Properties of the Initial Tobacco Mosaic Virus Infection Sites Revealed by Heating Symptomless Inoculated Tobacco Leaves

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ABSTRACT

Heat-induced tobacco mosaic virus (TMV) lesions in inoculated Turkish tobacco (*Nicotiana tabacum*) leaves proved usable in some common procedures requiring local lesions, as well as in studies on the characteristics of symptomless virus infections. In the range where lesion number was inversely proportional to inoculum dilution, quantitative assays of infectivity by the two-dilution method had the same level of accuracy usually observed with assays on conventional local lesion hosts. The susceptibility of Turkish tobacco leaves to TMV inoculation, in terms of heat-induced lesion number per unit leaf area, proved to be unaffected by leaf age or by trimming the plants to two leaves before inoculation. The size of the heat-sensitive area 2 or 3 days after inoculation indicated that cell-to-cell TMV movement in inoculated Turkish tobacco leaves is increased by increases in air temperature (20-30 C) and by trimming the plant before inoculation, but is unaffected by leaf age and by differences in light intensity (8,611-21,528 lux), day length (8-16 hours), or relative humidity (35-80%). The extraction of fewer infectious virus particles from heated tissue than from unheated tissue 12 hours after treatment, was the earliest indication that heating had altered treated tissue.

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The development of the starch-lesion technique (12) has facilitated the investigation of virus infection in symptomless tissue. As a research tool, starch lesions have been used to assay infectivity quantitatively (15, 16), to detect a virus which has no known local lesion host (1, 7, 15), to determine host susceptibility to inoculation (5, 6, 13, 15, 16, 29, 30), to measure cell-to-cell virus spread in inoculated leaves (5, 6), to study systemic virus movement (12, 13, 27), to demonstrate cross protection (22), and to accentuate small necrotic lesions (2, 8, 9, 10). Alterations in starch-lesion number caused by light (15, 16), water relations (16), temperature (5, 6, 16), plant nutrition (29, 30), the use of different plant tissues (13, 16, 29), inoculum constituents (16), different methods of inoculation (16), or previous infection with other viruses (22), have been documented. Changes in starch-lesion size by postinoculation temperatures have also been reported (5, 6).

After our preliminary results associated heat-induced lesions with the initial infection sites in symptomless Turkish tobacco (*Nicotiana tabacum* L.) leaves inoculated with tobacco mosaic virus (TMV) (3), other workers used this simple dependable technique to investigate symptomless virus infections. Ohashi and Shimomura (17) reported that there is no difference between the number of lesions in hypersensitive tobacco leaves and the number of heat-induced lesions in similar nonhypersensitive tobacco leaves inoculated with the same TMV inoculum. However, in these experiments, the heat-sensitive areas were always larger than the lesions in hypersensitive leaves at any given time after inoculation. When Takahashi (32) studied the influence of leaf age on the development of heat-induced TMV lesions in nonhypersensitive tobacco leaves from intact plants, he observed the greatest number of lesions per unit leaf area in middle leaves, and the largest lesions in the upper leaves. Additional studies were undertaken in our laboratory to investigate virus infection in symptomless leaves, and to further define the usefulness of the heat-treatment technique.

MATERIALS AND METHODS.—Experiments involved 7- to 9-week-old Turkish tobacco plants that were grown in the greenhouse and fertilized as previously described (4). Leaves when inoculated were rubbed with a suspension of 38-μm (400-mesh) Carborundum in a dilution of infectious crude juice obtained from Turkish tobacco leaves systemically infected with the common strain of TMV. At some time before heat treatment, each plant was decapitated just above the youngest desired leaf, and all unwanted leaves were removed. Each treatment within an experiment usually involved 10 leaves distributed as two leaves on each of five plants.

