Nature of the Inhibitory Effect of Bean Pod Mottle Virus on Local Lesion Production by Soybean Mosaic Virus on Bean

Yih-Shyong Lee and J. P. Ross

Graduate Assistant, Department of Plant Pathology, North Carolina State University; and Plant Pathologist, Plant Science Research Division, ARS, USDA, P.O. Box 5397, Raleigh, North Carolina 27607.

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ABSTRACT

Bean pod mottle virus (BPMV) causes partial inhibition of local lesion production by soybean mosaic virus (SMV) when bean leaves are inoculated with mixtures of the two viruses. Neither zones of SMV lesion inhibition nor a reduction in SMV lesion size was induced by BPMV. The effect of inactivated and infective BPMV and the sequence of virus inoculation on SMV lesion inhibition were studied. Infective BPMV caused more inhibition than BPMV inactivated by heat or ultraviolet irradiation. Sequential inoculations with the two viruses separated by various time intervals indicated that maximum inhibition of SMV lesions occurred when inoculations were simultaneous; SMV lesion inhibition was sharply reduced during the 1st-min interval between inoculations. Inhibition of SMV lesions by BPMV infection may be due to the unilateral competition for infectible sites.

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Simultaneous infection of a local lesion host by two unrelated viruses may increase, decrease, or have no effect on the number of lesions produced (5). *Phaseolus vulgaris* L. 'Kentucky Wonder' wax pole bean (KWP) is a local lesion host for soybean mosaic virus (SMV) and bean pod mottle virus (BPMV) (7, 8). Previous research (J. P. Ross, *unpublished data*) has shown that when KWP primary leaves are inoculated with dilutions of plant sap containing various concentrations of SMV and BPMV, the per cent inhibition ($\hat{Y}$) of SMV lesions caused by concentrations of BPMV causing various numbers (15-274) of local lesions (X) can be expressed by the regression equation $\hat{Y} = 19 + 0.2075$ (X). The size of SMV lesions was not affected, nor were there zones surrounding BPMV lesions where SMV lesions failed to develop. The purpose of this study was to examine the inhibition of SMV local lesion formation by BPMV in an attempt to understand the nature of the inhibition of local infection and of host response.

Partially purified preparations of SMV-1 (9) and BPMV were used as inoculum throughout (1, 2, 3, 8). Viruses were purified from leaves of soybean (*Glycine max* [L.] Merr. 'Hill' [SMV] and 'Ogden' [BPMV]) 2 months after inoculation. We inoculated Carborundum-dusted leaves by rubbing with cotton swabs soaked in virus suspensions. Virus assays and local lesion interaction studies were carried out on recently expanded primary leaves of KWP, one plant/4-inch pot. Each inoculated leaf was rinsed with water, excised, floated on 2% sucrose solution in a petri plate, and incubated in darkness at 34 and 20 C for SMV and BPMV assays, respectively.

Symptoms induced by SMV on excised KWP leaves appeared in 2 or 3 days as superficial, black, necrotic local lesions followed by necrosis of small veins (9). Symptoms induced by BPMV appeared in 4 days as subepidermal local lesions best observed with light from beneath the leaf. Both viruses cause local lesions when inoculated on the upper or lower leaf surface.

In a series of inhibition experiments, BPMV was inactivated either by exposure to ultraviolet light for 8 min ($BPMV_U$) or by exposure to 75 C for 10 min ($BPMV_T$). The number of SMV lesions caused by inoculation with mixtures of SMV + $BPMV_U$ or SMV + $BPMV_T$ were compared by the half-leaf method with the number of lesions produced by inoculation with mixtures of SMV + BPMV at the same virus concentrations. In several such experiments, BPMV inactivated by either method reduced the number of SMV lesions ca. 35%, whereas active BPMV reduced SMV lesions about 74% (Fig. 1).

The effective distance over which BPMV inhibited infection was examined by inoculating one-half of the lower leaf surface of KWP leaves with BPMV; the other half-leaves were rubbed on the lower leaf surfaces with buffer solution. Entire upper leaf surfaces were immediately inoculated with SMV. BPMV inoculum caused an average of 80 lesions/half leaf. An average of 148 and 144 SMV lesions developed on leaf surfaces opposite buffer and BPMV, respectively; these numbers were not statistically different.

The effect of inoculation sequence with the two viruses on SMV local lesion development was studied in two time series experiments. In one, entire leaf surfaces were inoculated with SMV, immediately rinsed with water, and dried with absorbent tissue paper. At various times after SMV inoculation, half of each leaf was reinoculated with BPMV. In the other series, half of each leaf was first inoculated with BPMV and the opposite half was rubbed with buffer; SMV was then inoculated on the entire leaf surface at various time intervals after BPMV inoculation. In both series, when the second virus was inoculated as soon as possible (15 sec), leaves were not rinsed or dusted with Carborundum between virus

inoculations. Each experiment also included inoculations with a mixture of both viruses. Inoculum concentrations of each virus were held constant.

