Effect of Aphid Saliva and Extracts of Aphid-Infested Leaves on the Infectivity of Tobacco Mosaic Virus and Some Stylet-Borne Viruses

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ABSTRACT

Crude extracts and resuspended alcohol precipitates of extracts of leaves infested with aphids were tested for their ability to inhibit the infectivity of tobacco mosaic virus (TMV) and four stylet-borne viruses: cucumber mosaic (CMV), tobacco etch (TEV), alfalfa mosaic, and turnip mosaic. Sucrose solutions containing aphid saliva were tested for their ability to inhibit purified TMV, CMV, and TEV. Infectivity tests, made by mechanical inoculation of local lesion hosts, showed that infectivity of the stylet-borne viruses was inhibited by extracts of aphid-infested leaves as rapidly and to as great an extent as TMV. Inhibition of TMV infectivity by saliva-containing solutions occurred in some tests, but was no greater than that of CMV or TEV.

The fact that preparations which contain saliva inhibit the infectivity of stylet-borne viruses to the same extent as they inhibit TMV infectivity makes questionable the conclusion of others, based on experiments with similar preparations, that inability of TMV to be transmitted by aphids is due to the inhibitory effects of saliva. Phytopathology 60:1657-1659.

Inhibition of the infectivity of tobacco mosaic virus (TMV) by aphid saliva has often been suggested to explain the inability of aphids to transmit this virus. Day & Irzykiewicz (1), the first to attempt to test this hypothesis, were unable to demonstrate inhibition of TMV by aphid saliva, although they, as well as Orlob (7), showed that saliva of other insects was inhibitory to TMV. Nishi (4) reported that infectivity of TMV was strongly inhibited by extracts of leaves infested by aphids, and suggested that inhibition was due to substances secreted by aphids in the feeding process. Nishi (5) later showed that resuspended alcohol precipitates prepared from infested leaves also inhibited infectivity of TMV. He also reported that infectivity of TMV was inhibited by sucrose solutions which had been fed on by a large number of aphids for 3 days (5). In a recent paper, Nishi (6) suggested that his data support the hypothesis that the inability of aphids to transmit TMV is due to inhibition of infectivity of this virus by aphid saliva. If this suggestion is valid, then preparations such as those reported by Nishi (4, 5) to inhibit TMV infectivity would not be expected to inhibit viruses which aphids are able to transmit. Experiments were designed to compare the effect of such preparations on TMV and some stylet-borne viruses. A preliminary report of these studies has been published (8).

MATERIALS AND METHODS.—The procedures used by Nishi (5) for production and extraction of inhibitor were followed as closely as possible, although in some instances details such as numbers of aphids per leaf or duration of infestation were not available. Mustard (*Brassica perviridis* Bailey 'Tendergreen') or tobacco (*Nicotiana tabacum* L.) plants grown at 22 C with 14 hr of light at 16,500 lumens/m^2 were infested with *Myzus persicae* (Sulz.). After 6-7 days, the infested leaves were covered with aphids. After another 6-7 days, the infested leaves were harvested and the aphids removed, and the leaves were ground in a mortar. The extract was centrifuged 10 min at 12,000 *g*, and the supernatant fluid was tested for inhibition of the viruses. Control extracts were from plants processed in the same manner, but which had not been infested with aphids. Mustard leaves used for preparation of alcohol precipitates of the inhibitor were obtained in the same manner. Alcohol precipitates of noninfested leaves were prepared for use as controls. The procedures described by Nishi (5) were used to prepare the alcohol precipitates. The yield was 8-10 mg of precipitate/ml of sap.

The stylet-borne alfalfa mosaic (AMV), cucumber mosaic (CMV), and tobacco etch (TEV) viruses were propagated in Havana 425 tobacco. Turnip mosaic virus (TuMV) was propagated in mustard. TMV inoculum was prepared by mixing purified virus with sap. Preliminary tests were made to detect whether there were any differences in the effect of the inhibitor on TMV extracted from Samsun tobacco and on purified TMV to which an aliquot of Havana 425 tobacco sap was added to give a concn equal to that in extracts of the stylet-borne virus-infected plants. There was no difference in inhibition. The purified virus was used in all subsequent tests to obtain greater consistency in lesion numbers between experiments.

