THE MOVEMENT OF TOBACCO MOSAIC VIRUS WITHIN THE PLANT

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(With Plate IV and 4 Text-figures.)

Within the last few years several papers have appeared dealing with different phases of the movement of viruses through plants. Most of the work has dealt with viruses of the tobacco mosaic group (including tobacco mosaic, yellow tobacco mosaic, aucuba mosaic of tomatoes, and the mixed virus streak of tomatoes, one constituent of which is tobacco mosaic), but some of the conclusions arrived at have been decidedly conflicting. There have been two main views advanced with regard to the method of movement of tobacco mosaic virus: (1) that of a progressive advance from the point of inoculation through the tissues of the plant at a more or less uniform rate, and (2) that of a very slow cell to cell movement via the plasmodesmen combined with a rapid distribution through the plant via the phloem.

The former view was given its most definite expression in the work of Böning (1928), and has recently been supported by Caldwell (1930, 1931). The following quotations from Caldwell's papers give the main substance of this view of virus movement: "These results, which confirm the data of Böning, seem to indicate that there is no rapid movement of the virus agent in any one direction in the plant, but rather that the agent moves slowly up and down the stem from the point of insertion of the inoculated leaf" (1931, p. 293). "The rates of movement of the virus agent in the tomato are practically the same upward or downward. The slightly greater rate of upward movement appears to be associated with the greater metabolic activity which occurs in the upper portion of the plant" (1931, p. 297). "There is strong presumptive evidence that the movement of the agent of aucuba disease of tomato can take place and does take place readily through any living tissue, and that the phloem is, in this case, not the main channel of movement in the normal plant" (1931, p. 296). "It is concluded that movement takes place in the living ground tissue of the plant" (1930, p. 443)[1].

[1] In a paper which appeared after this manuscript was submitted for publication Grainger (*Ann. Appl. Biol.* xx, 236) concluded that tobacco mosaic virus moves at a logarithmic rate, beginning slowly and later accelerating and also that spread seems to be independent of mechanical carriage by the transpiration or translocation streams.

On the other hand, the view of a rapid distribution of the virus through the plant via the phloem was expressed as early as 1898 by Beijerinck, and has recently gained much support from the experimental work of Holmes. Holmes (1930) found that there was an initial period of multiplication of the virus in local sites on the inoculated leaf, and then a comparatively sudden appearance of virus in all parts of the stem and root, and in the leaves at the top of the plant, the virus spreading later to lower leaves. Holmes pointed out that his technique using *Nicotiana glutinosa* might not be suitable for detecting very small traces of virus. But he presented a table showing measurements of virus in detached tomato stems which provided strong evidence that, if the virus had been present in one portion of the stem 2 or 3 days earlier than in another, his method would have detected it.

Holmes's work (1931, 1932) on the movement of mosaic within the inoculated leaf by means of the iodine test also showed an initial period when the virus was localised in primary lesions, and then a sudden appearance of virus all along the main vascular channel from the leaf. It was also shown that the first appearance of the virus in the systemically infected leaves was along the network of finer veins. Holmes made the suggestion that the restricted movement of the virus across the lamina of the inoculated leaf in comparison with its rapid movement down the mid-rib and through the stem might be explained on the basis of movement with food substances to dependent parts of the plant, and he pointed out that "if movement of virus with a particular food material could be proved the virus would serve as a useful indicator of the distribution of the substance." A number of his experiments on defoliation and the covering of leaves, and on inoculation of very young leaves, gave general support to these ideas.

It seems very likely that the cause of the discrepancy which exists between the experiments of Böning and Caldwell and those of Holmes is due to the fact that the former workers did not plan their experiments on a sufficiently large scale to be able to follow the movement of the virus during the first few days following inoculation. In the experiments quoted by Caldwell (1931, p. 292), for example, the position in which the virus was found by cutting up plants on the third day after inoculation may possibly have been assumed by movement from the inoculated leaf within a period only a few hours before the plants were cut up. Similarly, the majority of Böning's experiments are not inconsistent with Holmes's view of virus movement, but they are on too limited a scale to provide either confirmation or disproof. It seemed advisable, therefore, to under-

take a more extended series of experiments with cuttings in order to see whether the results would fall into line with those determined by Holmes, using *N. glutinosa* as a test plant for the presence of the virus.

No fuller treatment of the literature on the movement of tobacco mosaic virus is attempted, since the papers of Böning (1928), Caldwell (1930) and Henderson Smith (1930) contain summaries up to recent dates.

Experimental.

Comparison of methods.

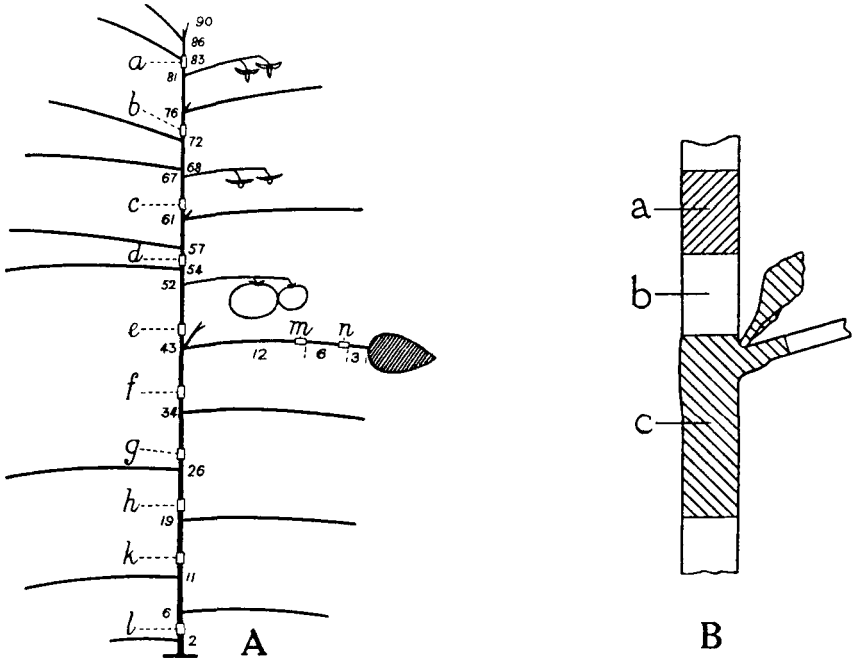
A test was first made of the reliability of the various methods which have been used for determining the presence of the mosaic virus at different points within the plant. Three main methods have been used: (1) cutting up the stems (usually of tomato plants) at various times following inoculation and rooting the portions as cuttings; the cuttings are then grown until the presence or absence of mosaic can be determined from the symptoms on the new leaves; (2) cutting up the inoculated plants into sections, grinding these up and using the juice to inoculate seedlings of tobacco or tomato, the presence or absence of virus being judged according to the systemic infections which result; and (3) cutting into sections as for (2), but inoculating on to *N. glutinosa* leaves, and judging the presence (and roughly also the concentration) of virus from the number of local lesions produced.

