CONCENTRATION OF TOBACCO MOSAIC VIRUS IN TOMATO PLANTS THROUGHOUT THE GROWING SEASON OF TWO GLASSHOUSE CROPS AND A FIELD CROP[1]

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INTRODUCTION

Commercial glasshouse tomato crops generally become 100 percent infected with green strains of the tobacco mosaic virus. Infection frequently becomes prevalent shortly after the plants are set in the soil. Field grown tomatoes are less universally infected, but where early infection occurs the losses are considerable. Alexander (1949, 1950, 1951), Heuberger and Moyer (1931), Heuberger and Norton (1933), Jones and Burnett (1935), and Selman (1942) have studied the effect of tobacco mosaic virus on the yield of tomatoes; and Alexander (1949), Heuber and Moyer (1931), and Heuberger and Norton (1933) have shown that the reductions of yield of early infected field tomatoes may be as high as 50 percent. These workers point out that decrease in yield is greatest when plants are infected early. Moreover, Alexander (1949, 1950, 1951), Jones and Burnett (1935), and Selman (1942) have shown that the reduction in yield of glasshouse tomatoes due to mosaic varies with the season, the decrease in yield appearing to vary from 10 to 25 percent. Selman (1942) first pointed out that fruit set was reduced at the time of infection. Alexander (1950, 1951) further emphasized this fact and, by a study of the average fruit set for each cluster throughout a glasshouse tomato crop, showed that tomato plants infected early with mosaic set few fruits on the early clusters and then made a remarkable recovery. In fact, the fruit set on later clusters was slightly above average.

A decrease in the virus content of the plants might be an explanation for the partial recovery. Accordingly, it was decided to follow the active virus concentration of infected plants throughout one or more growing seasons in order to determine whether there was a drop in the active virus content of the plants which correspond to the recovery of the plants.

METHODS AND MATERIALS

Experiments designed to study the effect of tobacco mosaic infection on tomatoes at different stages of growth are described in detail elsewhere (Alexander, 1949, 1950, 1951). Inasmuch as the same plants were used in this study, a brief description is repeated here. The tomato plants were grown in a glasshouse which was divided twice both lengthwise and crosswise, making a total of nine plots. The plants in three plots were inoculated January 18, and the plants in another three plots on March 28. The plants in the third set of plots were uninoculated and were intended to be kept healthy as controls. However, they accidentally become contaminated about the first of May. In 1950 one-half of each plot was planted with plants of the variety Ohio W–R Globe and one-half with plants of the variety Strain A Globe. Thirteen plants of each variety were used. In 1951, only the variety Ohio W–R Globe was used.

In 1951, the virus concentration of inoculated, field grown, Stokesdale plants was followed. The plants were unpruned and unstaked. Ten plants were used for each plot and the plots were replicated four times. The plants in one set of

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plots were inoculated June 15, the plants in another set on July 16, and the plants in a third set were left as healthy controls.

In all cases, the plants were inoculated by dusting three or more leaflets with carborundum and then rubbing them with juice from infected plants. In the glasshouse work, inoculum was prepared from a diseased plant that appeared to be infected with a normal green strain of the tobacco mosaic virus. In the case of the field experiments the initial inoculation was prepared in 1950 from a plant that appeared to be infected with a normal green strain of the virus, and in 1951 the initial inoculum was a purified virus suspension prepared from tomato plants that appeared to be infected by a normal green strain.

The virus concentration was estimated by the local lesion method described by Holmes (1929a, b) and Samuel and Bald (1933) using *Nicotiana glutinosa* as the assay plant. In the preliminary work of 1950 whole leaves were used, and five leaves on each of two plants were inoculated for each assay. In 1951, in order to insure a more even distribution of the 600 grit carborundum, a small Hudson hand duster was used. It was modified by replacing the quart dust container with a pint jar and shortening the discharge tube to approximately four inches. With this duster it was possible to get what appeared to be an even distribution of the carborundum on the leaves. The assay inoculum was rubbed over five half leaves on each of two plants.