The virus-infected leaves were triturated in a mortar, and the juice was diluted with 0.02 M phosphate buffer pH 7.0 to a concn which would produce 50-150 lesions/half-leaf of *Chenopodium amaranticolor* Coste & Reyn. with the control inocula. One ml of sap extract was added per ml of virus preparation. The alcohol-precipitated material was made up to a concn of 10 mg/ml in water, and this was also added to the virus preparations at a 1:1 ratio.

Sucrose solutions containing aphid saliva were obtained by allowing ca. 200 *M. persicae* to feed through a Parafilm membrane on 1 ml of 2% (w/v) sucrose for 2-9 days. The aphids were replaced every 2-3 days

in tests of over 2 days' duration. Control solutions were handled in a similar manner, but were not fed on by aphids.

Purified viruses were used to test the inhibitory effect of sucrose solutions which contained aphid saliva. TMV was purified by differential centrifugation, CMV by Scott's method (10), and TEV by the method of Purcifull (9). Virus concn were adjusted to produce 50-150 lesions/half-leaf of *C. amaranticolor* with the control inocula. The saliva solutions were added to the virus preparations in a ratio of 0.9 ml saliva:0.1 ml virus.

Since the effect of inhibitors may be influenced by the assay plant (2), *C. amaranticolor,* a local lesion host for all of these viruses was used for all direct comparisons. Other local lesion hosts used in tests with individual viruses were Havana 425 tobacco and *Nicotiana glutinosa* L.

Leaves of the local lesion host were dusted with Carborundum, and inocula applied with a cheesecloth pad. Half-leaves were inoculated with inhibitor-virus combinations immediately and 2 hr after mixing. The opposite half-leaves were inoculated with the appropriate control preparations. Each treatment was assayed on 3-4 leaves/experiment. Per cent inhibition was calculated by the formula: % inhibition =

$$\left[1 - \frac{\text{number of lesions produced by treatment}}{\text{number of lesions produced by control}}\right] \times 100\%.$$

The t-test for paired comparisons was used to analyze differences in numbers of lesions produced by control and treated inocula for each virus, as well as the difference in inhibition of infectivity of TMV and each of the stylet-borne viruses.

RESULTS.—*Effect of extracts of aphid-infested leaves.*—The averages of the results of six experiments with extracts of aphid-infested mustard leaves are shown in Table 1. As reported by Nishi (4, 5, 6), the infectivity of TMV was greatly reduced by extracts of aphid-infested leaves, both in mixtures tested immediately and those tested 2 hr after addition of extracts to the virus. Infectivity of the stylet-borne viruses, however, was reduced just as rapidly and to the same extent as TMV. Similar results were obtained when extract-virus mixtures were assayed on Havana 425 tobacco for TuMV and TMV in four experiments and *N. glutinosa* for TMV in three experiments. Results were also similar when the viruses were mixed with extracts of aphid-infested tobacco leaves and assayed on *C. amaranticolor.*

TABLE 1. Effect of crude extracts of aphid-infested mustard leaves on the infectivity of tobacco mosaic virus and some stylet-borne viruses[a]

Virus	% Reduction in infectivity after[b] 0 hr	2 hr
Tobacco mosaic	92[c]	92
Turnip mosaic	98	96
Cucumber mosaic	93	97
Alfalfa mosaic	93	98
Tobacco etch	90	97

[a] Averages of six experiments, three replications/experiment.

[b] Based on number of local lesions produced on half-leaves of *Chenopodium amaranticolor.* % Reduction of infectivity = $\left[1 - \frac{\text{number of lesions produced by treated}}{\text{number of lesions produced by control}}\right] \times 100\%$.

[c] Differences between control and treated highly significant ($P = < .01$) for all viruses. Differences between stylet-borne viruses and TMV not significant.

TABLE 2. Effect of alcohol-precipitated material from aphid-infested mustard leaves on the infectivity of tobacco mosaic virus and some stylet-borne viruses[a]

Virus	% Reduction in infectivity after[b] 0 hr	2 hr
Tobacco mosaic	44[c]	65
Turnip mosaic	41	62
Cucumber mosaic	54	69
Alfalfa mosaic	44	59
Tobacco etch	43	58

[a] Averages of eight experiments, three replications/experiment.

[b] Based on number of local lesions produced on half-leaves of *Chenopodium amaranticolor.* % Reduction in infectivity = $\left[1 - \frac{\text{number of lesions produced by treated}}{\text{number of lesions produced by control}}\right] \times 100\%$.