Heat treatment involved immersing the infected leaves of each plant for 40 seconds in a water bath containing 454 liters of tap water maintained at 50 C. Immediately after heating, five-to-seven plants were placed in each recovery chamber that was located in a 21 C growth chamber (4). After the treated plants were removed from the recovery chambers 2 days later, they were maintained in this growth chamber until tissue collapse in the lesion areas was complete.

Experimental results were usually recorded 3-6 days after the heat treatment. The lesion number per leaf was determined with a magnifying glass (× 1.2) and a lighted background. Lesion size was recorded as the average diameter of 100 randomly selected lesions distributed as 10 lesions on each of 10 leaves and measured by means of a stereoscopic microscope (× 10) equipped with an ocular micrometer. Each experiment was repeated at different times of the year.

RESULTS.—*The effect of leaf age on TMV infection of trimmed tobacco plants.*—Our knowledge about the influence of leaf age on the infection of tobacco leaves by rubbing with TMV inoculum has been obtained mostly from experiments with local lesions in hypersensitive tobacco. In a recent study, evaluating the effect of leaf age on the number and size of heat-induced TMV lesions in nonhypersensitive leaves from intact Samsun tobacco (*N. tabacum*) plants, Takahashi (32) counted the greatest number of lesions per unit leaf area in middle leaves with a leaf plastochron index of 5 to 8, and observed the largest lesions in the youngest leaves. We also investigated the relative susceptibility to TMV of nonhypersensitive tobacco leaves of different ages, and studied the relative rates of cell-to-cell movement of TMV in such leaves. Since decapitation has been reported (11) to reduce the variation of the number of TMV lesions in leaves at different leaf positions on plants of *Nicotiana glutinosa* L., we decided to compare leaves on trimmed plants in this and subsequent studies.

On the day before TMV inoculation, the growing points and all but two leaves were removed from Turkish tobacco plants that had been grown in the greenhouse. A plant having two young developing leaves was paired with a nearby plant containing two older fully expanded leaves, so that the differently treated plants were arranged in a checkerboard pattern on the greenhouse bench. Young leaves on plants trimmed a day before inoculation were usually thinner, appeared lighter in color, and expanded slightly faster than older leaves of plants trimmed at the same time. In the susceptibility studies, leaves to be inoculated were traced on calibrated graph paper (3.94 squares/cm) just before inoculation in order to record the size of the leaves at that time. Leaves were rubbed with a TMV inoculum capable of inducing 10-500 initial infection sites per leaf. A pair of young leaves on one plant was always rubbed just before the pair of old leaves on the next plant. The practice of alternating plants with young and old leaves was also used during the standard heating procedure 2 or 3 days after inoculation, and in the arrangement of the treated plants in each recovery chamber.

TABLE 1. The number and size of lesions in Turkish tobacco leaves differing in age when inoculated with tobacco mosaic virus 2 or 3 days prior to the standard heat treatment[a]

Leaf age group[b]	Lesion number		Diameters (mm) of lesions induced 2 and 3 days after inoculation		Increase in diameter of sensitive area per day (mm)
	Lesions per leaf	Lesions per cm^2	2 days	3 days	
Young	304	1.95	1.14	2.39	1.25
Old	414	1.91	1.17	2.38	1.21
Difference[c]	110	0.04	0.03	0.01	0.04

[a]The two types of leaves were on different paired plants that were each trimmed to two leaves a day before inoculation and maintained in the greenhouse before and after inoculation. Each number is an average from data of three experiments, each involving 10 leaves per age group. The diameters of 10 lesions per leaf were measured.

[b]At the time of inoculation, young emerging leaves averaged 167 cm^2 in area, and older expanded leaves averaged 241 cm^2 in area.

[c]Differences between young and old leaves were assessed by the *t*-test and proved not to be significant ($P = 0.05$) in each of three experiments involving lesion counts and in two of three cases involving the same type of lesion measurement.

