

## Mechanisms of Resistance in Corn to Maize Dwarf Mosaic Virus

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### ABSTRACT

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The mechanisms of resistance in corn to maize dwarf mosaic virus strain B (MDMV-B) were studied by using two resistant (Pa405, Bsq) and one susceptible (Ma5125) inbreds and their F<sub>1</sub> hybrids. MDMV-B replicated to high titers and spread locally in the inoculated leaves of all genotypes, but spread systemically only in the susceptible genotype. The infectivity of virus extracted from resistant plants was comparable to that from susceptible plants, which suggests that resistance is not due to virus inhibition on inactivation. When uninoculated leaf tissues of previously inoculated resistant plants were challenge-inoculated, high concentrations of MDMV-B were produced in the challenged tissues, suggesting that no induced virus inhibitors were present in these areas. The infection process of MDMV-B was analyzed by immunofluorescent staining. The numbers of infection

loci developing in mechanically inoculated leaves were higher in Bsq than in Pa405 and Ma5125, suggesting that resistance is not due to fewer infection sites. The rate of expansion of the localized infected area in Pa405 and Bsq was either higher than or equal to that in Ma5125. Many secondary infection loci developed in the susceptible inbred, whereas the asymptotically infected areas in inoculated leaves of Pa405 and Bsq were generally continuous. In inoculated leaves of susceptible plants the virus spread faster towards the proximal than the distal end of the leaf, whereas in inoculated leaves of resistant plants the virus spread slowly and in similar rates towards both ends. The pattern of virus spread in the inoculated leaves of resistant plants suggests that the plant inhibits the virus from traveling through the leaf vascular system.

Maize dwarf mosaic virus (MDMV) is one of the most important viruses affecting corn in the continental United States and, since the mid-1960's, has caused widespread economic losses (3). The genetics of resistance of corn to MDMV are not understood clearly, but most studies indicate that resistance to MDMV is probably conditioned by relatively few major genes and a population of minor genes (4,6,9,11,13). By this research we hoped to elucidate some of the mechanisms of disease resistance of corn to MDMV and thereby facilitate more accurate studies of the genetics of its disease resistance.

Tu and Ford (18) reported that MDMV-A replicated to similar amounts in inoculated leaves of resistant (Illinois A) and susceptible (Seneca Chief) varieties but mosaic symptoms rarely developed in the resistant variety. They suggested that resistance of maize to MDMV was due to inhibition of virus movement in the vascular system (18). Free amino acids in the new leaves of inoculated plants increased only in Seneca Chief in which systemic movement of the virus occurred. However, the exact cause and consequences of such changes are not known. Tu and Ford (17) reported that no inhibitor of virus replication was found in the resistant variety but their assay was, in fact, detecting inhibition of infection, because the inhibitor was added to the inoculum during inoculation. Jones and Tolin (5) reported that a high concentration of virus was extracted from chlorotic bands occasionally developed in inoculated leaves of the resistant hybrid T8 × Oh7B, whereas the dark green part of leaves remained free of virus. They suggested that the mechanism of resistance in T8 × Oh7B is not against virus infection or multiplication, but against movement of the virus in the host. Clearly, more information is needed to determine more conclusively the mechanism(s) of corn resistance to MDMV.

Initial experiments were carried out to test our plant materials and virus isolate in regard to the reported (or assumed) immunity of resistant plants (18). It was soon determined, however, that MDMV-B replicated to high titers at the points of inoculation but did not move to the new leaves of inoculated plants (7).

Experiments were then designed to investigate the number of infection loci and the profile of virus spread after mechanical inoculation. The possibility of acquired immunity in the new leaves was also studied.

