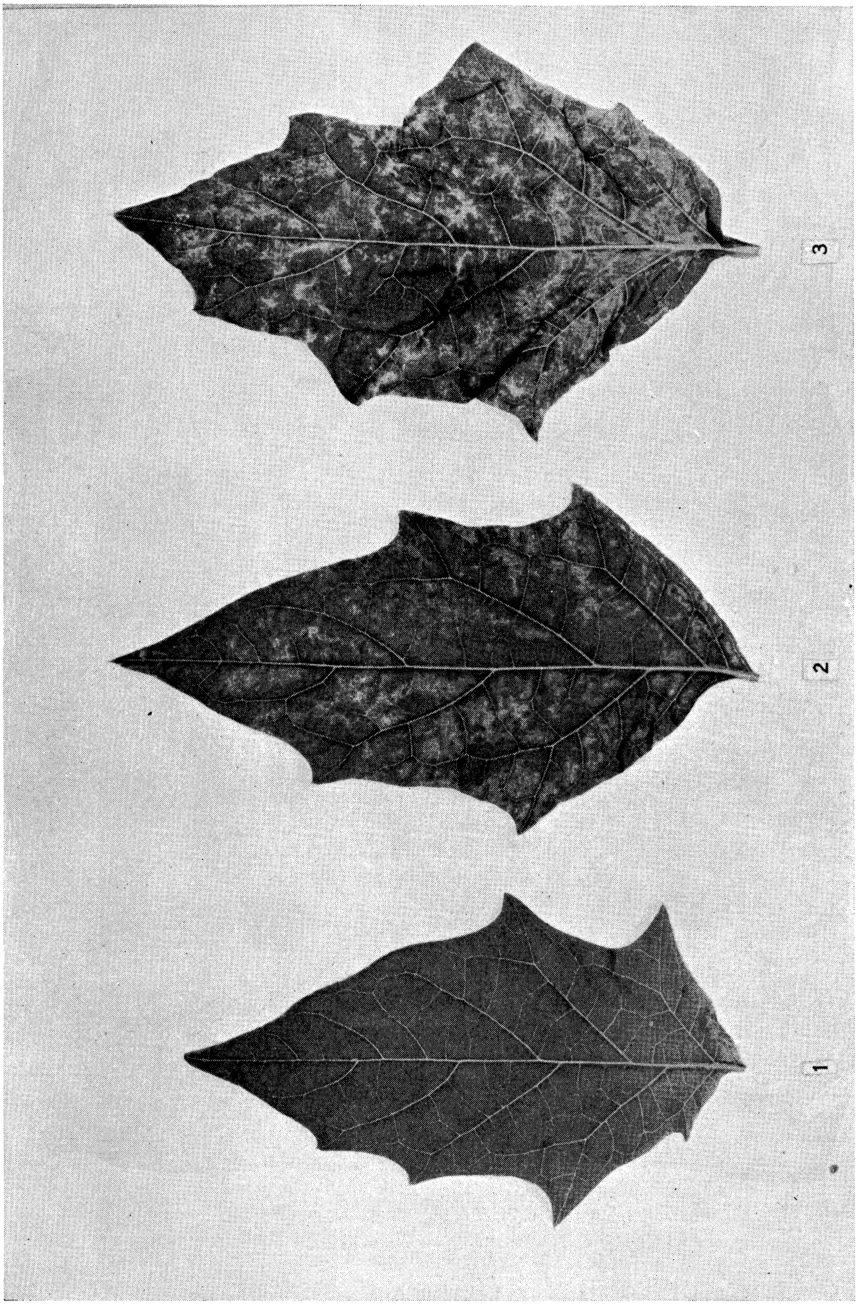
MUTATION OF VIRUS X STRAINS



Leaves of globe amaranth showing numbers and types of lesions produced by avirulent X strains 31-45, 50-58, and 50-57 and virulent 25-144.

MUTATION OF VIRUS X STRAINS



D. stramonium leaves illustrating the development of a mutant virulent X strain from the avirulent 50-58.