There seems little doubt that method (1) is entirely reliable, and that however small the amount of virus which gains entrance to a portion of a plant taken for a cutting, it will multiply and eventually be determinable from symptoms at the growing point. It is by no means certain, however, that a very small amount of virus in a portion of stem could be detected by the grinding up and inoculating methods (2) and (3). This was pointed out by Holmes (1930), but was apparently not considered by Böning (1928) in some of his experiments.

The matter was accordingly tested by using all three methods on the same series of plants. Ten tomato plants (*A–L*), each 100–120 cm. high and with about 20 leaves up the stem, were inoculated on the end leaflet of a leaf about half-way up the plant. Two plants were then cut up each day from the second to the sixth day according to the plan in Text-fig. 1 A. Before cutting up, a sketch of each plant was made and the heights of all the leaves recorded. A fresh sterile scalpel was used for cutting each sample, and each sample was handled only with a small square of clean newspaper in the hand, so that at no time was any portion of the plant

touched with the fingers. The pot containing the base of the stem, with one or two buds left, was always kept and grown on to determine whether the virus had passed beyond the lowest cut, probably into the roots.

Cuttings were made from five portions of the stem above the insertion of the inoculated leaf and from five portions below. Each cutting necessarily



Text-fig. 1 A. Working plan for plant 32 in Table III, showing height in cm. of every leaf and fruit truss, and the positions (*a–l*) from which portions of the stem were taken for tests for the presence of virus. Petiole samples from *m* and *n*, with distances from the inoculated leaflet. Similar plans were made for every plant cut up.

Text fig. 1 B. Plan for one node of the stem in the case of the plants in Tables I and II, showing (*a*) portion taken for the "test-tube" method, (*b*) portion taken for an immediate expressed sap test on tobacco or *N. glutinosa*, and (*c*) portion rooted as a cutting.

contained an axillary bud, with about 2 cm. of stem below (Text-fig. 1 B, *c*); the sketch-plan gave the distances between each. Each cutting was immediately planted in a (5 : 1) sand-soil mixture in a 4 in. pot, and comparatively few cuttings failed to root and grow. In cases when the cutting did fail to grow, a grinding-up test with inoculation to *N. glutinosa* revealed the presence or absence of virus.

Expressed sap tests for the presence of the virus were made by taking small pieces of stem about 1 cm. long from immediately above the

portions used for cuttings (Text-fig. 1 B, *b*). These were ground up in a mortar with five drops of water, and inoculated with a small glass spatula on to two leaves of small tobacco seedlings and on to single leaves of *N. glutinosa* plants.

During the course of the experiment a fourth method was thought of and applied to plants *G* to *L* in addition to the other methods. This consisted in taking a further series of 1 cm. lengths of stem, adjacent to the other samples (Text-fig. 1 B, *a*), and placing them in sterile test-tubes with a little moistened cotton-wool at the bottom. The samples were kept for a week in a well-lighted room, when they were ground up and inoculated on to single leaves of *N. glutinosa* plants. It was considered that a very small amount of virus which had just arrived in a portion of stem might be missed by methods (2) and (3), but that it would have an opportunity to multiply during the week in a test-tube and would then be easily detectable by the *N. glutinosa* test. The principle is much the same as that used in the method of cuttings, but it has the advantages (1) that the necessity of taking an axillary bud and a moderate length of stem (2–3 cm.), as necessary for the cuttings, is dispensed with; (2) that there is a great saving in time, since the results are available in about 10 days, whereas with cuttings it is often necessary to grow them on for 6 weeks or more before all the results can be recorded; and (3) there is thus a considerable saving in greenhouse space. This fourth method is in essence a "tissue culture," and was previously used by Holmes (1930) in tests on the rate of multiplication of virus.

The results of the whole experiment are presented in Table I. It is evident that the method of cutting up the stem into small portions, which are immediately ground up and inoculated on to tobacco plants or on to *N. glutinosa* plants, does not give a reliable indication of whether virus had reached the particular parts under test or not. In plant *L*, for example, no sign of virus was detected by the expressed sap tests, either on *N. glutinosa* or on tobacco, and yet the cutting tests later showed that the virus was present throughout the whole of the stem. Apparently the number of virus particles present could be so small that either the chance of their introduction to a suitable inoculation wound on the test plant was almost nil, or they were rendered ineffective by adsorption effects when ground up with the whole bulk of tissue. In plant *K* a few positive tests resulted (one on *N. glutinosa*, four on tobacco), but here again the virus was actually present throughout the whole of the stem. It had probably been present for 1, or possibly for 2 days, and had multiplied sufficiently in certain places to be detected by expressed sap tests. (The

number of local lesions on the *N. glutinosa* leaves in these cases was very low.) It is seen, on the other hand, that the results from keeping the stem samples in test-tubes for a week before crushing them to test for the presence of virus on *N. glutinosa* leaves gave results identical with those given by the cuttings.

Table I.

A comparison of four methods for determining the presence of the first small traces of tobacco mosaic virus which penetrate the stem of a tomato plant following inoculation on a leaf about half-way up the stem.

Plant no.	Days after inoculation	Stem sample (see Text-fig. 1) *a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*k*	*l*
Expressed sap tests on *N. glutinosa* (Method 3).											
C	3	–	–	–	–	–	–	–	–	–	–
D	3	–	–	–	–	–	–	–	–	–	–
E	4	–	–	–	–	–	–	–	–	–	–
F	4	–	–	–	–	–	–	–	–	–	–
G	5	–	–	–	–	–	–	–	–	–	–
H	5	–	–	–	–	–	–	–	–	–	–
K	6	–	–	–	–	–	–	–	–	–	+
L	6	–	–	–	–	–	–	–	–	–	–
Expressed sap tests on *N. tabacum* (Method 2).											
C	3	–	–	–	–	–	–	–	–	–	–
D	3	–	–	–	–	–	–	–	–	–	–
E	4	–	–	–	–	–	–	–	–	–	–
F	4	–	–	–	–	–	–	–	–	–	–
G	5	–	–	–	–	–	–	–	–	–	–
H	5	–	–	–	–	–	–	–	–	–	–
K	6	–	–	–	–	–	+	–	+	+	+
L	6	–	–	–	–	–	–	–	–	–	–
Tests by rooting cuttings (Method 1).											
C	3	–	–	–	–	–	–	–	–	–	–
D	3	–	–	–	–	–	–	–	–	–	–
E	4	–	–	–	–	–	–	–	–	–	–
F	4	–	–	–	–	–	+	+	+	+	+
G	5	–	–	–	–	–	+	+	+	+	+
H	5	–	–	–	–	–	+	+	+	+	+
K	6	+	+	+	+	+	+	+	+	+	+
L	6	+	+	+	+	+	+	+	+	+	+
Test-tube samples 1 week old on *N. glutinosa* (Method 4).											
G	5	–	–	–	–	–	+	+	+	+	+
H	5	–	–	–	–	–	+	+	+	+	+
K	6	+	+	+	+	+	+	+	+	+	+
L	6	+	+	+	+	+	+	+	+	+	+