Young and old leaves differing by 50-100 cm^2 in area were compared. The young leaves consistently contained fewer heat-induced TMV lesions per leaf than did the older leaves, but the differences were not statistically significant (Table 1). Neither were the differences significant when the data were calculated as the numbers of lesions per unit leaf area. Lesions induced in both types of leaves on the same day after inoculation were similar in average size (Table 1). Therefore, leaf age did not significantly affect the rate of cell-to-cell movement of TMV in the leaves of our trimmed nonhypersensitive tobacco plants.

Influence of trimming the tobacco plant on TMV infection.—Since the removal of the growing tip and unwanted leaves before inoculation was a routine practice in this research, the effects of this practice on heat-induced lesion size and number were studied. Leaves on trimmed tobacco plants became larger in size, firmer in texture, lighter in color, and more resistant to heat injury than leaves on intact tobacco plants. In the only reported study of this kind with a nonhypersensitive host, removal of the apical growing points of cucumber plants before inoculation did not alter the susceptibility of the cotyledons of those plants to TMV (16).

Turkish tobacco plants grown in the greenhouse were arranged on a greenhouse bench so that plants trimmed 1 day before, or just before, inoculation alternated with intact plants in a checkerboard pattern. In the susceptibility studies, the leaves to be inoculated were traced on calibrated graph paper (3.94 squares/cm) just before inoculation, so that the leaf areas at this time could be calculated. Trimmed and intact plants were alternately inoculated with a TMV inoculum capable of inducing 10-500 initial infection sites per leaf. Trimmed and intact plants also were alternated during the standard heating procedure 2 or 3 days after inoculation. Just before the hot-water exposure, the intact plants were trimmed in order to standardize the manipulation of the plants to be compared. Each recovery chamber contained plants of each treatment.

Comparisons between the differently treated leaves on the basis of the average lesion number per unit leaf area

TABLE 2. The number and size of lesions in Turkish tobacco leaves on trimmed and untrimmed plants inoculated with tobacco mosaic virus and heated 2 or 3 days after inoculation[a]

Plants					
Kind	Time before inoculation when trimmed[b] (hours)	Number of lesions per cm^2	Diameters (mm) of lesions induced 2 and 3 days after inoculation		Increase in diameter of sensitive area per day (mm)
			2 days	3 days	
Intact		0.51	1.27	2.34	1.07
Trimmed	0-1	0.49	1.42	2.76	1.34
Difference		0.02[c]	0.15[d]	0.42[d]	0.27[d]
Intact		0.51	1.33	2.44	1.11
Trimmed	24	0.54	1.47	2.74	1.27
Difference		0.03[c]	0.14[d]	0.30[d]	0.16[e]

[a]Paired plants were maintained in the greenhouse before and after inoculation, heated for 40 seconds at 50 C, and subsequently maintained under identical conditions. Data are averages of results from three experiments each involving 10 leaves per treatment. Ten lesions per leaf were measured to determine the average lesion diameter. Differences were assessed by the *t*-test.

[b]The growing point and all but two expanded leaves were removed from each trimmed plant.

[c]Differences in three experiments were not significant ($P = 0.05$).

[d]Differences in three experiments were significant ($P = 0.05$).

[e]In two of three experiments, the enlargement of the heat-sensitive area per day in trimmed plants was not significantly different ($P = 0.05$) from that in intact plants.

TABLE 3. The size of heat-induced lesions in leaves of trimmed Turkish tobacco plants inoculated with tobacco mosaic virus and then maintained for the next 2 days in various environmental conditions[a]

Temperature (C)	Light intensity (lux)	Day length (hours)	Relative humidity (%)	Lesion diameter[b] (mm)
20	8,611	16	50-60	0.80[c]
25	8,611	16	50-60	1.55[c]
30	8,611	16	50-60	2.49[c]
25	8,611	16	50-60	1.59
25	21,528	16	50-60	1.64
25	21,528	8	50-60	1.64
25	21,528	16	50-60	1.67
25	8,611	16	35-45	1.56
25	8,611	16	70-80	1.59

[a]Five plants, each with two inoculated leaves, were placed in each growth chamber during comparisons of environments differing by one factor. All inoculated leaves were heated for 40 seconds at 50 C 2 days later. Ten lesions on each heated leaf were measured.