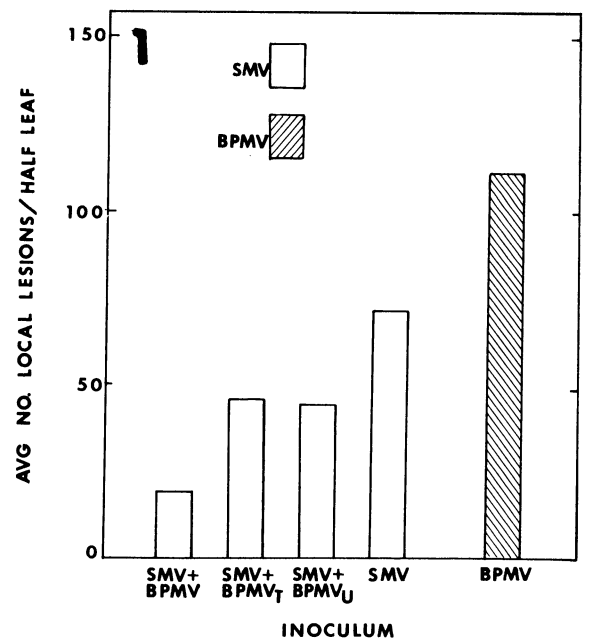
SMV lesions were inhibited most when inoculations were made simultaneously with mixtures of BPMV + SMV, and inhibition of SMV lesions was inversely related to the time intervals between inoculations (Fig. 2). The decrease of SMV lesion inhibition was greatest during the 1st-min interval between inoculations, and this rate was greater when BPMV was inoculated before SMV than when the inoculations were reversed.

Results obtained from these experiments suggest that inhibition of SMV local lesions by BPMV infections in simultaneously inoculated bean is in large part due to a unilateral competition for infectible sites. The failure of SMV to inhibit BPMV lesions suggests that establishment of SMV infection is more sensitive than that of BPMV. Since infective SMV cannot be recovered from its local lesions whereas infective BPMV is easily recovered from its lesions, this indicates that bean is a better host for BPMV than for SMV. Hence, multiplication of BPMV at infection sites in leaves simultaneously inoculated with SMV + BPMV may exert inhibitory effects on initial SMV infection processes in a host such as KWP which is not normally conducive to SMV multiplication.

Since SMV lesions were not inhibited when BPMV was inoculated on the opposite leaf surface, the diameters of SMV lesions were not reduced on simultaneously inoculated leaves, and there were no inhibition zones to SMV infections around BPMV lesions, it appears that BPMV does not exert its inhibitory action through leaf cells or tissues.

Infective BPMV is necessary for maximum SMV lesion inhibition, since greater numbers of SMV lesions developed from inoculations with mixtures of SMV and inactivated BPMV than did from inoculations with mixtures of SMV + BPMV. The slight inhibition of SMV lesions exerted by $BPMV_U$ may be similar to inhibition of infection due to protein interference (4), and indicates that BPMV protein and its infective nucleic acid each play a role in inhibiting SMV infection and/or lesion formation. The inhibition caused by BPMV protein in $BPMV_U$ and $BPMV_T$ was about half that of infective BPMV. These results are similar to those of Moed & Veldstra (6) who studied the inhibition of alfalfa mosaic virus lesions by turnip yellow mosaic virus on bean. They concluded that both the protein and intact virus are important in the inhibitory phenomenon.

Results of the inoculation interval series indicate that competition for infectible sites is time-dependent, and that sites change rapidly (< 5 min); this was especially noticeable in the rapid decrease in the inhibition of SMV lesion formation when BPMV inoculations preceded those of SMV.



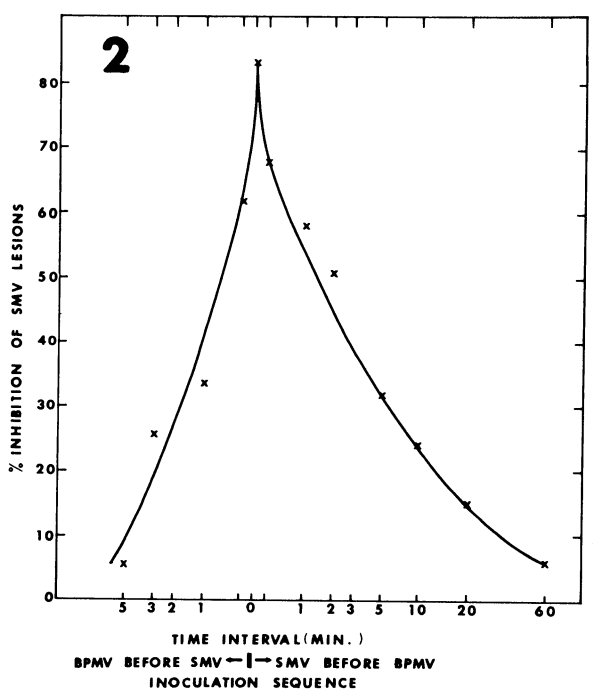


Fig. 1-2. 1) Effect of ultraviolet- ($BPMV_U$) and heat- ($BPMV_T$) inactivated bean pod mottle virus (BPMV) on inhibition of local lesions of soybean mosaic virus (SMV) when primary leaves of Kentucky Wonder wax pole beans were inoculated with mixtures of the viruses. Comparison was made between inoculum of SMV + BPMV and each of the other inocula containing SMV. In each of three comparison experiments, 16 half-leaves were used. 2) Effect of time interval between inoculation with soybean mosaic virus (SMV) and bean pod mottle virus (BPMV) on inhibition of SMV local lesion formation on Kentucky Wonder wax pole bean primary leaves. BPMV inoculum alone caused 196 lesions and SMV inoculum alone caused 44 lesions/half leaf. Abscissa axis is logarithmic.

These results support Siegel's opinion that infectible sites have finite lifetimes (10), and suggest that the infectible sites for SMV and BPMV on KWP are either closely associated or perhaps identical.

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