Effect of Bean Leaf Detachment on Susceptibility to Tobacco Mosaic Virus Infection

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ABSTRACT

The number of lesions developed on bean leaves detached 18 hr before inoculation with tobacco mosaic virus (TMV) was twice the number that developed on attached leaves. Fewer lesions developed on leaves detached 48 hr prior to inoculation than on inoculated attached leaves. The number of lesions on leaves detached 1 hr after inoculation was 54% more than developed on leaves left on the plant. Thirty per cent more were present when detachment after inoculation was delayed 2-18 hr. Since the lesions developed on the leaves detached 6 hr after inoculation were nearly the same as those on the leaves detached 24 hr after inoculation, leaf detachment probably affects lesion formation and virus multiplication, but not the establishment of infective centers. Moderate leaf senescence before and after inoculation stimulates the leaf susceptibility to TMV infection and TMV multiplication within the lesions. Phytopathology 61:354-356.

In a previous study (2), detachment of leaves promoted senescence of leaf tissues, and multiplication of tobacco mosaic virus (TMV) was increased by leaf senescence associated with changes in nucleic acid and protein metabolism. In bean leaves, detachment of the leaf immediately after inoculation decreased susceptibility to TMV infection, whereas TMV concn within lesions increased and lesion size enlarged. However, the nature of susceptibility to TMV infection in leaves detached before inoculation, and the effect of time between inoculation and detachment on local lesion formation, are still unknown. This paper reports the increased susceptibility of bean leaves to TMV infection by leaf detachment before and after inoculation.

MATERIALS AND METHODS.—*Plant materials and virus.*—Bean plants (*Phaseolus vulgaris* L. 'Kairyo Otebo') were grown in 10-cm diam pots in a greenhouse. The ordinary strain of TMV was purified using differential centrifugation. Two primary leaves of plants, 10- to 12-days old, were dusted with 600-mesh Carborundum and rubbed with 10 μg/ml of TMV suspended in 0.10 M phosphate buffer at pH 7.0 by means of an artist's brush. Inoculated leaves were rinsed with tap water, and half leaves were detached at various intervals after inoculation by splitting along the midrib. The detached half leaf was floated on distilled water in a petri dish. The rest of the half leaf remained attached to the plant as a control.

For testing the effect of leaf senescence before inoculation, the previously detached half-leaf and the corresponding opposite half-leaf attached to the plant were inoculated and floated on distilled water in a petri dish. These petri dishes were placed in a growth chamber under continuous fluorescent light (ca. 5,000 lux) at about 25 C. Lesion numbers were determined with transmitted light using an ocular micrometer 2 days after inoculation.

RESULTS.—*Effect of leaf detachment before inoculation on the susceptibility to TMV infection.*—Whether susceptibility to TMV infection is influenced by the physiological conditions of leaves before inoculation was investigated. In Fig. 1, the ratio of lesion number on detached leaves to that on attached leaves (D/A) is shown at intervals of 1, 3, 6, 18, 24, and 48 hr before inoculation. A remarkable increase in the ratio occurred when leaves were detached 18 hr before inoculation, but decreased sharply when leaves were detached and incubated for a longer period before inoculation. Lesions were slightly larger on leaves detached 6 to 18 hr before inoculation, whereas they were comparatively smaller on those detached 48 hr before inoculation.

Effect of length of time between inoculation and leaf detachment on susceptibility to TMV infection.—We reported previously that 33% fewer lesions developed on leaves detached immediately after inoculation as compared with those which developed on attached leaves, whereas TMV concn within each lesion on the detached leaves was 4.3 times higher than that of attached leaves (2). For testing the possibility that local lesion formation was influenced by time between inoculation and detachment, half leaves were detached at intervals of 0.5, 1, 2, 3, 6, and 18 hr after inoculation. Although the number of local lesions was slightly less on leaves detached 0.5 hr after inoculation, the number of leaves detached 1 hr after inoculation was 54% greater and the ratio of increase in lesion number was 30% greater for leaves detached from 2 to 18 hr after inoculation (Fig. 2).

To analyze more precisely the susceptibility of leaves to lesion formation, one half leaf was detached 6 hr after inoculation, and floated on distilled water in a petri dish. The opposite half leaf remained attached for 24 hr after inoculation, and was then detached and incubated as described above for 24 hr. The lesion numbers on both attached and detached leaves were compared 24 and 48 hr after inoculation (Table 1). Visible necrosis appeared a few hr earlier on the detached leaves than on the attached leaves 20-22 hr after inoculation. The ratio of lesion numbers on the detached leaves to those on the attached leaves was 1.5 and 1.2 on an average for 24 hr and for 48 hr incubation, respectively, whereas the ratio of lesion appearance in 48 hr and 24 hr after inoculation was 1.9 and 1.5 on the attached and detached leaves, respectively. Thus, on the attached leaves for 24 hr after