[b]Differences were assessed by the *t*-test.

[c]Each of these diameters were significantly different ($P = 0.05$) from each of the other two in this comparison.

revealed no consistent or significant differences (Table 2). However, the average size of heat-induced lesions in leaves of trimmed plants heated 2 or 3 days after inoculation was consistently and significantly greater than the size of lesions in similarly treated leaves of intact plants. The increase in the size of the heat-sensitive area per day was always statistically different when plants trimmed just before inoculation were compared to intact plants, but this was not always so when plants trimmed a day before inoculation were involved in the comparison. These differences between leaves of trimmed and intact plants tended to be greater when plants were trimmed just before inoculation, than when the plants trimmed earlier were used. The rate of cell-to-cell virus spread was probably stimulated in rapidly expanding leaves of recently trimmed plants; as the expansion of the infected leaves on trimmed plants slowed, however, the rate of virus spread in these leaves approached that in leaves of intact plants.

The effect of postinoculation environmental conditions on the size of heat-induced TMV lesions.—When Turkish tobacco plants maintained in the greenhouse were heated 2 days after inoculation with TMV, the average size of the resulting lesions in the inoculated leaves varied considerably throughout the year. From the only reported study of environmental effects on the rate of virus spread in symptomless inoculated leaves, Gonzalez and Pound (5, 6) concluded that the change in the average size of starch lesions obtained on different days in leaves of *N. glutinosa* infected with turnip mosaic virus was not affected by air temperature despite the fact that the average size of the starch lesions in leaves at 28 C was larger than those in leaves at 20 C at any given time after inoculation. The light intensity, day length, and relative humidity during this temperature study were not specified. With the improved control of environmental conditions in growth chambers, the influence of air temperature, light intensity, day length, and relative humidity on the size of heat-induced TMV lesions in Turkish tobacco leaves could be determined.

Turkish tobacco plants grown in the greenhouse were decapitated and trimmed of all but two large expanding leaves just before inoculation. After the attached leaves were rubbed with an inoculum that would induce 10-100 TMV infection sites per leaf, similar plants were transferred to growth chambers adjusted to environmental conditions differing by one factor. During the 2 days of exposure in the growth chambers, each plant was watered but not fertilized so that variations in nutrient uptake due to the different environmental conditions would be minimized. An alternating sequence of heating the plants maintained in environments differing by one factor insured that the standard heat treatment was not affecting the final results. Each recovery chamber contained plants that had been exposed to each environment being compared.

The indicated changes in light intensity, day length, or relative humidity at the constant temperature of 25 C did not significantly affect the average size of TMV lesions induced by heating 2 days after inoculation (Table 3). Progressively higher air temperatures increased the final diameter of the heat-sensitive areas. None of the tested environmental conditions substantially altered the appearance of heat-induced lesions in the treated leaves.

TABLE 4. The relative infectivities of tobacco mosaic virus "unknown" inocula compared to "standard" inocula as calculated from two-dilution assays involving heat-induced lesions[a]

Experiment number	Percentage potency of "unknown" relative to that of "standard"		Error	Dilution curve slope[d]
	Actual[b]	Estimated[c]		
1	50	46	8%	1.13
2	50	42	16%	1.04
3	50	54	8%	0.89
4	50	53	6%	0.78

[a]For each assay, two different half-leaves on each of 12 plants were inoculated with each dilution 2 days before the plants were heated at 50 C for 40 seconds.

[b]Actual potency calculated from the extent to which the "standard" was diluted in the preparation of the "unknown." Percentage potency of the "standard" inoculum was considered to be 100 in each test.

[c]Calculated from the experimental data by means of Sherwood's (28) formula.

[d]Calculated from the denominator of the fraction in Sherwood's (28) formula.