Leaf samples for assaying were first collected from the tops of the tomato plants approximately two weeks after inoculation. Thereafter, samples were collected every two weeks. It seemed desirable to follow the virus content of the first leaves sampled; therefore, lower leaves were assayed as well as the top leaves. Somewhat later, as the plants approached maturity, the leaves in the middle of the plants were also assayed.

In the preliminary work of 1950, the assay inoculum was prepared by grinding 0.6 g of tomato leaf in 6.0 ml of water. In the more exact experiments of 1951, the assay inoculum was prepared from ten cm^2 of tomato leaf tissue. A leaf punch of one cm^2 area was used to obtain a sample from ten different plants. The ten cm^2 of leaf tissue were thoroughly ground with nine ml of water. This solution was then diluted 1:100, making an approximate dilution of the inoculum of 1:1,000. One half of each of five *Nicotiana glutinosa* leaves was inoculated with this inoculum. This dilution of the inoculum gave well distributed lesions.

The other half of the five leaves of each *Nicotiana glutinosa* plant was inoculated with a purified green strain of the tobacco mosaic virus complex. Precipitation of the virus complex was carried out at the isoelectric point, pH 3.4, following the work of Best (1936, 1948). Tissue macerated in a ball mill was diluted one to five with water and centrifuged at 1000 x g for about 20 min and then recentrifuged at 1500 x g for about two hr. The precipitate, consisting of plant debris, was discarded and the supernatant liquid was diluted with an equal volume of standard buffer, pH 3.4, giving a dilution of 1:10. The addition of the 3.4 pH buffer to the clarified juice gave a pH of approximately 4.0. Final adjustment to pH 3.4 was made with 0.1 N hydrochloric acid. The clarified juice was again centrifuged at 2000 x g for 30 min. The precipitated virus complex was diluted 1:10 with standard pH 7.0 buffer and was highly infective but was not infective at pH 3.4. This is in accord with the recent work of Takahashi (1949). The virus complex was reprecipitated, using 5.0 M glacial acetic acid to adjust to the isoelectric point. However, since it required undue amounts of acetic acid to attain a pH below 4.0, the final adjustment to pH 3.4 was made with 1.0 N hydrochloric acid. The precipitated virus complex was diluted to the original juice volume with a standard pH 4.0 buffer and stored in a refrigerator. When used, the virus suspension was diluted 1:1,000 with pH 7.0 buffer. Comparisons between the purified virus complex diluted 1:1,000 and freshly ground virus infected tomato leaves diluted 1:1,000 gave very similar numbers of local lesions on *Nicotiana glutinosa*.