Other points of interest in Table I are (1) that there was no movement of virus into the stem before the fourth day, and (2) that when virus first appeared in the stem it went downwards towards the roots very quickly, and then, about a day later, went equally quickly up to the top of the stem. The number of plants was certainly small, but in none was the

virus intercepted when it had only travelled a part of the way up or down the stem.

Returning to the question of methods, because of the success of the test-tube method and the saving in time and glasshouse space made possible by its use, a confirmatory test was carried out, the results of which are presented in Table II.

Table II.

A further examination of the "test-tube method" for detecting the first traces of virus entering the tomato stem. Plants cut up 5 days after inoculation.

Plant no.	Stem sample (see Text-fig. 1) *a*	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*k*	*l*
Stem samples tested on *N. glutinosa* on day of cutting up.										
M	−	−	−	−	−	−	−	−	−	−
N	−	−	−	−	−	−	−	−	−	−
P	−	−	−	−	−	−	−	−	−	−
Q	−	−	−	−	−	−	−	−	−	−
R	−	−	−	−	−	−	−	−	−	−
Stem samples preserved in test-tubes 1 week and then tested on *N. glutinosa*.										
M	+	+	+	+	+	+	+	+	+	+
N	+	+	+	+	+	+	+	+	+	+
P	+	+	+	+	+	+	+	+	+	+
Q	+	+	+	+	+	+	+	−	+	+
R	−	+	+	+	−	+	+	+	+	−
Cuttings rooted from the nodes.										
M	+	+	+	+	+	+	+	+	+	+
N	+	+	+	+	+	+	+	+	+	+
P	+	+	+	+	+	+	+	+	+	+
Q	+	+	+	+	+	+	+	+	+	+
R	+	+	+	+	+	+	+	+	+	+

This test once again showed the inadequacy of results obtained from crushing samples of stem at the time of the cutting up of plants as a method of determining the presence or absence of the first traces of virus in the portions taken. In none of the samples taken immediately on crushing could any virus be detected, yet it must certainly have been present since in the adjacent stem samples kept in test-tubes for 1 week before crushing abundant virus was found in nearly every case.

There were, however, four cases of negative results from the test-tube samples, whereas all the cuttings gave positive results. It is difficult to see why a negative result should have been obtained if there was any virus present in the sample taken, for the portions of stems in the test-tubes were under just as favourable conditions as the cuttings and the great majority of them showed multiplication of virus. It is just possible that in these cases the virus for some reason did not move out from the

phloem to the mesophyll tissue. The only other explanation is that these samples were actually free from virus particles when taken, and that there was no virus there to multiply. There is further evidence to support this last conclusion, and the matter is discussed again later. It may be noted here, however, that the samples which gave negative results were from internodes, whereas the cuttings necessarily included nodes, and it seems possible that the vascular twists at the nodes would have a greater tendency towards the temporary arrest of widely spaced virus particles passing down vascular channels such as the phloem.

MOVEMENT OF VIRUS FROM THE INOCULATED LEAF INTO THE STEM.

Experiment 1.

After the experience gained from the preliminary experiments it was decided to use the test-tube method for the first larger experiment. Forty tall tomato plants (Dwarf Champion variety), growing in 8 in. pots and 70–90 cm. in height, were inoculated with mosaic on the end leaflet of a leaf about half-way up the plant (Text-fig. 1 A). The inoculations were done at 9–10 p.m. at night, and then commencing at 9–10 a.m. on the next day, but one group of four plants was cut up into sections at intervals of 12 hours. Again ten samples were taken at various distances up the stem, five above the inoculated leaf and five below. The samples were 1 cm. portions of stem cut with sterile scalpels and placed in sterile test-tubes on moist cotton-wool for 1 week. The same methods of handling were adopted as previously described, with the addition that in this case two samples (*m* and *n*) were taken at measured distances down the petiole below each inoculated leaflet. All samples were ground up 7–10 days later and inoculated on to single *N. glutinosa* leaves. If virus was present in a stem sample, some twenty to eighty local lesions were found on the inoculated *N. glutinosa* leaf, otherwise there were none. The results of the experiment are given in Table III.

The following are the main points of interest in Table III:

(1) With one exception (at $3\frac{1}{2}$ days) no virus moved out of the inoculated leaf into petiole or stem until the fourth day.

(2) In six plants (23, 25, 27, 28, 31, 35) the virus travelled down to the roots immediately it left the inoculated leaf.

(3) In three other plants (32, 33, 36), as well as moving down to the roots it also moved up one or two internodes. In plant 32, where it moved up one internode, a developing fruit truss was situated one internode above the insertion of the inoculated leaf (the diagram in Fig. 1 A

refers to this plant), and in plants 33 and 36, in which it moved up two internodes, developing fruit trusses were situated two internodes above the insertion of the inoculated leaf. On the other hand, certain of the six plants in which the virus only moved downwards had also developing fruit trusses one internode (plant 35) or two internodes (plants 25, 27) above the insertion of the inoculated leaf. It would therefore seem that the normal path of the virus on leaving the leaf is down to the roots, but that the presence of a developing fruit truss a few nodes above the insertion of the inoculated leaf may sometimes exert a pull causing part of the virus to move upwards as far as this point in addition to the normal downward movement.

Table III.

Results of cutting-up tests on tomato plants inoculated with tobacco mosaic on a leaf about half-way up the stem, showing determinations for the presence of the first traces of virus in the stem by the test-tube method. Dwarf Champion plants, 70–90 cm. high, bearing several fruit trusses.