[c] Differences between control and treated highly significant ($P = < .01$) for all viruses. Differences between stylet-borne viruses and TMV not significant.

Effect of alcohol-precipitated material from aphid-infested leaves.—The results of eight experiments with two different preparations are shown in Table 2. A consistent reduction in the infectivity of all viruses occurred immediately upon addition of the alcohol-precipitated material obtained from infested leaves. The stylet-borne viruses were inhibited as rapidly and to as great an extent as TMV. A further reduction in infectivity was observed after 2 hr with all viruses. Similar results were obtained when assays were made on tobacco for TuMV and TMV, and on *N. glutinosa* for TMV.

Effect of solutions containing aphid saliva.—Numerous salivary secretions were evident in the sucrose solutions which had been fed on by the aphids. Three initial tests of TMV mixed with solutions on which aphids had fed for 2 days, assayed on *N. glutinosa* or Havana 425 tobacco, revealed no decrease in the infectivity of TMV.

Solutions on which aphids had fed for periods of 3-9 days were tested for effects on the infectivity of purified TMV, CMV, and TEV. All viruses were assayed on *C. amaranticolor.* A significant reduction in infectivity of TMV and CMV occurred in four of nine experiments, and in that of TEV in two of five experiments. In no case was the infectivity of TMV reduced more rapidly or to a greater extent than that of either of the stylet-borne viruses (Table 3).

DISCUSSION.—Although the inhibition of TMV infectivity by extracts of aphid-infested leaves has been

TABLE 3. Effect of sucrose solutions which contained aphid saliva on the infectivity of tobacco mosaic virus (TMV) and the stylet-borne cucumber mosaic (CMV) and tobacco etch (TEV) viruses[a]

Experiment	Length of time aphids fed (days)[c]	% Reduction in infectivity of[b]		
		TMV	CMV	TEV
1	3	18	14	
2	4	5	7	
3	5	+2	+15	
4	8	22[d]	25[d]	
5	5	12	16	5
6	8	31[d]	27[d]	18
7	8	+6	+ 9	10
8	9	27[d]	29[d]	34[d]
9	7	23[d]	44[d]	17[d]

[a] Four replications/experiment.

[b] Based on number of local lesions produced on half-leaves of *Chenopodium amaranticolor*. % Reduction in infectivity $= \left[1 - \frac{\text{number of lesions produced by treated}}{\text{number of lesions produced by control}}\right] \times 100\%$.

[c] Aphids fed through a Parafilm membrane on 2% sucrose solutions.

[d] Differences between control and treated significant ($P = < .05$). Differences between stylet-borne viruses and TMV not significant in any experiment.

attributed by Nishi (6) to the action of aphid saliva, it seems equally possible that the inhibition may be due to the action of some constituent of the leaves which is formed in response to aphid feeding. Even if one accepts the suggestion that aphid saliva is the inhibitory substance in extracts of aphid-infested leaves, the data in Tables 1 and 2 indicate that such extracts inhibit the infectivity of stylet-borne viruses to the same extent as they do TMV. If the inability of aphids to transmit TMV is due to inhibition by saliva, then aphid-transmitted viruses would not be expected to be inhibited to the same extent.

The data presented in this report conflict with those of Nishi (6), who reported that while inhibition of TMV occurred immediately, that of TuMV and AMV occurred gradually over a period of 2 hr. In Nishi's experiments, however, each virus was assayed on a different local lesion host. Thus, his failure to get similar inhibition with all three viruses might reflect differences in susceptibility of these plants to the inhibitor, a situation well documented for inhibitors of plant origin (2).

The attribution of the inhibitory effects of the sucrose solutions to aphid saliva is also open to question, because microbial activity in the saliva-containing solutions might be higher than that in the controls. Again, aside from this consideration, inhibition of TMV was not significantly greater than that of CMV or TEV in any of the experiments (Table 3). Inhibition of CMV by such a system has also been reported by Nakazawa (3).

The fact that preparations which contain saliva inhibit the infectivity of stylet-borne viruses to the same extent as they inhibit TMV infectivity makes questionable Nishi's (6) conclusion, based on experiments with similar preparations, that inability of TMV to be transmitted by aphids is due to the inhibitory effects of saliva.

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