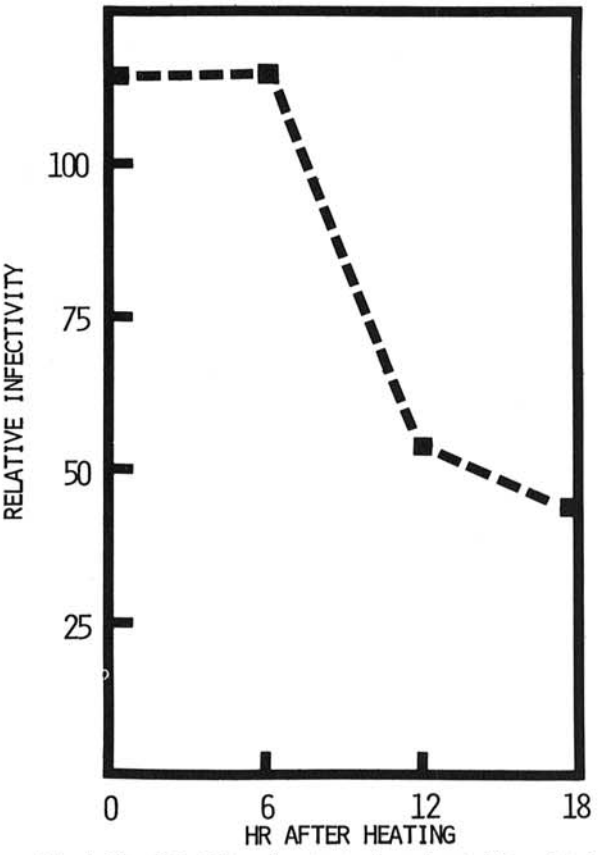

Fig. 1. The infectivity of extracts from heated inoculated tissue relative to that of extracts of comparable unheated inoculated tissue. Half-leaves of tobacco mosaic virus-inoculated Turkish tobacco leaves were heated at 50 C for 40 seconds 2 days after inoculation. The heated and unheated tissues of 10 leaves were then sampled at each 6-hour interval. Each point represents the average of three two-dilution assays made on Samsun NN tobacco with extracts taken at the same interval after heating.

Use of heat-induced lesions for the determination of the relative infectivity of two TMV inocula.—Many workers (20, 23, 25, 26, 31) have compared designated "unknown" solutions of known potency to a "standard" inoculum on suitable local lesion hosts by the two-dilution assay method (31). The determination of the relative infectivity of two known virus solutions revealed the minimal difference between inocula detectable by this technique, and also helped estimate the variability of assay results. In the following experiments, this type of comparison was used to evaluate whether the accuracy of assays using heat-induced TMV lesions was similar to previous results from assays on conventional local lesion hosts.

Each assay involved 12 uniform Turkish tobacco plants that were grown in the greenhouse and trimmed of all but the four youngest expanded leaves 12-24 hours before virus inoculation. The "standard" inoculum was prepared by diluting crude extract from systemically infected Turkish tobacco leaves with enough distilled water to provide a preparation capable of inducing 100-500 lesions per leaf. A portion of this preparation was diluted with an equal volume of distilled water to obtain the "unknown" inoculum. The two remaining inocula necessary for the two-dilution assay were prepared as $10^{-0.5}$ dilutions of the "standard" and the "unknown." After the addition of equal amounts of 38-μm (400-mesh) Carborundum to the inocula, each was rubbed on the appropriate half-leaves according to the scheme originally used by Rochow et al. (25) for another host-virus combination. The inoculated plants were exposed 2 days after inoculation to the standard treatment for the production of heat-induced TMV lesions.

Sherwood's formula (28) was used to calculate the "percentage potency" of the "unknown" relative to the "standard" and the slope of the dilution curve in the area of the comparison. The estimated potencies were within the acceptable 20% variation (21) of the actual potencies, and the slope of each dilution curve was approximately unity (Table 4). When the designations of "unknown" and "standard" were reversed, the error was still less than 20% in each of the comparisons.