Plant no.	Days after inoculation	Stem sample (see Text-fig. 1)										Base of plant	Petiole	
		a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*k*	*l*		*m*	*n*
1–4	1½	–	–	–	–	–	–	–	–	–	–	–	–	–
5–8	2	–	–	–	–	–	–	–	–	–	–	–	–	–
9–12	2½	–	–	–	–	–	–	–	–	–	–	–	–	–
13–16	3	–	–	–	–	–	–	–	–	–	–	–	–	–
17–19	3½	–	–	–	–	–	–	–	–	–	–	–	–	–
20	3½	–	–	–	–	+	–	–	–	–	–	–	–	–
21	4	–	–	–	–	–	–	–	–	–	–	–	–	–
22	4	–	–	–	–	–	–	–	–	–	–	–	+	+
23	4	–	–	–	–	–	+	+	+	+	–	+	+	–
24	4	–	–	–	–	–	–	–	–	–	–	–	–	–
25	4½	–	–	–	–	–	+	–	+	–	+	+	–	–
26	4½	–	–	–	–	–	–	–	–	–	–	–	–	–
27	4½	–	–	–	–	–	+	+	+	–	–	+	–	–
28	4½	–	–	–	–	–	+	+	+	+	+	+	+	+
29	5	–	–	–	–	–	–	–	–	–	–	–	–	–
30	5	+	+	+	+	+	+	+	+	+	+	+	+	+
31	5	–	–	–	–	–	+	+	+	+	+	+	+	+
32	5	–	–	–	–	+	+	+	+	+	+	+	+	+
33	5½	–	–	–	+	+	+	+	+	+	+	+	+	+
34	5½	+	+	+	+	+	+	+	+	+	–	+	+	+
35	5½	–	–	–	–	–	+	+	+	+	+	+	+	+
36	5½	–	–	–	+	+	+	+	+	+	+	+	+	+
37	6½	–	+	+	+	+	+	+	+	+	+	+	+	+
38	7½	+	+	+	+	+	+	+	+	+	+	+	+	+
39	8½	+	+	+	+	+	+	+	+	+	+	+	+	+
40	9½	+	+	+	+	+	+	+	+	+	+	+	+	+

(4) In one case (plant 20) the virus moved upwards one internode, where there was a developing fruit truss, before it moved downwards.

(5) In plant 37, where the virus was not found in the highest sample, the top of the plant had been scorched one hot day, and the main growth

was being resumed at a side-shoot just below where the highest sample was taken.

(6) In plants 23, 25, 27 and 34 negative tests were obtained on stem-samples situated *between* positive tests, suggesting that the virus must have passed *through* these portions of stems without affecting them. Similar results were obtained in a number of cases with cuttings, and there seems little doubt that the suggested explanation is correct. The subject is further discussed below.

(7) The same applies to the petiole tests in plants 23, 25 and 27.

(8) In one case only (plant 22) was virus found in the petiole but not in the stem or roots. In all other cases when once the virus had left the inoculated leaflet it was found (occasionally at intermittent points only) right down to the roots, and about a day later up to the top of the stem as well. It must therefore have moved with considerable speed.

(9) No differences could be observed between the morning and night samplings, but the number of plants in which any such differences could have been observed was very few.

(10) It may fairly be concluded from the results in Table III, combined with similar results from several other experiments, that plants showing virus present throughout the whole stem (such as plants 30, 34, 37, 38, 39 and 40) have already passed through the initial stage when virus moved downwards only.

Experiment 2.

Because of the unexpected nature of the results of the preceding experiment, and especially because certain stem samples gave negative results when the virus must have passed through them, it was decided to repeat the experiment using the method of planting the stem sections as cuttings. It was considered that, if the two methods gave similar results, there would be little doubt as to the reality of the happening.

In this case nine plants were cut up on the third, fourth and fifth day and five plants only on the sixth day after inoculation. The plants were of the tall variety Sensation, 100–140 cm. high. The cuttings were necessarily nodal, with 2–3 cm. of stem, and each was grown in a separate 4 in. pot as before. The results of this experiment are given in Table IV.

In this case it was only in certain plants cut up on the fourth day after inoculation that the early stages of invasion of the stem were intercepted. (Possibly this was due to the fact that this was the most vigorously growing batch of plants used.) Once again the tendency for the virus first to pass downwards towards the roots was evident (plants 13, 14 and

15). In plants 15 and 16, in which the virus was also found to have moved upwards some distance, small developing fruit trusses were situated just above the highest positive record, but this may have been only a coincidence. In two cases (plants 14 and 15) the virus was intercepted before it had reached the lowest section of the stem or the roots.

Table IV.

Results of cutting-up tests on tomato plants inoculated with tobacco mosaic on a leaf about half-way up the stem, showing determinations for the presence of the first traces of virus in the stem by the method of rooted cuttings. Sensation tomato plants, 100–140 cm. high, bearing several fruit trusses.

Plant no.	Days after inoculation	Stem sample (see Text-fig. 1)										Base of plant	Petiole*	
		a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*k*	*l*		*m*	*n*
1–9	3	−	−	−	−	−	−	−	−	−	−	−	−	−
10	4	−	−	−	−	−	−	−	−	−	−	−	−	−
11	,,	−	−	−	−	−	−	−	−	−	−	−	−	+
12	,,	−	−	−	−	−	−	−	−	−	−	−	−	−
13	,,	−	−	−	−	−	+	−	−	−	+	Died	−	+
14	,,	−	−	−	−	−	+	+	+	+	−	−	−	−
15	,,	−	−	−	+	−	+	+	−	+	−	−	−	−
16	,,	−	+	+	+	+	+	−	−	−	−	+	+	+
17	,,	+	+	+	+	+	+	+	+	−	+	+	+	−
18	,,	+	+	+	+	+	+	+	+	+	+	+	−	−
19–27	5	+	+	+	+	+	+	+	+	+	+	+	+	+
28–32	6	+	+	+	+	+	+	+	+	+	+	+	+	+

* The tests on the two portions of the petioles were done by the test-tube method.

In plants 13, 15, 16 and 17 there was again definite evidence of the virus having passed through sections of stem without causing any infection. There was no doubt about these negative records, for in six such cases the cuttings were transplanted to richer soil after having rooted in the sand, and when they still appeared healthy after having grown to a height of about 9 in. a final test with *N. glutinosa* was made upon them. In the case of the negative result for cutting *k*, plant 17, there seems no other conclusion possible than that the virus had passed through this section of the stem without infecting it. In the case of plant 16, where the virus had passed through four consecutive samples without infecting them and yet was found in the base of the plant, it would be reasonable to suspect the accidental infection of the basal section. Every possible precaution, however, was taken to avoid accidental infections, and it is not believed that this is the explanation. The frequency with which the virus was found to have passed through one or two consecutive segments without infecting them, not only in this but in other experiments, would make it not

unreasonable to extend the same view of virus movement to cover this case of plant 16. The distance between the bases of samples *f* and *l* in this plant was 50 cm., but each cutting was only 2½ cm. long, which means that only 10 of the 50 cm. of stem was actually tested for the presence of virus. It is possible that virus would have been found at intermediate points if the whole 50 cm. had been tested. Also it is again noticeable how frequently tests on the petioles of the inoculated leaves (by the test-tube method) gave negative results when the virus was already in the stem.

