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Effects of Oxalate and Bentonite on Aphid Transmission of Tobacco Mosaic Virus

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由　崎　俊　道：タバコモザイク・ウイルスのアブラムシによる伝搬に及ぼすシユウ酸塩およびベントナイトの影響

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Abstract

An addition of oxalate to the inoculum gave rise to an increase in mechanical infections of tobacco mosaic virus (TMV) or its ribonucleic acid (TMV-RNA) in *Nicotiana glutionsa* L.. Bentonite also caused a similar increase in infections of TMV-RNA in *N. glutinosa.* When TMV preparations containing oxalate or bentonite were sprayed on the leaves of *N. glutinosa* and the leaves were then infested with aphids (*Myzus persicae* Sulz.), no effect was found on the infections of TMV. An increase in the infectivity of TMV-RNA was found on the leaves which were sprayed with TMV-RNA preparations containing oxalate or bentonite before aphid infestation ; oxalate promoted the infection of TMV-RNA by about 1.8 times that of the controls, bentonite promoted it by about 2.8 times while the mixture of these chemicals showed the same degree of infectivity of TMV-RNA as that of bentonite alone. These increased rates were lower than those due to of mechanical inoculation.

Introduction

It has been reported that the infection of tobacco mosaic virus (TMV) in *Nicotiana glutinosa* L. occurs when aphids are given access to leaves that had previously been sprayed with TMV or its ribonucleic acid (TMV-RNA) preparation (Teakle and Sylvester, 1962 ; Lojek and Orlob, 1969, 1972a, 1972b ; Pirone, 1969, 1971, 1972 ; Bradley and Harris, 1972 ; Harris and Bradley, 1973a, 1973b). It is also known that the addition of oxalate or bentonite to the inoculum increases the infectivity of TMV or TMV-RNA in *N. glutionsa* by mechanical

inoculation (Singer and Fraenkel-Conrat, 1961 ; Yoshizaki, 1975, 1980a, 1980b, 1981). The present study was designed to examine whether oxalate and bentonite might be able to increase the infectivity of TMV or TMV-RNA in *N. glutionsa* when the virus containing them was sprayed on the leaves prior to infestation with aphids (*Myzus persicae* Sulz.).

Materials and Methods

The ordinary strain of TMV was propagated in tobacco plants (*N. tabacum* L. cv. White Burley). TMV was purified by differential centrifugation (Steere, 1959) and TMV-RNA was prepared from purified TMV by use of the bentonite-phenol method (Fraenkel-Conrat *et al.*, 1961). The concentration of TMV and TMV-RNA was determined spectrophotometrically (Takahashi, 1951). The bentonite suspension was prepared according to the procedure of Fraenkel-Conrat *et al.* (1961). The infectivity of TMV or TMV-RNA in the case of mechanical inoculation was determined by the half-leaf method (Holmes, 1929).

The green peach aphid (*Myzus persicae* Sulz.) propagated on turnips (*Brassica rapa* L. cv. Yorii) was used for the infection experiments. All experiments were carried out with apterous aphids. Detached leaves of *N. glutionsa* were sprayed with the virus preparations and allowed to dry slightly. Each of the leaves was placed on a rubber stand in a petri dish filled with water, and aphids were dropped on the leaves at a rate of 100 per leaf. After infestation with aphids for 6 hours, the aphids were killed with a nicotine sulfate solution. The leaves were incubated on a moistened filter paper under fluorescent lights. The local lesions produced were counted after a lapse of 3 days.

Results

1) The effects of sodium oxalate and bentonite on TMV or TMV-RNA infection by mechanical inoculation

Purified TMV (0.1 μg/ml) or TMV-RNA (10.0 μg/ml) was prepared in a phosphate buffer solution (0.1 M, pH 7.0) containing sodium oxalate at a concentration of 0.02 M, or bentonite at a concentration of 10 mg/ml. As a control, TMV or TMV-RNA was likewise prepared in phosphate buffer solutions. The preparations were inoculated to the leaves of *N. glutionsa*. The total number of local lesions produced is shown in Table 1. The oxalate mixture caused an increase of the local lesions infected with TMV or TMV-RNA by about 3 or 4 times that of the controls. The bentonite mixture increased the local lesions of TMV-RNA infection by about 16 times that of the controls, but failed to increase TMV infections. The addition of oxalate and bentonite to the inoculum induced the greatest increase of the lesions of TMV-RNA infection, as is shown in Table 1.

2) The effects of sodium oxalate and bentonite on TMV infection by aphid inoculation

Four kinds of TMV preparations were prepared as follows ; TMV (5 mg/ml) was suspend-

Table 1. Effects of oxalate and bentonite on the infectivity of TMV and TMV-RNA in mechanical inoculation

Addition of oxalate or bentonite to inoculum[a]	Virus[b]	Number of local lesions[c]		
		Addition	Control	% of control
Oxalate	TMV	684	211	324.1
	TMV-RNA	872	238	366.4
Bentonite	TMV	189	208	90.9
	TMV-RNA	2297	144	1595.1
Oxalate plus bentonite	TMV	553	196	282.1
	TMV-RNA	3497	184	1900.5

a) Sodium oxalate and bentonite were added to the viruses at a concentration of 0.02 M and 10 mg/ml, respectively.

b) TMV and TMV-RNA were used at a concentration of 0.1 μg/ml and 10 μg/ml, respectively.

c) Total number of local lesions produced on 24 half-leaves of *N. glutinosa* is shown.

ed in a phosphate buffer only ; in a phosphate buffer containing sodium oxalate at a concentration of 0.02 M ; in a phosphate buffer containing bentonite at a concentration of 8.33 mg/ml ; and in a phosphate buffer containing sodium oxalate and bentonite at the same concentrations as for the above preparations. Each of the TMV preparations was sprayed on the detached leaves of *N. glutinosa*. After aphids had infested the leaves for 6 hours, the leaves were incubated on moistened filter papers for 3 days. As a control, the leaves sprayed with TMV preparations, but without aphid infestation, were also incubated on moistened filter papers. The average number of local lesions produced is shown in Table 2. As a result, it was found that local lesions were produced on the leaves sprayed with TMV preparations and then infested with aphids, while on the control leaves which were sprayed with each of TMV preparations without aphid infestation few lesions developed. No obvious effect of oxalate

Table 2. Effects of oxalate and bentonite on the inoculation of TMV by aphids (*Myzus persicae* Sulz.)