### MATERIALS AND METHODS

**Virus and plants.** The virus and plant materials were essentially the same as reported previously (8). In brief, the B strain of MDMV (MDMV-B) was maintained in young plants of Golden Cross Bantam sweet corn and used throughout these experiments. Two resistant inbreds, Pa405 and Bsq (an inbred obtained from a cross between B68 and Silver Queen), one susceptible inbred, Ma5125, and two F<sub>1</sub> hybrids between the resistant and the susceptible inbreds were used in these experiments. The genetics of resistance of Pa405 and B68 to MDMV have been studied by several investigators, most recently by Mikel et al (10). All plants were kept in a greenhouse, maintained near 25 C, until use. For several experiments the plants were transferred to a growth chamber and were kept at 27 C and 16-hr day period, at about 9,000 lx. Virus inoculum was prepared by grinding young symptomatic leaves in inoculation buffer (0.05 M sodium phosphate buffer, 10 mM sodium diethyldithiocarbamate, pH 7.4). Carborundum was mixed with the plant sap and leaves were inoculated by rubbing with cotton swabs dipped in virus containing plant sap.

**ELISA.** The double-sandwich method of enzyme-linked immunosorbent assay (ELISA) was performed according to the method of Clark and Adams (1) as modified by Converse (2), with additional minor modifications. The same batch of MDMV-B antiserum described in the previous paper (8) was used. Specific antibody to MDMV-B was coated to polystyrene plates at 1 µg/ml for 2 hr at room temperature. Leaf samples were ground in the virus buffer (phosphate-buffered saline, pH 7.4, plus 0.05% Tween 20, 2% polyvinyl pyrrolidone [MW 10,000], and 0.2% egg albumin). Samples were incubated in the plates for 3 hr at 37 C. The alkaline phosphatase-conjugated antibody (1:1,000) was incubated for 3 hr at 37 C. Substrate was allowed to react at room temperature and the reaction was stopped after 30 min. At the beginning of the experiments, known concentrations of purified MDMV-B (19) were added to healthy plant extract (1:10, w/v in virus buffer) to obtain a correlation of A<sub>405nm</sub> readings and virus concentration. The minimum detection level of our procedure was

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60 ng of MDMV-B per milliliter of plant sap. A quantitative relationship between  $A_{405nm}$  readings and concentration of MDMV has previously been demonstrated by Reeves et al (12), and was also observed in our experiments.

**Distribution and concentration of MDMV-B.** One 4-cm leaf section per plant near the middle of the first fully developed leaf was marked with small dots of indelible ink and was then inoculated. At 3-day intervals, four 4-cm leaf sections were collected, one containing the area of inoculation, two the stem-side adjacent leaf sections, and one from the youngest leaf of each plant. Each leaf section was ground in 1 ml of virus buffer and each undiluted sample was assayed for MDMV by ELISA. After samples were taken, the inoculated plants were further maintained for up to 30 days after inoculation and the new leaves were similarly tested for MDMV by ELISA.

**Field evaluation of MDM resistance.** Plants of the three inbreds and two  $F_1$  hybrids were inoculated at the five-leaf stage. The first experiment was carried out during June–September 1982. Each plot consisted of 10 plants of one cultivar planted in a row. The treatments were: one inoculation of MDMV-B at the five-leaf stage, four inoculations of MDMV-B at weekly intervals, and uninoculated control. The inoculation was carried out by rubbing the first three young leaves with sap from virus-infected leaves. There were two plots for each treatment and the total of 30 plots was completely randomized.

The second experiment was carried out during June–September 1983. A randomized block design was adopted. The treatments were: two inoculations at a 7-day interval starting at the five-leaf stage and uninoculated control. A total of 10 plots were completely randomized in a block. Five blocks were arranged parallel to each other. Plants were evaluated weekly for disease incidence and severity. The disease severity index of 0–4, as defined in Table 2, was a modification of the disease severity index of Scott and Rosenkranz (14).

**Infectivity of MDMV-B from leaves of resistant plants.** Four-centimeter sections of the two youngest fully opened leaves of Pa405 and Bsq plants at the six- to seven-leaf stage were inoculated near the middle of the leaf. The inoculated leaf sections were collected 7 or 12 days after inoculation. Twenty leaf sections were pooled from 10 plants. For ELISA, the inoculated leaf sections were ground and diluted in a 10-fold series in virus buffer. For bioassay, the inoculated leaf sections were ground and similarly diluted in inoculation buffer and each dilution was inoculated onto 25 seedlings of Gold Cup sweet corn. The inoculated seedlings were observed for symptom development for up to 30 days. The highest dilutions, which resulted in a positive reaction in ELISA ( $A_{405nm} \geq 0.1$ , which was greater than twice the  $A_{405nm}$  value of healthy sap), or symptomatic plants in the bioassay were recorded as dilution end points in the corresponding tests.