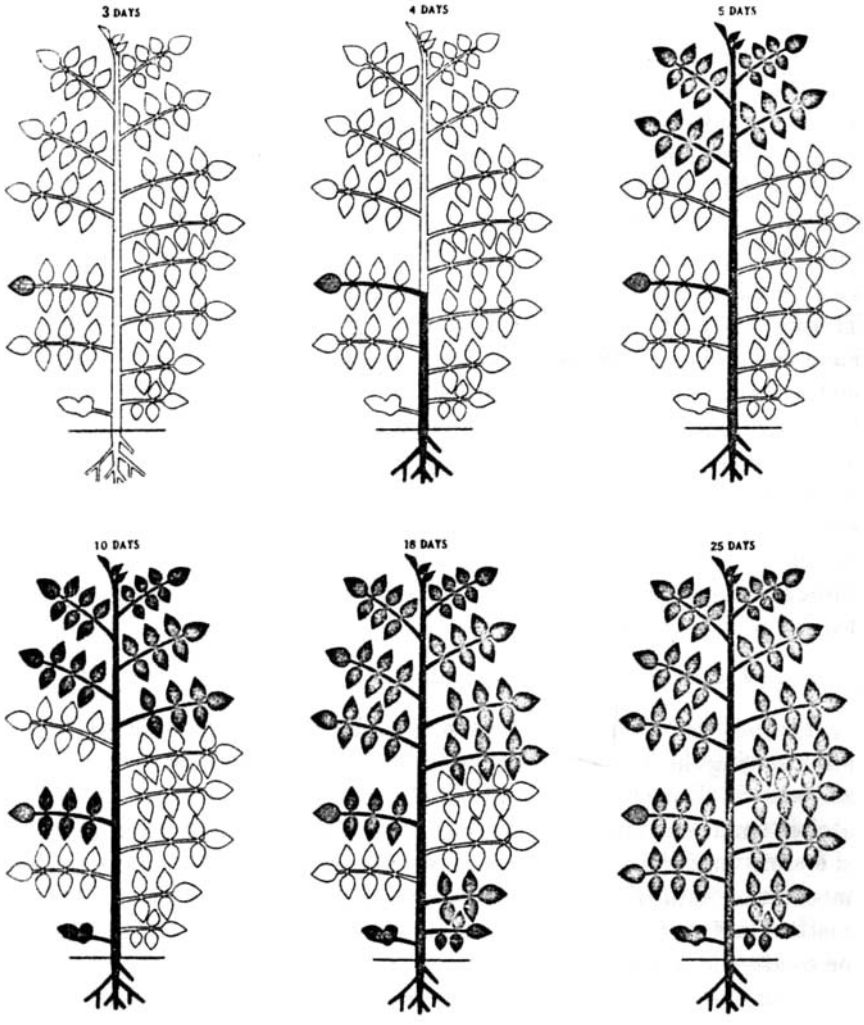
Several further experiments were done both by the cutting and by the test-tube method. These gave essentially the same results, except in one case. In this instance, which occurred in one of the test-tube series, the virus was found in four of the top five samples, but not in the lower five. This was the only case out of some dozens in which the virus was found to have moved first upwards in the stem instead of downwards, there being no fruit trusses on the plant. It is just possible that there was a mistake in the ticketing of the *N. glutinosa* plants used, but that is not thought likely. The plant was in a series of young Sensation tomatoes averaging 50 cm. high and not yet flowering; they were in 6 in. pots and commencing to suffer from lack of nitrogen. (There was no movement of the virus out of the inoculated leaves in this series until 5–7 days.) One other plant in the same series gave the usual initial downward movement from the inoculated leaf.

MOVEMENT INTO THE LEAVES.

Holmes (1930) has already presented tables showing that the virus, after leaving the inoculated leaf a few days following inoculation, appears suddenly in the stem, in the leaves at the top of the plant, and in the roots, almost simultaneously, but that it does not invade the central leaves on the stem until later. These tests were done by crushing samples and inoculating immediately on to *N. glutinosa*, and, as previously shown, this method suffers from the disadvantage of only detecting the virus a day or so after it is actually there.

That this disadvantage actually does apply to leaf, as well as to stem samples, was proved by taking opposite leaflets from several leaves towards the top of a plant shortly after systemic infection had occurred but was not yet visible, and testing one lot on *N. glutinosa* leaves immediately, while the other lot was kept in test-tubes for a week before testing. It was found that the lot kept in test-tubes showed the virus to be present on the average one leaf lower down the plant than could be detected by the test done immediately on sampling.

It was also proved by taking every leaflet from several leaves about the level where invasion was just occurring, that when the virus moves

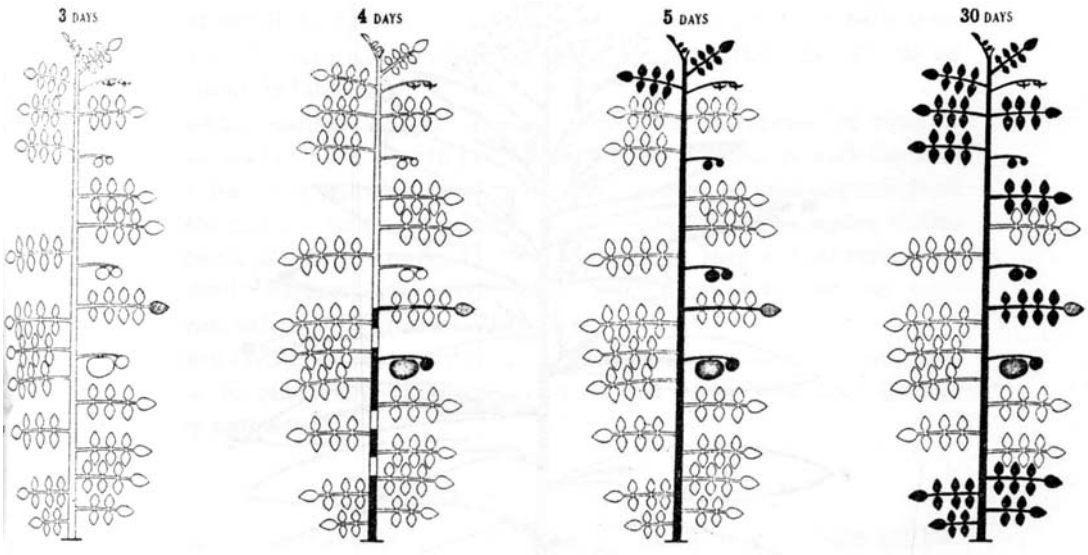


Text-fig. 2. Diagram to show the progress of the spread of mosaic (in black) through a medium young tomato plant. Based on tests of Dwarf Champion tomato plants about 15 in. high, growing in 6 in. pots in an unheated greenhouse. Inoculated leaflet shaded.

into a leaf it moves into all the leaflets almost simultaneously (within about a day). This made it legitimate, in tests for the distribution of virus throughout the leaves of a plant, to take a single leaflet from each

leaf, thus permitting determinations to be made again later on the same plants, and more than once, if necessary. The rate of invasion of the leaves in the middle of the stem could thus be followed on a single plant.