Influence of heating on the extractable TMV infectivity of inoculated tissue.—Previous studies comparing the amount of extractable infectious virus in collapsing and symptomless infected tissue indicated that virus multiplication may be stimulated (24), reduced (19), or unaffected (24) during the necrotic reaction. In our investigation, the concentration of extractable infectious TMV in heated inoculated tissue was compared to that in unheated inoculated tissue at intervals between heating and lesion formation. The method used was to compare the amount of infectious virus extracted from heated half-leaves with the amount extracted from the opposite unheated half-leaves.

The growing point and all leaves except one symmetrical expanded leaf were removed from each Turkish tobacco plant a day before inoculation. In order to minimize variations in the numbers of infections due to differences in susceptibility, an inoculum (crude TMV extract diluted 1:100) capable of inducing the maximum number of initial infections was used. Two days later, one half of each leaf was given the standard heat treatment, and the plants were placed in recovery chambers. At 6-hour intervals after heating, 10 leaves were excised and the midribs removed. The treated leaves were selected so that each sample of heated or unheated tissue consisted of five half-leaves from the right of the midvein and five half-leaves from the left of the midvein. Each sample was then ground in a separate sterilized meat grinder, and the resulting extract was squeezed through cheesecloth. The entire crude extract for each treatment was diluted with distilled water until the most concentrated inoculum could be expected to cause 50-400 lesions per half-leaf of Samsun NN tobacco (*N. tabacum*). At each interval after heating, the relative infectivity of the extract from heated leaves was compared to that of the extract from the unheated leaves by the two-dilution assay procedure (31) on Samsun NN tobacco. Since the first indications of lesion formation were evident in these tests 18-24 hours after heating, sampling was discontinued after 18 hours to avoid any complicating effects of necrosis on virus extraction. Observations on 10 extra leaves confirmed that small solid spots developed only in the heated half-leaves.

A decrease in the extractable TMV content of heated leaves relative to that of unheated ones occurred between 6 and 12 hours following heat treatment (Fig. 1). The relative amounts of virus infectivity extracted from heated tissue continued to decrease at least until heat-induced lesions appeared. The data are not sufficient to indicate whether the reduction in infectious virus content of heated tissue was due to heat-induced alterations in virus synthesis per cell prior to collapse of the affected cells, or to slower than normal cell-to-cell movement of virus after heating.

DISCUSSION.—At least with the host-virus combination used, lesions resulting from the heat-induced collapse of local infections appear to be as generally useful as local lesions with other systems. The work reported on here clearly demonstrates, for example, that use of heat-induced lesions can give as reliable results as do regular local lesions in quantitative assays of infectivity, in measuring the effect of different variables on susceptibility to infection, and in studies on cell-to-cell movement of the virus. In addition, our preliminary unpublished results with procedures similar to those used by Jones (14) indicate that heat-induced lesions may be useful in cross-protection tests. Other pointers to the general utility of this technique are the data reported here and earlier (4) indicating that heat-induced lesions can be produced under a wide variety of environmental conditions. Our results (4) and those of others (14, 18, 33) provide reason for believing that the technique can be used with many other host-virus combinations.

Our research provided new information on some aspects of TMV infection in nonhypersensitive tobacco. For the first time, data have been obtained on the effects of trimming this type of plant, on the alterations of virus multiplication in heated inoculated tissue, and on the responses of virus movement in nonhypersensitive tissue to differences in light intensity, day length, or relative humidity. These experiments not only provided information about symptomless virus infections but also suggested additional lines of inquiry.

The decrease in the infectious TMV content of heated leaves relative to that of unheated ones 6-12 hours after treatment was the earliest change we were able to detect following the heat treatment of inoculated leaves.