**Resistance to infection.** Resistance to infection was tested by comparing the number of infection loci following mechanical inoculation of susceptible and resistant corn plants. A 4-cm-long section of the first fully opened leaf of each plant was inoculated. The infection loci were detected by the immunofluorescent method previously described (8). The numbers of infection loci were adjusted for leaf width to give number of loci per square centimeter. The first two experiments were carried out during January 1984 and after inoculation the plants were incubated in a growth chamber. The third experiment was carried out during April 1984 and the plants were kept in the greenhouse throughout the experiment.

**Resistance to cell-to-cell spread.** Resistance to cell-to-cell spread was studied by comparing the rate of cell-to-cell spread of MDMV-B in leaves of susceptible and resistant corn plants. The same samples that were used to determine resistance to infection were also used to determine resistance to cell-to-cell spread. The length of each infection locus, i.e., its dimension parallel to the veins of the leaf, was measured with an ocular micrometer. All or up to 10 random loci per leaf were measured.

**Resistance to long-distance spread.** Resistance to long-distance spread of MDMV-B was studied by monitoring with the immunofluorescent staining method the spread of the virus from

an initial inoculated point. MDMV-B was inoculated by applying infected sap with a 2-mm-wide plastic applicator onto the upper side of a 2-mm-wide leaf strip perpendicular to the leaf axis and across the full width of the leaf in the middle of the leaf. One strip per plant was inoculated. Four-centimeter-long leaf sections, containing the inoculated strip in the middle of the section, were collected at 2- or 3-day intervals and the spread of the virus was monitored by the immunofluorescent staining method. The distance of the spread of the virus from the inoculated strip to either end of each leaf section, as well as the pattern of spread of the virus, were recorded.

**Tests for acquired resistance.** Four-centimeter leaf sections were inoculated with MDMV-B to elicit the production of potential antiviral substances. Four-centimeter sections of the emerging new leaves were challenge-inoculated 7 days after the first inoculation. The inoculated leaves from the second inoculation were collected 7 days after challenge-inoculation and the virus concentrations in the challenge-inoculated sections were determined by ELISA. Plants inoculated with healthy sap and then challenge-inoculated with virus were used as controls. In some experiments, challenge inoculation was made on new leaves and on leaf sections adjacent to the area of first inoculation. In these experiments, one set of inoculated plants was left unchallenged to check the extent of spread of the virus from the first inoculation. In all experiments, 4-cm leaf sections were ground in 1 ml of virus buffer and undiluted samples were used in ELISA to determine the concentration of viral antigen in the leaves.

## RESULTS

**Distribution and concentration of the virus.** Both resistant and susceptible corn genotypes produced high concentrations of MDMV-B antigen, corresponding to high concentrations of virus, in the inoculated leaf areas. Bsq and Ma5125  $\times$  Bsq produced significantly higher concentrations of virus in inoculated sections than the other genotypes (Table 1).

TABLE 1. Spread of maize dwarf mosaic virus strain B in mechanically inoculated leaves of susceptible and resistant corn genotypes as measured by ELISA<sup>1</sup>