In the preliminary work of 1950, the data are expressed as the number of lesions per square inch of leaf surface.   However, in the work of 1951, the data are presented as the number of lesions per square centimeter of leaf surface of *Nicotiana glutinosa*.   The area of the *N. glutinosa* leaves was obtained by measuring the breadth of the leaves at their widest point and the length of the leaves from the tip to the base of the blade.   The product of these measurements does not give the exact area of the leaves, but a series of measurements by use of a planimeter on 49 leaves indicated that the actual area was approximately 86 percent of the
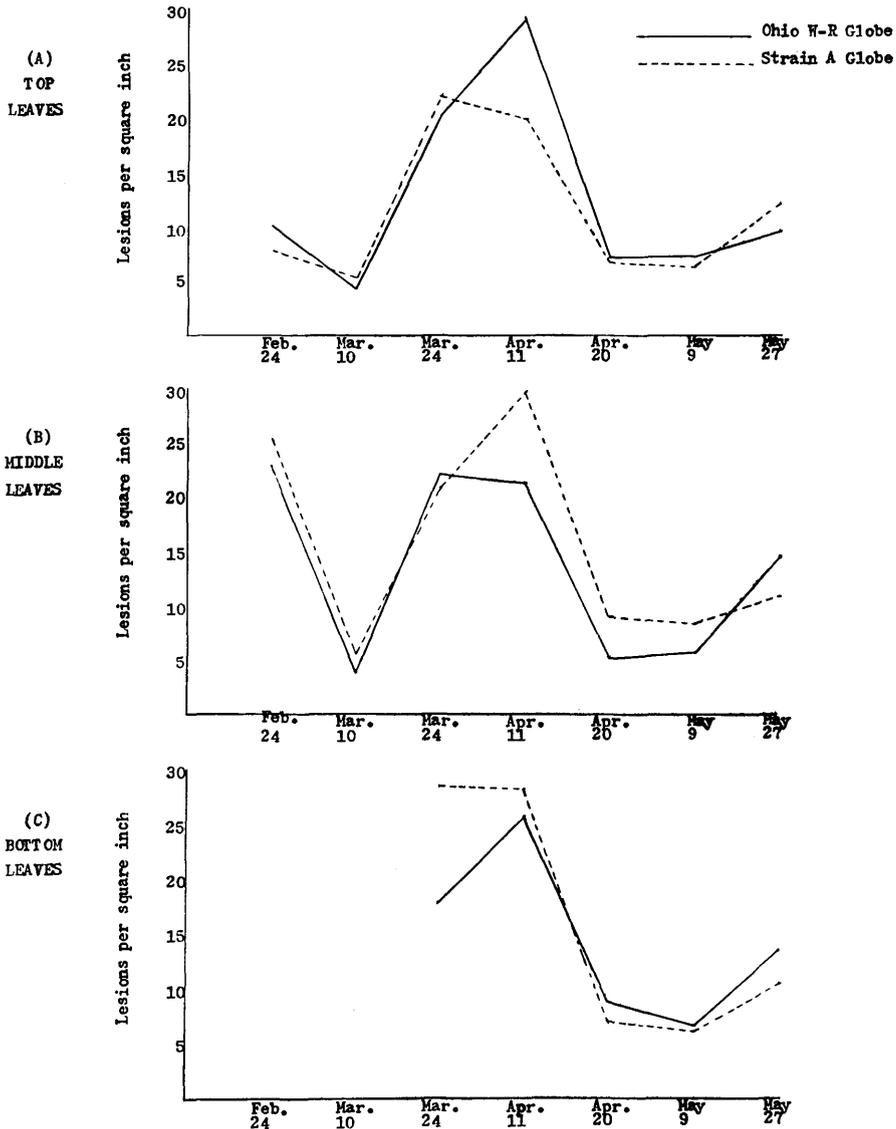

FIGURE 1.   Tobacco mosaic virus concentration in tomato leaves expressed as average number of lesions per square inch for two tomato varieties.   Inoculated January 18, 1950.   Sam Dean's Glasshouse.

calculated area.   However, since the correction would be a constant and the data are relative, the area was assumed to be the product of the width by the length.

RESULTS

Several methods of evaluating the effects of tobacco mosaic diseases on tomatoes have been used.   In this investigation the number of fruit set per cluster is used as an indication of the effects of the disease.   In order to accomplish this, a record of the number of fruits which were set per cluster was made for the entire growing season.   This fruit-set record covers the same period as the virus assay.

During the analysis of the plants in 1950 the number of lesions per square inch which occurred on leaves of *Nicotiana glutinosa* was used as the index of virus content of the plants.   However, in 1951 in order to refine the technique, the number of lesions per square centimeter on half leaves was expressed in terms of the number of lesions on the other half leaves inoculated with a purified virus preparation.   This was done in order to eliminate, at least in part, the variability of different lots of assay plants.

TABLE 1

Influence of the tobacco mosaic disease on the number of fruits set per cluster for two greenhouse tomato varieties.   Sam Dean's Glasshouse—Spring, 1950.