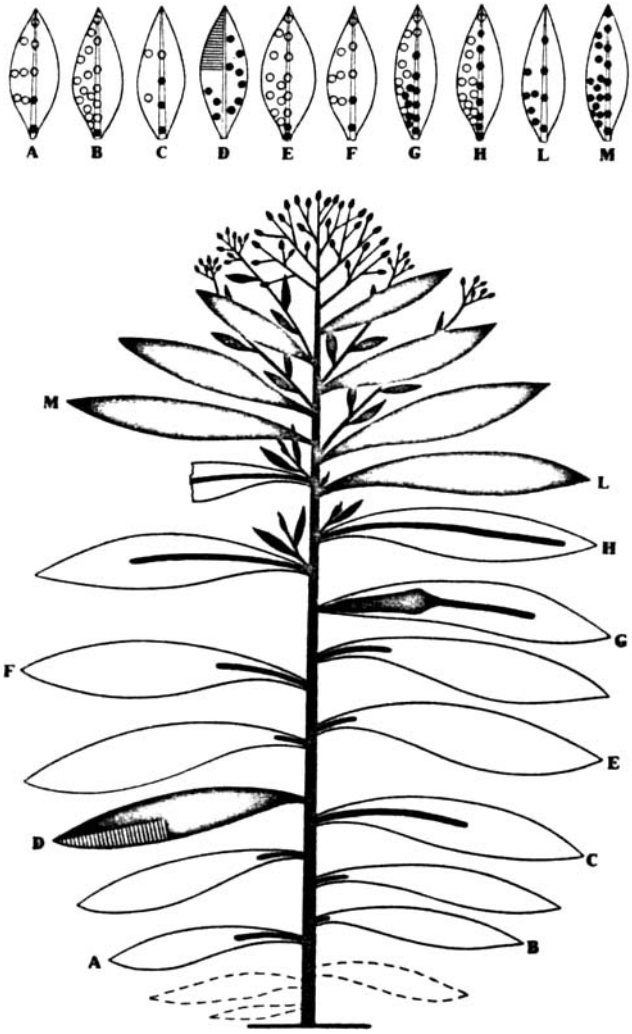
The results obtained from sampling every leaf of tomato plants of various ages confirmed entirely the work of Holmes. They are therefore not given in detail but are summarised graphically in Text-figs. 2 and 3.



Text-fig. 3. Diagram to show the progress of the spread of mosaic (in black) through an older fruiting tomato plant. The intermittent blackening at 4 days merely expresses the fact that the earliest virus particles to pass out from the inoculated leaflet are found at intermittent distances down the stem. The stem, of course, is not infected right through in these places; a mental picture at such a stage should imagine the infective particles (represented by the black areas) moving slowly down to the roots and gradually becoming more and more numerous. Similarly, although the fruit is entirely blackened, in reality only very few virus particles would have yet entered it. Inoculated leaflet shaded.

Young plants are completely invaded by the virus very soon after the virus becomes systemic; medium plants are often not fully invaded until some 3 weeks after inoculation (Text-fig. 2); and old plants may not be fully invaded 1 or 2 months or more after inoculation (Text-fig. 3).

Tests made on field plants, which were much larger, showed that there was still less distribution of virus throughout the plants than occurred in the oldest of the plants in pots. Text-fig. 4 shows the approximate distribution of virus in a tobacco plant *three months* after inoculation, and it is



Text-fig. 4. Diagram to show the approximate distribution of mosaic virus (in black) in a field tobacco plant 3 months after inoculation. 150 samples to test for the presence or absence of virus were taken with sterile cork borers at various points; the position of most of these is indicated by circles in the set of leaves above, the circles being blackened where virus was found to be present. The tobacco plant was 6 ft. high and most of the leaves 2 ft. in length. Inoculated quarter-leaf shaded.

seen that, beyond a slight creeping out into the basal parts of the mid-ribs, there appears to have been no tendency whatever for the virus to move into the mature leaves. This plant was inoculated before flowering, and the capsules were nearly ripe at the time the tests were made. Tests made on other field plants, both tobacco and tomato, confirmed this result, and showed that when the plant inoculated was already a few feet in height, invasion of the stem and of the leaves at the growing point occurred within 12 days, but that the mature leaves (apart perhaps from some near the top) remained free from virus until the time of testing some 2 months later.

It seems possible that the difference between the spread of virus in field plants as compared with pot plants may be connected with the fact that the leaves of pot plants are rarely fully grown, so that organic food materials may continue entering them for some time. The leaves of the field tobacco plant in Text-fig. 4 were from 20–26 in. long, so that they were undoubtedly mature, and movement of assimilates in the phloem may have been entirely away from the leaves.

Nelson (1932) has recently drawn attention to the fact that systemic infection of plants by viruses may often not be so complete as was formerly supposed.

MOVEMENT INTO THE FRUIT.

As shown in Text-fig. 3, the virus was found to move into all the developing fruit trusses up the stem of a tomato plant immediately on invasion of the stem. This was in marked contrast to the long time taken for invasion of the adjacent leaves.

DISCUSSION.

The results of the foregoing experiments are definitely in favour of the phloem theory of distribution of the virus of tobacco mosaic through the plant.

Speed of movement. It is evident from Table III that the virus travels from the inoculated leaf down to the roots in considerably less than 12 hours. Using the methods employed in this work it would be almost impossible to determine the exact speed of transport, since, by the method of mechanical inoculation of mesophyll cells, the time at which the virus enters the phloem is never known precisely. If a method could be obtained for injecting the virus directly into the phloem, the speed of movement should be easily ascertained. The fact that *Myzus pseudosolani*

will transmit tobacco mosaic from tomato (Hoggan, 1931) offers a promising starting-point for work of this type.