Although the data were insufficient to pinpoint this reduction as a result of decreased virus synthesis, we believe this to be the case. It seems logical to expect a lethally damaged cell to lose its ability to synthesize virus sometime prior to complete cell collapse. Confirmation of reduced virus multiplication in affected cells would be possible if normally symptomless tissue could be detected and sampled before it collapsed.

Certain observations made during this research suggested that the rate of leaf expansion during cell-to-cell virus movement may affect the size of heat-induced lesions. Leaves on trimmed plants attained a larger size, and contained larger heat-sensitive areas, than leaves on intact plants. When Takahashi (32) measured the size of heat-induced lesions in different leaves from intact plants, he observed larger lesions in the upper leaves than he did in leaves which usually do not expand as rapidly as the younger leaves. Arranging the experiment so that young leaves on a trimmed plant were compared to older leaves on another trimmed plant insured similiar expansion of both types of leaves and helped equalize the enlargement of the heat-sensitive area. In future experiments designed to measure the direct effect of some factor on cell-to-cell virus movement, a correction for the influence of the factor on the rate of leaf expansion may be advisable.

Experiments with heat-induced lesions may provide new insight into TMV infection of hypersensitive tobacco plants in which the final lesion size is affected by factors that alter either the cell-to-cell virus movement or the localization reaction. Parallel tests with tobacco cultivars differing only in the *N* gene should make it possible to determine whether a given factor affects virus movement or the localizing reaction. For example, factors which affect the final size of TMV lesions in hypersensitive tissue without affecting the size of the heat-sensitive area in nonhypersensitive tissue probably act on the localization reaction.

Research with heat-induced lesions has the potential of providing information unattainable by present procedures. Some viruses that are difficult to study could be easily and rapidly detected or quantitatively assayed by the use of heat-induced lesions. Other studies on the susceptibility of a host to virus infection, the movement of virus within leaf tissue, and the final distribution of virus in a plant, are possible with plants that do not exhibit symptoms during virus infection.