Treatment							Cluster number						Average fruits per cluster
		1	2	3	4	5	6	7	8	9	10	11	
						Strain A Globe							
Inoculated	1/18/50	1.9	4.0	4.6	5.8	5.0	4.7	5.0	4.6	3.6	3.9	3.4	4.2
Inoculated	3/28/50	3.9	4.8	5.2	5.1	4.3	4.2	4.3	4.6	3.9	4.1	3.8	4.4
Healthy		4.3	4.8	5.1	5.9	4.2	4.7	5.0	4.3	3.4	3.7	3.0	4.4
						Ohio W-R Globe							
Inoculated	1/18/50	2.2	4.5	4.7	5.2	4.7	4.9	5.4	4.1	3.7	3.6	3.6	4.2
Inoculated	3/28/50	4.1	4.8	4.8	5.0	4.3	4.2	5.2	5.4	4.1	4.0	4.0	4.5
Healthy		3.8	5.4	4.9	5.4	4.8	5.2	5.3	4.5	3.6	3.9	4.1	4.6

Fruit set record for 1950 and 1951.—In previous work Alexander (1950, 1951) showed that as glasshouse-grown tomato plants made a visible recovery following mosaic infection, the setting of fruit tended to approach normal or in some instances to exceed that of healthy plants.   The fruit set data for the varieties, Ohio W–R Globe and Strain A Globe, grown in the spring of 1950 are shown in table 1.

The number of fruits set on the early clusters of the Ohio W–R Globe plants, inoculated, the middle of January, at the time of transplanting them into the permanent ground beds, was reduced (tables 1 and 2).   Fruit set was improved on the second and third clusters and reached what appeared to be normal in the fourth and succeeding clusters.   Similarly, fruit set on the variety, Strain A Globe, was reduced on the first clusters following inoculation (table 1).   It is also shown (tables 1 and 2), for the variety Ohio W–R Globe and table 1 for the variety Strain A Globe, that a marked reduction in fruit set occurred following the second inoculation made approximately ten weeks after the first.   This reduction in fruit set occurred on the fifth, sixth, and seventh clusters for both varieties in the spring of 1950.   In the spring of 1951, variety Ohio W–R Globe, the sixth and seventh clusters were severely affected following inoculations.   In the later clusters there was a general decline in fruit set on the plants of all plots. This general decline in fruit set may possibly be explained by the high temperatures of May and June.

Virus concentration, Glasshouse, 1950.—The active virus concentration in the two varieties, Ohio W–R Globe and Strain A Globe, was similar throughout the season (fig. 1 and 2). The concentration of tobacco mosaic virus in the plants inoculated January 18, 1950, appeared to fluctuate throughout the season. The data are shown in figure 1. The number of lesions per square inch of leaf of assay plants produced by inoculum secured from the top leaves for the first two sample periods, February 24 and March 10, varied between five and ten. The virus

TABLE 2

Influence of tobacco mosaic virus on the number of fruits per cluster, Ohio W-R Globe. Sam Dean's Glasshouse—Spring, 1951.

Treatment	Cluster number											Average fruits per cluster
	1	2	3	4	5	6	7	8	9	10	11	
Inoculated January 17, 1951.	1.58	2.77	3.46	5.82	4.41	4.34	4.99	4.89	3.93	3.63	2.45	3.84
Inoculated March 28, 1951.	4.53	5.44	4.60	4.44	4.17	2.69	1.93	3.58	3.61	3.82	3.33	3.83
Healthy	3.90	5.30	4.74	4.59	5.35	4.71	4.67	4.42	4.07	3.36	2.90	4.36

content of all leaves sampled during the next two sample periods, March 24 and April 11, increased markedly. Later in the season, the virus content again decreased. However, in this case, increase in virus content occurred at about the time the plants were exhibiting symptoms of recovery and fruit set was returning to normal. The concentration of the virus throughout the plant in the March 24 and later sample periods, as represented by samples from the top, middle, and bottom leaves, appeared to be about the same.