At all events it appears probable that the rate of movement of tobacco mosaic virus within the plant may well fall into line with the high rates of movement already determined for sugar-beet curly top and maize streak. Severin (1924), working with the curly top virus, determined a speed of 7 in. (17·8 cm.) in half an hour at a temperature of 39·7° C. Storey (1928) found in one experiment that the virus of maize streak travelled 40 cm. in 2 hours at a temperature of 30° C. It is generally agreed that the rapid rate of movement of these insect-inoculated viruses from the moment of inoculation is probably due to the virus being injected directly into the phloem. It is significant that the evidence we possess on the rate of movement of metabolites through the phloem gives figures of the same order. Calculations on the rate of movement through the phloem which would be required to account for the observed increase in dry weight of developing fruits and tubers (Dixon, 1923; Mason and Lewin, 1926; Crafts, 1931) have given figures ranging from 20 to 50 cm. or more per hour. Crafts, moreover, determined a rate of about 0·3 cm. per min. (18 cm. per hour) from phloem exudation measurements, using cut stems of Cucurbitaceae, and Mason and Maskell (1928) found that changes in sugar concentration in the leaf could be reproduced within a period of 2 hours in the bark at a minimum distance in one experiment of about 50 cm.

Transport in the early stages as separated particles. The distances which must occasionally separate some of the earliest particles of virus that travel down the phloem are of interest. Since in the present experiments only $2\frac{1}{2}$ cm. samples were taken at 5–10 cm. intervals down the stem, it is not possible to state the maximum distance (beyond $2\frac{1}{2}$ cm.) which separated particles of virus in any particular case. As an extreme case it seems quite conceivable, however, that under certain conditions a few virus particles might escape into the phloem and be carried down as far as the roots without infecting any of the tissues on the way, before the next particles entered the phloem.

Escape of virus from the phloem. At present we have no tests delicate enough to detect the presence of the virus while it is still in the phloem, and before it has escaped into the adjacent mesophyll cells apart from the method of taking cuttings or test-tube samples from the stem. Plate IV, fig. 1, shows a leaf inoculated with yellow tobacco mosaic just above a gap in a lateral vein. The leaf was killed and stained with iodine 6 days after inoculation, and systemic symptoms developed the following day on

the plant from which it was removed. The virus must therefore already have passed from the primary lesion through numerous small veins round the gap and then down the mid-rib into the stem, but no sign of it could be detected by the iodine test in the inoculated leaf, beyond the primary lesion. Nor could it be detected in certain other similar cases by direct sampling of the tissue below the gap, with subsequent inoculation. The virus could only be detected when it had already passed out of the phloem, presumably through lateral sieve plates, and had multiplied for a little while in the adjacent mesophyll cells (Plate IV, figs. 2, 3).

The passing out of the veins from the phloem may frequently occur at fairly widely separated points. This may be seen in the small isolated spots of virus multiplication which are visible in iodine stained leaves: (1) in early stages of systemic invasion of young leaves (Holmes, 1931, Fig. 2G); (2) when the inoculation point on a leaf has been between the main lateral veins instead of near them (Holmes, 1931, Fig. 2D); (3) when the inoculation point has been over a lateral vein, but the virus has been forced by cuts to take a path through small veins (Holmes, 1932, Fig. 3D, and this paper, Plate IV, figs. 2, 3, 4); and (4) when *N. glauca* is inoculated with yellow tobacco mosaic. In this case, although abundant infection can be obtained on the inoculated leaves when sand is used in the inoculum (Samuel and Bald, 1933) and the virus multiplies considerably in the primary lesions, the virus appears to be unable to move out of the inoculated leaf except on rare occasions. In the few cases in which escape does occur the virus becomes visible on the upper leaves in small yellowish spots situated in intimate relation with some of the smaller veins (Plate IV, fig. 5). It can be shown that these spots are local sites of multiplication of the yellow tobacco mosaic, and that no virus is detectable in the intervening tissues of the leaf. This unusual case could be understood if an exceptional penetration of a few virus particles from the primary lesions into the phloem occurred, these, when carried up to higher leaves, passing out into mesophyll cells where it is known that they can multiply readily. A somewhat similar type of case is dealt with below when discussing the effect of temperature on viruses which produce necrosis.

Effect of temperature on virus movement. No extended discussion of the effect of temperature on the movement of virus can be undertaken here, but it is desired to refer to one case only, namely, that of tobacco mosaic on *N. glutinosa*. It is well known that at ordinary temperatures the primary lesions formed by tobacco mosaic on this host are necrotic, and that the virus does not occur in the intervening green parts of the leaf, nor become systemic in the plant. The virus apparently causes such a

severe necrosis in the cells that they collapse and die before there is much multiplication of the virus, or before there is any possibility of its entering the phloem. Nevertheless, as recorded previously (Samuel, 1931), at a temperature of 35° C. the primary lesions are not necrotic, there is abundant multiplication of virus, and systemic infection takes place in the normal manner. This relation between low temperatures and increased necrosis, and *vice versa*, applies also to other cases among plant virus diseases.

Sometimes when *N. glutinosa* plants are inoculated with tobacco mosaic in the greenhouse there is no movement of virus from the inoculated leaves for some time (beyond a very slow spread of the necrosis down the petioles to the basal part of the stem), and then suddenly a small portion of the stem, or of an upper leaf, will become necrotic as if the virus had travelled upwards without affecting the intermediate tissue. Caldwell (1932) recorded the same thing for aucuba mosaic on *Datura stramonium*. A possible explanation of this is that a rise in temperature, such as might occur in a glasshouse on a sunny day, would allow of multiplication of virus without necrosis in some cells bordering a sieve-tube; a few virus particles might then enter the phloem and be carried upwards; on passing out into mesophyll cells a short period of multiplication might occur, and then necrosis supervene following a fall in temperature.

In cases such as the above, no virus can usually be detected in the stem between the upper and the lower necrotic patches, probably because no virus is there; the virus particles may have passed into and along the phloem only during a short period when the temperature made it possible.

The evidence which has been gathered above is very definitely in favour of the long distance transport of tobacco mosaic virus through the plant being accomplished via the phloem. This would bring the tobacco mosaic virus into line with many other viruses, especially insect-transmitted viruses, which are already fairly generally recognised as being distributed via the phloem (Quanjer, 1931, p. 581).

Since tobacco mosaic is in many ways a particularly easy virus to work with, it offers attractive possibilities as an aid in the study of phloem movements, and in this way may contribute a chapter to plant physiology which would be very welcome. The observation that the virus almost invariably moves down to the roots first, except when developing fruit are situated a little above the inoculation point, is of particular interest. If it is a true indication of the path of movement of metabolites from the leaf, it is a fact which could have been determined in no other way as yet.

The subsequent movement to the top of the plant would also suggest that the metabolites used at the growing point came, not directly from the leaves, but via the roots.