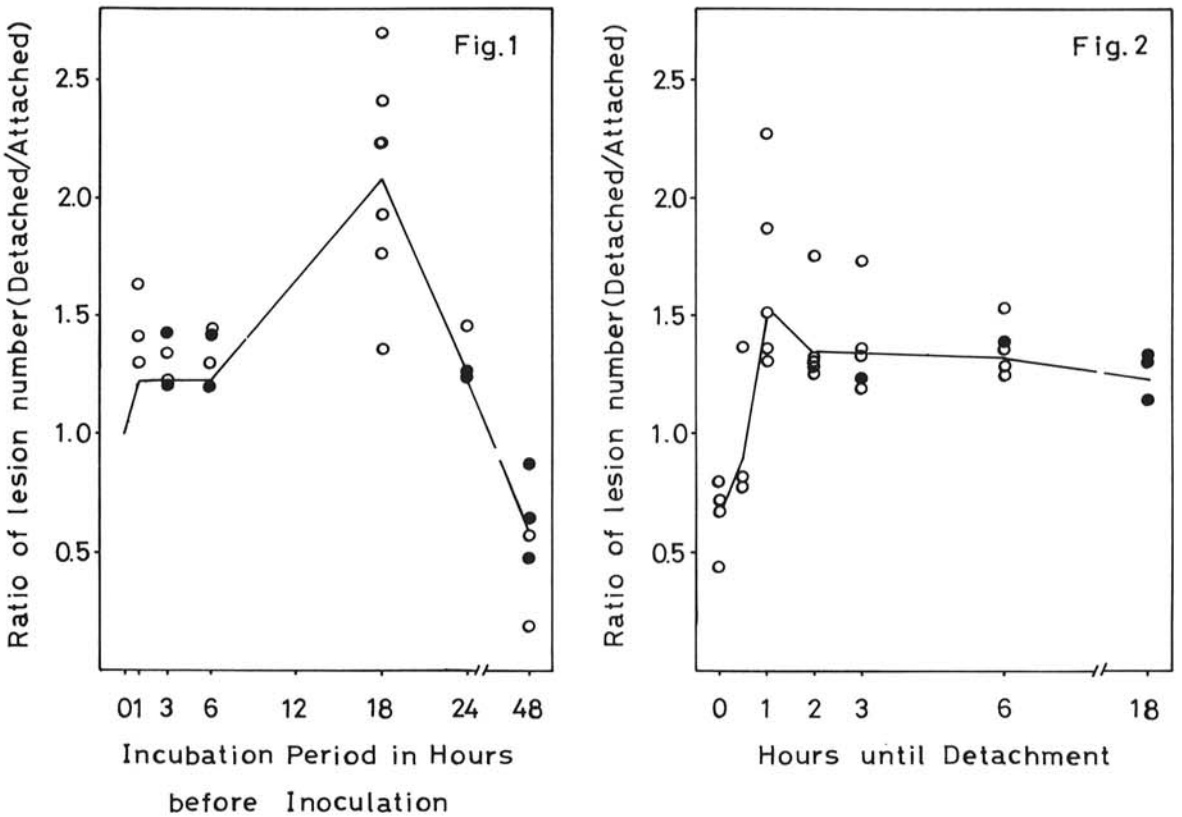

Fig. 1-2. 1) The effect of time between detachment of bean half leaves (degree of senescence) and inoculation of tobacco mosaic virus: ratio of number of local lesions on detached leaves to those on attached leaves. The experiment was repeated 6 times, using 10 half leaves/assay. Significant levels are indicated by ○ (0.01) and ● (0.05). 2) The effect of time between inoculation and detachment of bean half leaves (degree of senescence); ratio of number of local lesions on detached leaves to those on attached leaves. The experiment was repeated 6 times, using 10 half leaves/assay. Significant levels are indicated by ○ (0.01) and ● (0.05). The ratio of lesion numbers immediately after inoculation was cited from Nakagaki & Hirai (2).

inoculation, the lesion appearance was remarkably activated by leaf detachment. Figure 3 shows lesions on both attached and detached leaves during incubation.

DISCUSSION.—These results suggest that the leaf susceptibility to TMV infection and TMV multiplication within lesions were influenced by the physiological conditions of plant tissues. In a previous study (2), since TMV multiplication in tobacco leaves was promoted by leaf senescence associated with changes in nucleic acid and protein metabolism in leaves detached before inoculation, it was considered that moderate leaf senescence induced by leaf detachment before inoculation enhanced the leaf susceptibility to TMV infection. In the present study, on the other hand, the increased leaf susceptibility to TMV infection was also observed by leaf detachment after inoculation, as shown in Fig. 2. Siegel & Wildman (6) and Rappaport & Wu (4) demonstrated that TMV particles introduced into host cells establish the infective centers during 2.5-6 hr after inoculation. In Table 1, although D/A after 24 hr was 1.5, D′/A′ after 48 hr became near 1. Accordingly, it is unlikely that there are differences in the infection process on the detached and attached leaves. If establishment of the infective centers in the detached leaves occurred in larger number than those in the attached leaves, D′/A′ after 48 hr should be far larger than 1. Accordingly, it is more probable that lesion formation and virus multiplication on detached leaves occurred more effectively than on attached leaves. Thus, there are two possible explanations of the susceptibility of detached leaf to TMV infection: (i) Some of the infective centers in the attached leaves are latent and fail to form visible necrotic lesions; these latent infective centers are converted into the visible lesions by leaf senescence induced by leaf detachment; and (ii) some of the infective centers on the attached leaves develop into microlesions invisible to the unaided eye (1), and these microlesions develop into visible macrolesions by leaf senescence induced by leaf detachment. It is well known that lesion size is influenced by numerous factors; e.g., temp, light, and leaf age. The increase in lesion number and size by leaf detachment reported here seems to be in agreement with results of heat treatment (7, 8), wherein heat treatment after inoculation increases the number and size of lesion de-