The concentration of the virus in the plants inoculated March 28, 1950, fluctuated somewhat but remained at a lower level than that attained in the first inoculation (fig. 2). The effect of this inoculation reduced fruit set (table 1). The plants inoculated March 28, 1950, were approximately five ft tall, and in this case the virus concentration, though present in the lower leaves, did not increase greatly. The concentration of the virus in the center portion of the plants approached that in the tops of the plants. This might be expected because the leaves in the center portion of the plant were immature at the time of inoculation.

Virus concentration, Glasshouse, 1951.—The virus content of Ohio W–R Globe plants was followed throughout the growing season of 1951. *Nicotiana glutinosa* was again used as the assay plant. However, the data are expressed as lesions per square centimeter of half leaf surfaces after correction according to the number of lesions produced by the purified virus on the adjoining half leaves.

The data are shown graphically in figure 3. As in 1950, the virus concentration in the tops of the plants inoculated January 17 was approximately the same as in the bottoms of the plants (fig. 3a). Again there did not appear to be any relationship between the virus concentration and the recovery by the plant, judging from its ability to set a normal number of fruits per cluster.

The virus concentration of the plants infected March 28 was much less uniform (fig. 3b). The results differed in that the virus concentration in the lower leaves remained low and then increased rapidly.

Virus concentration, Field tomatoes, 1951.—Using the same methods, the virus content of plants of the variety Stokesdale, field grown and unstaked, was followed for a growing season. The plants were transplanted to the field May 31.

The first inoculation was made on June 15 and the second on July 16.   Ten plants were used per plot with three replications.   The data are shown graphically in figure 4.   Inadvertently, assays were not made for the first two periods on the tops of the plants inoculated June 15.   The virus concentration increased in the bottom leaves until July 30, then it declined.   The virus content in the top leaves of the plants infected July 16 was high, whereas the virus content of the bottom leaves was low initially but gradually increased.
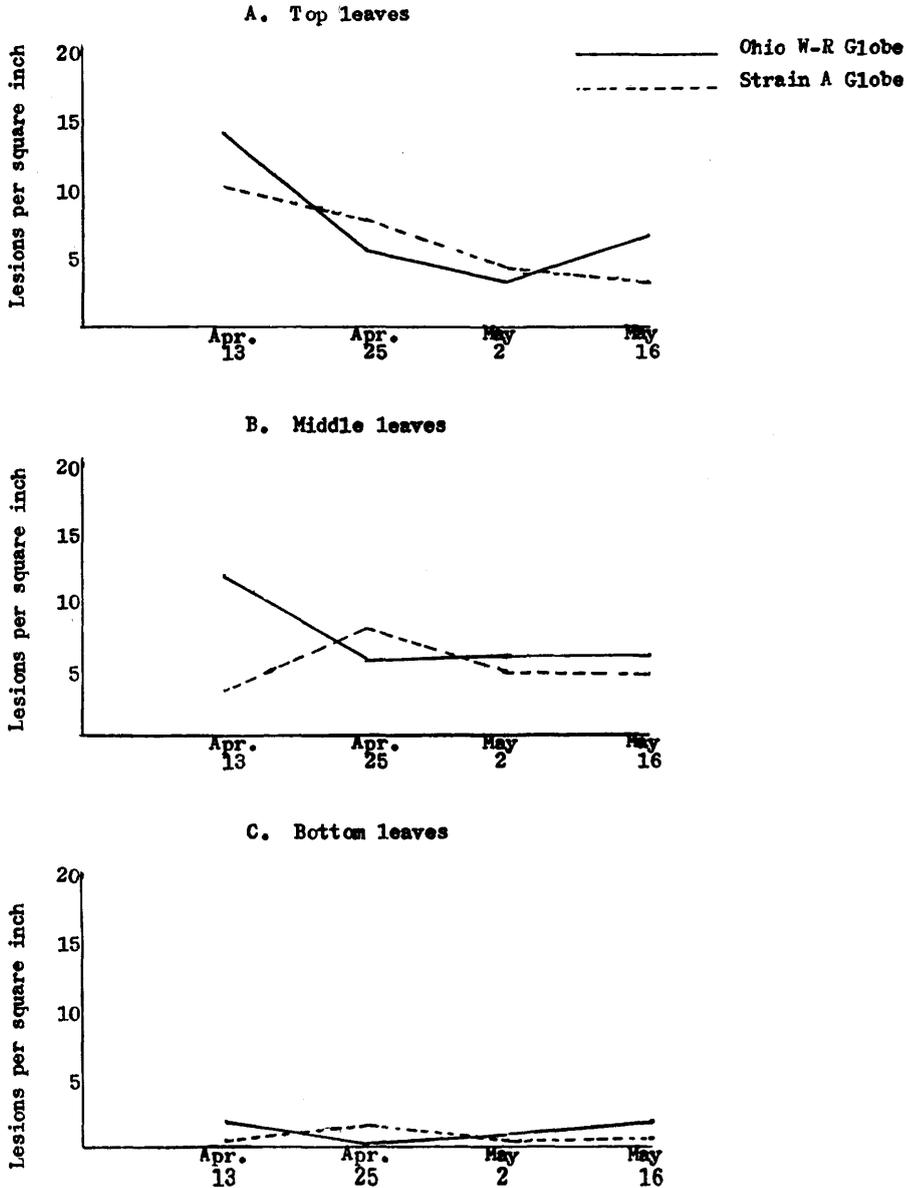