These results can only provide a stimulus for further work, both with tobacco mosaic and other viruses. More complete studies on the movement of the numerous potato viruses would be particularly valuable, but it is obvious that to be of use these must be planned on a much more extensive scale than has been done in the past, including adequate determination of the position of the virus in the early stages following inoculation. Kunkel (1930) recorded that the virus of peach yellows moves down the stem about ten times as fast as it moves upwards. Full details of these experiments have not yet appeared, but there would seem to be some other factor than ploem movements concerned in this case. Possibly particle size of the virus is important. It is these cases which apparently cannot be explained purely on the basis of phloem transport that are the most interesting.

At first sight it would seem that what have been called the different rates of travel of the two constituents of certain mixed viruses (such as tomato streak) might prove rather difficult to explain by the phloem theory of transport. But it has not yet been satisfactorily demonstrated that there actually *are* different rates of travel of the two constituents of these mixed viruses. It has only been demonstrated that there are different times of arrival at a point at some distance from the inoculation point. Differences in rate of multiplication of the two viruses in the primary lesion, or of size of particles, might determine very different times of entry of each into the sieve-tubes.

SUMMARY.

By means of extensive cutting-up tests on tomato plants inoculated with tobacco mosaic the following points have been determined:

1. Confirming the work of Holmes, there is no movement of virus from the inoculated leaf for the first 3 or 4 days. This period is slightly less or considerably more according to the greater or less activity of growth of the plant.

2. When the virus passes out from the inoculated leaf it travels first to the roots of the plant with such speed that it can seldom be intercepted at intervening positions.

3. Usually about a day later it travels with equal rapidity to the top of the plant.

4. In the earliest stages of entering the stem virus particles may be separated by considerable distances (at least several centimetres), since successive samples taken from the stem may yield lengths of $2\frac{1}{2}$ cm. (the length of the cuttings) free from infection, interspersed irregularly between portions containing the infection.

5. The presence of developing fruit trusses on the stem may cause part of the virus to travel upwards as far as these trusses at the same time that the initial downward movement is occurring.

6. The virus enters developing fruits at the same time as it travels through the stem, whereas adjacent leaves remain uninfected for days or weeks.

7. In pot plants, after the initial rapid infection of the developing leaves at the top of the plant, the more mature leaves become successively invaded from the top downwards and from the bottom upwards until the plant is completely invaded by the virus. Complete invasion occurs very quickly in small vigorously growing plants; it may take 3 weeks or more in medium sized plants (Text-fig. 2); and as much as 2 months in large fruiting plants (Text-fig. 3).

8. Complete invasion never occurs when large field plants of tobacco or tomato bearing a number of mature leaves are inoculated. The mature leaves remain free from virus, apart from a limited movement along the mid-ribs, for periods of more than 3 months following inoculation (Text-fig. 4).

It is considered that these facts favour the theory of a slow cell to cell movement of the virus via the plasmodesmen, combined with a rapid distribution through the plant via the phloem, and the value of tobacco mosaic virus as an indicator of phloem movements is emphasised.

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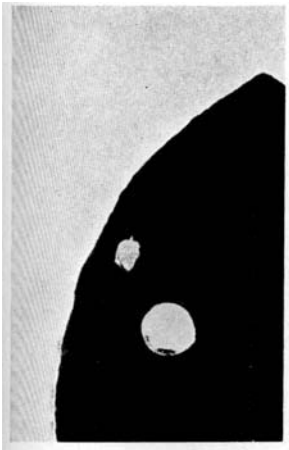

Fig. 1.

Fig. 2.

Fig. 3.

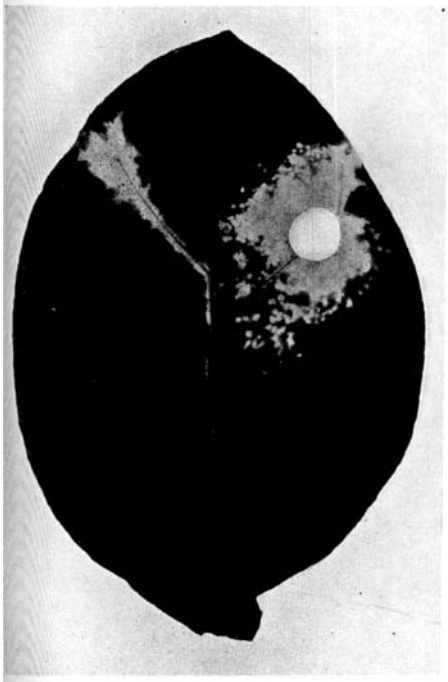

Fig. 4.

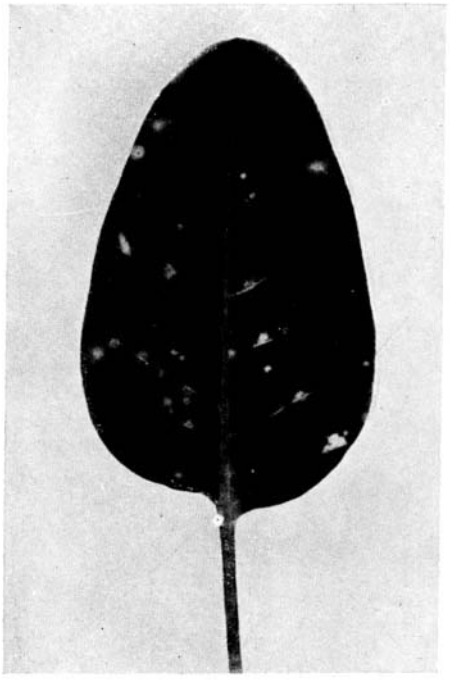

Fig. 5.

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EXPLANATION OF PLATE IV.

Figs. 1–3. Leaves of Blue Pryor tobacco, inoculated over a lateral vein near the margin with yellow tobacco mosaic. Prior to inoculation a disc of tissue was removed with a cork borer, interrupting the lateral vein about 1 cm. below the point of inoculation. Leaves removed after various intervals, killed in steam, decolorised with alcohol, and stained with iodine. Fig. 1, 6 days after inoculation; systemic symptoms just appearing. Fig. 2, 8 days after inoculation; note the single spot of virus multiplication (opposite X) which has appeared in the mesophyll below the vascular interruption. Fig. 3, 11 days after inoculation.

Fig. 4. Similar to the above, but inoculated on both sides of the leaf, the lateral vein being interrupted below the point of inoculation on one side only. 14 days after inoculation.

Fig. 5. Upper leaf of a *N. glauca* plant inoculated 6 weeks previously with yellow tobacco mosaic. Leaf taken from one of the three plants, out of thirty inoculated, which showed symptoms on leaves above the inoculated leaves. The symptoms consist only of localised yellow spots, in most cases definitely associated with the veins of the leaf.

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