LITERATURE CITED

1. DESJARDINS, P. R., and J. M. WALLACE. 1962. Cucumber, an additional herbaceous host of the infectious variegation strain of citrus psorosis virus. Plant Dis. Rep. 46:414-416.
2. EVANS, I. R., and F. W. ZETTLER. 1970. Aphid and mechanical transmission properties of bean yellow mosaic virus isolates. Phytopathology 60:1170-1174.
3. FOSTER, J. A., and A. F. ROSS. 1968. Local lesion development in Turkish tobacco leaves heated after inoculation with tobacco mosaic virus. Phytopathology 58:1050 (Abstr.).
4. FOSTER, J. A., and A. F. ROSS. 1975. The detection of symptomless virus-infected tissue in inoculated tobacco leaves. Phytopathology 65:600-610.
5. GONZALEZ, L. C., and G. S. POUND. 1962. The effect of temperature on multiplication and movement of cabbage virus A in Nicotiana glutinosa L. Phytopathology 52:733-734 (Abstr.).
6. GONZALEZ, L. C., and G. S. POUND. 1963. The response to temperature of cabbage virus A infection in Nicotiana glutinosa. Phytopathology 53:1041-1045.
7. GRANT, T. J., and M. K. CORBETT. 1960. Mechanical transmission of the infectious variegation virus of citrus. Nature (Lond.) 188:519-520.
8. HELMS, K. 1962. Masking of local lesions and the biology of infections of tobacco mosaic virus on Pinto bean. Virology 18:546-552.
9. HELMS, K., and G. A. MC INTYRE. 1962. Studies on size of lesions of tobacco mosaic virus on Pinto bean. Virology 18:535-545.
10. HELMS, K., and G. A. MC INTYRE. 1964. Heat-induced variations in number and size of lesions of tobacco mosaic virus on Pinto bean. Virology 23:565-572.
11. HOLMES, F. O. 1929. Local lesions in tobacco mosaic. Bot. Gaz. 87:39-55.
12. HOLMES, F. O. 1931. Local lesions of mosaic in Nicotiana tabacum L. Contrib. Boyce Thompson Inst. 3:163-172.
13. HOLMES, F. O. 1932. Movement of mosaic virus from primary lesions in Nicotiana tabacum L. Contrib. Boyce Thompson Inst. 4:297-322.
14. JONES, R. A. C. 1973. Effects of heat shock treatment on symptom development in tobacco leaves inoculated with potato mop-top virus. Ann. Appl. Biol. 74:349-358.
15. LAWSON, R. H. 1968. Cineraria varieties as starch lesion test plants for chrysanthemum stunt virus. Phytopathology 58:690-695.
16. LINDNER, R. C., H. C. KIRKPATRICK, and T. E. WEEKS. 1959. Some factors affecting the susceptibility of cucumber cotyledons to infection by tobacco mosaic virus. Phytopathology 49:78-88.
17. OHASHI, Y., and T. SHIMOMURA. 1971. Necrotic lesion induced by heat treatment on leaves of systemic host infected with tobacco mosaic virus. Ann. Phytopathol. Soc. Jap. 37:22-28.
18. OHASHI, Y., and T. SHIMOMURA. 1971. Induction of local lesion formation on leaves systemically infected with virus by a brief heat or cold treatment. Ann. Phytopathol. Soc. Jap. 37:211-214.
19. OTSUKI, Y., T. SHIMOMURA, and I. TAKEBE. 1972. Tobacco mosaic virus multiplication and expression of the N gene in necrotic responding tobacco varieties. Virology 50:45-50.
20. PRICE, W. C. 1945. Accuracy of the local-lesion method for measuring virus activity. IV. Southern bean mosaic virus. Am. J. Bot. 32:613-619.
21. ROBERTS, D. A. 1964. Local-lesion assay of plant viruses. Pages 194-210 *in* M. K. Corbett and H. D. Sisler, eds. Plant virology. University of Florida Press, Gainesville. 527 p.
22. ROCHOW, W. F. 1956. Interference with tobacco mosaic virus infection by cucumber viruses 3 and 4. Phytopathology 46:133-137.
23. ROCHOW, W. F. 1959. Chenopodium hybridum as a local-lesion assay host for brome mosaic virus. Phytopathology 49:126-130.
24. ROCHOW, W. F., and A. F. ROSS. 1955. Virus multiplication in plants doubly infected by potato viruses X and Y. Virology 1:10-27.
25. ROCHOW, W. F., A. F. ROSS, and B. M. SIEGEL. 1955. Comparison of local-lesion and electron-microscope particle-count methods for assay of potato virus X from plants doubly infected by potato viruses X and Y. Virology 1:28-39.
26. ROSS, A. F. 1953. Physalis floridana as a local lesion test plant for potato virus Y. Phytopathology 43:1-8.
27. SAMUEL, G. 1931. Some experiments on inoculating methods with plant viruses, and on local lesions. Ann. Appl. Biol. 18:494-507.

28. SHERWOOD, M. B. 1947. Simple formulas for calculating percentage potency in three- and four-dose assay procedures. Science 106:152-153.
29. SPENCER, E. L. 1935. Effect of nitrogen supply on host susceptibility to virus infection. Phytopathology 25:178-191.
30. SPENCER, E. L. 1935. Influence of phosphorus and potassium supply on host susceptibility to yellow tobacco mosaic infection. Phytopathology 25:493-502.
31. SPENCER, E. L., and W. C. PRICE. 1943. Accuracy of the local-lesion method for measuring virus activity. I. Tobacco-mosaic virus. Am. J. Bot. 30:280-290.
32. TAKAHASHI, T. 1972. Studies on viral pathogenesis in plant hosts. III. Leaf age-dependent susceptibility to tobacco mosaic virus infection in 'Samsun NN' and 'Samsun' tobacco plants. Phytopathol. Z. 75:140-155.
33. YARWOOD, C. E. 1958. Heat activation of virus infections. Phytopathology 48:39-46.