FIGURE 2.   Tobacco mosaic virus concentration in tomato leaves expressed as average number of lesions per square inch for two tomato varieties.   Inoculated March 28, 1950. Sam Dean's Glasshouse.

These data, however, do not indicate a relationship between virus content and recovery of the ability to set fruit. Fruit set data are not available but yield data previously published (Alexander, 1949, 1950, 1951; Heuberger and Moyer, 1931; Heuberger and Norton, 1933) have all shown that early infections result in the greatest loss. Alexander (1949 and unpublished data) secured yield data indicating that infections which occurred as late as July 15 did not result in great reductions in yield.

DISCUSSION

Many studies have been made of the active virus content of tomato and tobacco plants, but we have not found any references to studies pertaining to virus content throughout the entire productive life of tomato plants. Following the findings of Alexander (1949, 1950, 1951) that inoculated tomato plants made a recovery and set as many or more fruits per cluster as healthy plants on later clusters, it seemed desirable to follow the active virus content of plants over a period of months. As shown, it could not be demonstrated by the local lesion assay method on *Nicotiana glutinosa* that there was any marked change in active virus content at the time of recovery. It is recognized that this assay method will not detect
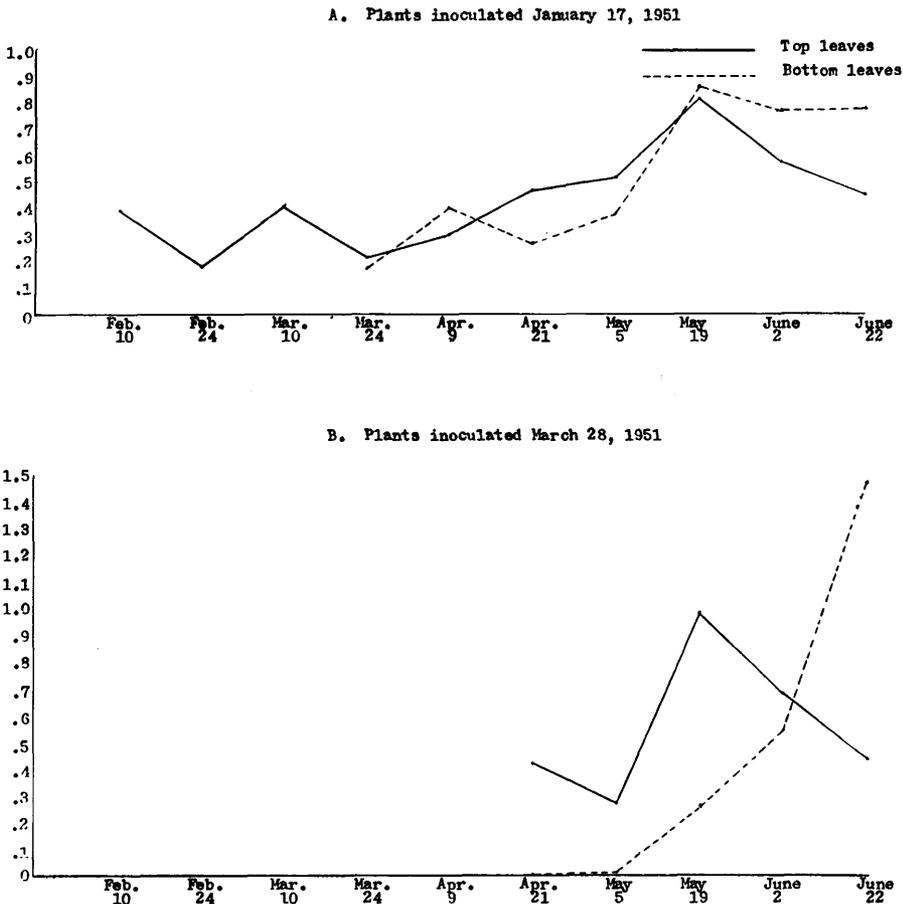

FIGURE 3. Tobacco mosaic virus concentration in leaves of the variety, Ohio W-R Globe, expressed as average number of lesions per square centimeter of half leaves. Sam Dean's Glasshouse. Spring 1951.

small differences (Beale, 1934; Spencer and Price, 1943).   However, it was thought
that a definite change would be reflected in a curve which was based on biweekly
assays.

The curves for virus concentration were erratic and while they tended to show
changes, they did not reflect changes at the time of recovery.   Following inocula-
tion at early stages, the concentration of active virus was generally uniform
throughout the plants.   In later inoculated plants the virus concentration tended
to remain at a low level.   These findings are in accord with those of Samuel (1934)
who found that the mature leaves of large plants remain free from virus for as
long as three months after the initial inoculation.