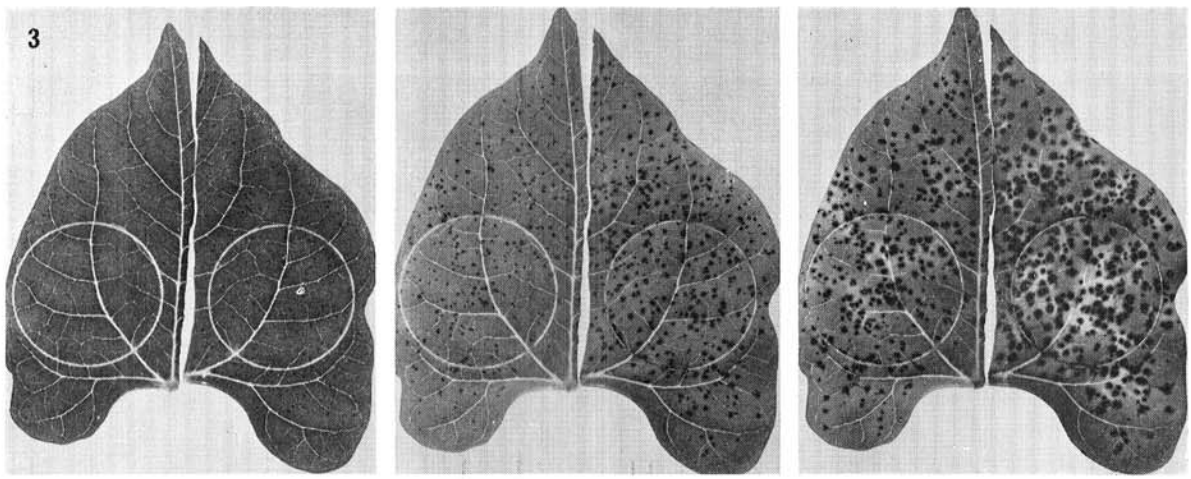

Fig. 3. Necrotic local lesions on attached bean leaves (left) and on detached leaves (right). Circular areas (20 mm in diam) were used for lesion count. Photographed from left to right at intervals of 1, 2, and 3 days after inoculation.

veloped on TMV inoculated bean leaves. If heat activation in virus infection was associated with heat injury to the host (8), it is likely that the increased leaf susceptibility to TMV infection corresponds to the effect of senescence induced by heat injury. Nakagaki et al. (3) and Ross & Williamson (5) reported that ethylene was produced by leaf detachment and formation of necrotic local lesions, and that leaf senescence was promoted by ethylene. Therefore, we consider that the effective increase in susceptibility to TMV infection and in TMV multiplication is closely associated with moderate leaf senescence which was induced by ethylene produced endogenously during incubation of detached leaves.

TABLE 1. Activation of tobacco mosaic virus infection by bean leaf detachment at 6 hr or 24 hr after inoculation[a]

No.	Ratio of lesion no. on the detached leaves to the attached leaves[b]		Rate of lesion appearance	
	24 hr	48 hr		
	D/A	D′/A′	A′/A	D′/D
1	1.38*	1.14	2.12	1.75
2	1.74**	1.28**	2.58	2.11
3	1.78**	1.25	2.12	1.50
4	1.70**	1.22*	2.19	1.57
5	1.31*	1.22**	1.43	1.34
6	1.35*	1.16	1.43	1.23
7	1.29*	0.99	1.50	1.16
8	1.56*	1.14	1.73	1.26
Avg	1.51*	1.18	1.89	1.49

[a] At 6 hr after inoculation, one half leaf was detached and floated on distilled water in a petri dish; 24 hr (D) and 48 hr (D′) after inoculation, lesions on the detached half-leaf were counted; 24 hr after inoculation, lesions on opposite half leaf attached to the plant were counted (A) and then the half leaf was detached and incubated as described above; 48 hr after inoculation, lesions on the detached half leaf were counted (A′).

[b] Ten half leaves were used/assay. Lesions on a leaf disc 20 mm in diam (Fig. 3) were counted.

Significant levels are indicated by * (0.05) and ** (0.01).

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