TMV in the presence of oxalate or bentonite[a]	Total number of lesions /leaves tested = average number of lesions per leaf	
	Leaves infested with aphids[b]	Control leaves (not-infested with aphids)[c]
Oxalate	101/15=6.7	4/15=0.3
Bentonite	106/15=7.1	2/15=0.1
Oxalate and bentonite	92/15=6.1	6/15=0.4
None	89/15=5.9	2/15=0.1

a) TMV (5 mg/ml) in the presence of sodium oxalate (0.02 M) or bentonite (8.33 mg/ml) and both were sprayed on the leaves of *Nicotiana glutinosa* L.

b) Aphids were infested on the TMV-sprayed leaves at a rate of 100 per leaf for 6 hours.

c) The leaves of *N. glutinosa* were sprayed with TMV suspension, but not infested with aphids.

and bentonite on the inoculation by aphids was found by comparing the number of lesions produced on the leaves sprayed with TMV containing these chemicals to those sprayed with TMV only.

3) The effects of sodium oxalate and bentonite on TMV-RNA infection by aphid inoculation

Detached leaves of *N. glutinosa* were sprayed with TMV-RNA (0.87 mg/ml) suspended in a phosphate buffer, TMV-RNA (0.87 mg/ml) suspended in a phosphate buffer containing sodium oxalate (0.02 M), or containing bentonite (8.33 mg/ml), or containing both sodium oxalate and bentonite in the same proportion as in the earlier experiments. The leaves were infested with aphids for 6 hours. The control leaves were treated similarly, except that they were not infested with aphids. The leaves were incubated in the same manner as in the earlier experiments. Lesions were counted after 3 days. The results are shown in Table 3. The number of lesions counted in aphid infested leaves was obviously higher than that of the control leaves to which aphids had no access. Further, it was found that TMV-RNA infection by aphids increased by the addition of sodium oxalate or bentonite, or both, to the inoculum. It was shown that oxalate promoted the number of lesions by about 1.8 times, bentonite promoted it by about 2.8 times of that of TMV-RNA only, while the mixture of these chemicals showed the same infection of TMV-RNA as that of bentonite alone. However, these increased rates of infectivity were considerably lower than that of mechanical inoculation, as is shown in Table 1.

Table 3. Effects of oxalate and bentonite on the inoculation of TMV-RNA by aphids (*Myzus persicae* Sulz.)

TMV-RNA in the presence of oxalate or bentonite	Total number of lesions / leaves tested = average number of lesions per leaf	
	leaves infested with aphids[b]	Control leaves (not-infested with aphids)[c]
Oxalate	17/25=0.7	1/25=0.0
Bentonite	28/25=1.1	4/25=0.2
Oxalate and bentonite	29/25=1.1	1/25=0.0
None	11/25=0.4	3/25=0.1

a) TMV-RNA (0.87 mg/ml) in the presence of sodium oxalate (0.02 M) or bentonite (8.33 mg/ml), or both were sprayed on the leaves of *Nicotiana glutinosa* L.

b) Aphids were infested on the RNA-sprayed leaves at a rate of 100 per leaf for 6 hours.

c) The leaves of *N. glutinosa* were sprayed with TMV-RNA suspension, but not infested with aphids.

Discussion

Bradley and Harris (1972) found that the removal of the claws of aphids prevented virus inoculation and that no infection resulted from virus induced by parts of the integument other

than the claw. They suggested that aphids do not inoculate TMV into plants by probing or feeding, as some previous reports of TMV infection by aphids had suggested, by attributing inoculation to the mouthparts (Hoggan, 1931, 1934 ; Teakle and Sylvester, 1962 ; Lojek and Orlob, 1969) ; inoculation is probably the result of aphid clawing. Pirone (1972) also reported data which he interpreted as a considerable weakening of the case for the transmission of TMV by aphid stylets, and suggested that transmission resulted from the movement of aphids on the leaves. Harris and Bradley (1973a) reported that the susceptibility of *N. glutinosa* to aphid transmission of TMV is due to injury to the foliar hair of the host brought about by aphid clawing. Further, Lojek and Orlob (1972a) have reported that lesions are produced on leaves covered with TMV or TMV-RNA and other viruses, when those leaves were subsequently exposed to aphids. Similar results were obtained in the present experiments. The addition of oxalate to the inoculum caused the increase of TMV and TMV-RNA infections by about 3.24 and 3.66 times that of the controls given mechanical inoculation, whereas oxalate did not increase TMV infection, and only increased TMV-RNA infection in aphid inoculation slightly. Bentonite or the mixture of bentonite and oxalate caused a substantial increase of TMV-RNA infection by mechanical inoculation to about 16.0 or 19.0 times that of the controls. Though bentonite and the mixture of bentonite and oxalate increased TMV-RNA infection in aphid inoculation by about 2.8 times that of the controls, the rate of increase was considerably lower than those obtained by mechanical inoculation. We therefore suspect that the mechanism of virus infection by aphids is different from that brought about by mechanical inoculation.

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