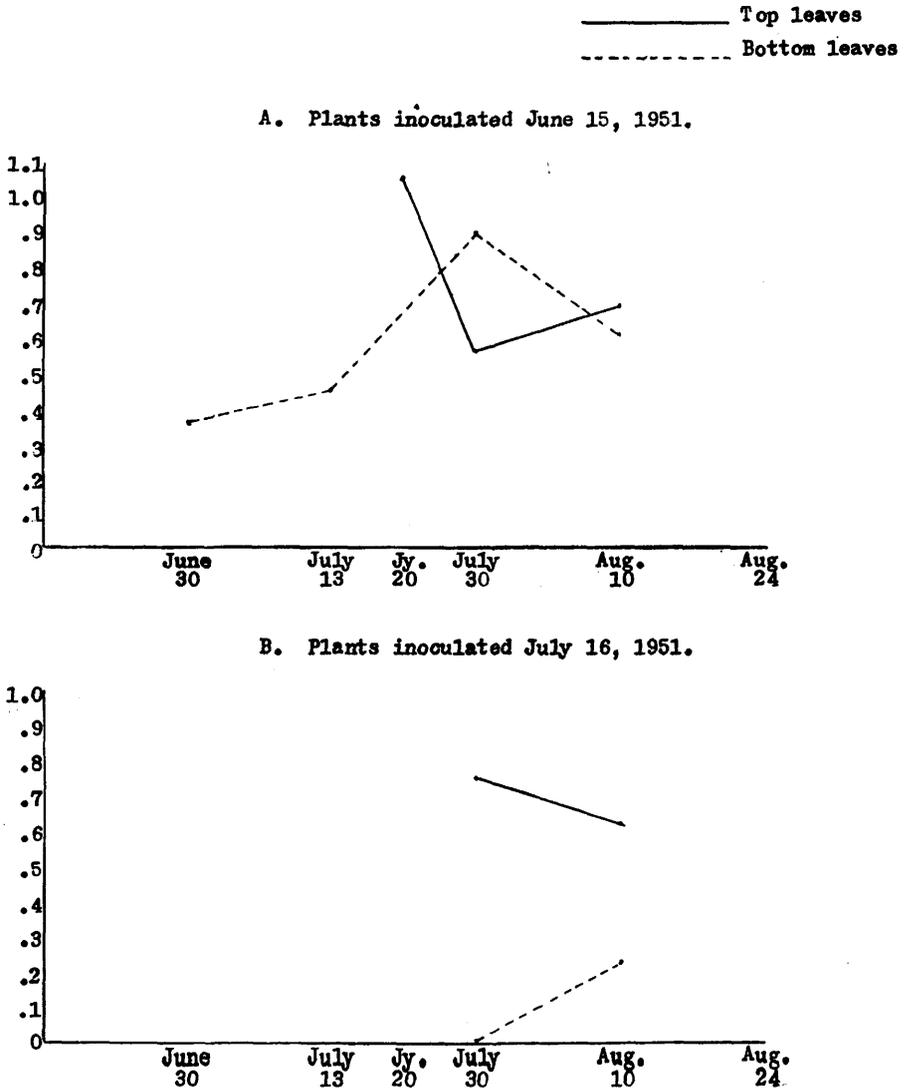In our experiments the inoculations were made on leaves near the growing

FIGURE 4.   Effect of the age of field grown Stokesdale plants inoculated at two different dates
on their tobacco mosaic virus content expressed as number of lesions per square
centimeter of half leaves.   Summer 1951.

point. Kunkel (1939) reported that tobacco mosaic virus moved after at least a 44-hr inoculation period. His findings and those of Capoor (1949) indicated that the virus moves in all directions at about the same time. Thus, other parts of the plants used in these experiments should have received the virus within a few days after inoculation. However, it should be expected that the greatest concentration of virus would be found in the rapidly growing tissue at or near the apex of the plants.

In many instances the active virus content of the plants fluctuated from one sampling period to the next. An unsuccessful attempt was made to correlate these fluctuations with changes in temperature. The effect of nutrition has been shown to have some effect on the virus concentration of plants. Spencer (1935a) reported that virus concentration in leaves could be increased by increased nitrogen supply, and Spencer (1935b) also reported that increased supplies of phosphorous affected the susceptibility of tobacco as the increase benefited plant growth. Bawden (1950) reported that both nitrogen and phosphorous affected susceptibility. The findings of these workers may explain, in part, the fluctuation in active virus concentration of leaves observed in this work because supplementary sidedressings of fertilizer were made at irregular intervals during the growth of these tomato plants.

SUMMARY

The number of tomato fruits set per cluster decreased following infection with the tobacco mosaic virus. Usually the decreased fruit set does not persist beyond the second or third cluster after infection.

There was no apparent relationship between the virus concentration within the plants and recovery of the plants, either from the standpoint of growth or fruit set.

The varieties, Ohio W–R Globe and Strain A Globe, reacted similarly to the virus from the standpoints of virus concentration, a lack of correlation between virus content and return to normal growth, and fruit setting.

The virus concentration of plants inoculated ten weeks after they were set in permanent beds in a glasshouse tended to remain lower than the virus content of plants inoculated at the time of transplanting. This tendency was greatest in the lower parts of the plants.

There was no correlation between the virus content of field-grown, unstaked Stokesdale plants and the recovery of their ability to set fruits.

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