Corn genotypes <sup>2</sup>	Day <sup>3</sup>	Absorbance (405 nm) of leaf sections			
		Inoc. <sup>4</sup>	0–4 cm <sup>5</sup>	4–8 cm <sup>5</sup>	NL <sup>6</sup>
Pa <sup>x</sup>	3	0.17 <sup>y</sup> ab <sup>z</sup>	0.04 a	0.00 a	0.00 a
Bsq	3	0.69 c	0.18 b	0.03 b	0.02 a
Pa $\times$ Ma	3	0.18 ab	0.09 ab	0.03 b	0.06 a
Ma $\times$ Bsq	3	0.24 b	0.06 a	0.04 b	0.02 a
Ma	3	0.10 a	0.00 a	0.00 ab	0.35 b
Pa	6	0.44 a	0.03 a	0.02 a	0.02 a
Bsq	6	1.44 bc	0.11 a	0.07 a	0.06 a
Pa $\times$ Ma	6	0.73 a	0.08 a	0.06 a	0.06 a
Ma $\times$ Bsq	6	1.57 b	0.24 a	0.06 a	0.06 a
Ma	6	0.95 ac	0.74 b	0.61 b	1.55 b
Pa	9	1.38 ab	0.09 a	0.05 a	0.04 a
Bsq	9	2.60 c	0.67 b	0.10 a	0.09 a
Pa $\times$ Ma	9	1.16 a	0.23 a	0.08 a	0.07 a
Ma $\times$ Bsq	9	2.57 c	2.09 bc	0.83 b	0.08 a
Ma	9	1.43 ab	1.51 c	1.39 b	1.62 b

<sup>1</sup> One of three similar experiments performed during June–September 1981 with 10 plants per treatment, and one 4-cm leaf section inoculated and four 4-cm leaf sections harvested per plant.

<sup>2</sup> Resistant inbreds: Pa405 and Bsq. Susceptible inbred: Ma5125.

<sup>3</sup> Days after inoculation.

<sup>4</sup> Inoculated leaf section.

<sup>5</sup> Centimeter from the inoculated leaf section.

<sup>6</sup> The youngest new leaf.

<sup>x</sup> Pa = cultivar Pa405, Bsq = an inbred developed from B68  $\times$  Silver Queen, and Ma = cultivar Ma5125.

<sup>y</sup> ELISA values at  $A_{405nm}$  of individual 4-cm-long leaf sections ground in 1 ml of virus buffer. Average ELISA values of healthy sap were 0.05.

<sup>z</sup> Numbers followed by the same letter are not significantly different at each date and on each type of leaf section according to Duncan's multiple range test at  $P = 0.05$ .

MDMV-B was detected in the uninoculated new leaves of Ma5125 plants 3 days after inoculation of a 4-cm section on the first fully developed leaf, whereas virus was still not detected in the leaf section adjacent to the inoculated one. At 6 days after inoculation, the virus was detected in all sample areas of Ma5125, although it was found only occasionally (in 2 of 10 leaves) outside the inoculated areas of the Ma × Bsq hybrid and not at all in the other inbreds or hybrids. At 9 days after inoculation, among the resistant inbreds the virus had spread the farthest in Ma5125 × Bsq, to 4–8 cm away from the inoculated section, whereas in Bsq and Pa405 × Ma5125 the virus had spread to 0–4 cm away from the inoculated section and in Pa405 the virus was still restricted to the inoculated section (Table 1).

MDMV-B was consistently detected by ELISA in new leaves of Ma5125 3–6 days after inoculation, the  $A_{405nm}$  values ranging from 0.35 to 1.80; however, the virus was never found in the new leaves of any of the resistant inbreds ( $A_{405nm}$  values ranged from 0.001 to 0.05). Even 30 days after inoculation, MDMV-B was found only in the new leaves of Ma5125.

**Field evaluation of MDMV-B resistance.** Pa405, Bsq, and Pa405 × Ma5125 were highly resistant to MDMV-B (Table 2). No plants of these inbreds ever showed any symptoms of MDMV infection. The hybrid Ma5125 × Bsq appeared to be intermediately resistant in the 1982 experiment (Table 2) when summer (mid-

June–July) was unusually cool. The same hybrid, however, appeared highly resistant in the 1983 experiment (Table 2) when the summer was hot. Symptoms on Ma5125 × Bsq plants consisted of narrow bands of chlorotic tissue in otherwise dark green leaves. In both experiments, all inoculated plants of the susceptible inbred Ma5125 developed symptoms (Table 2) and their disease severity rating was the highest possible (Table 2). The uninoculated control plants never developed any symptom.

**Infectivity and ELISA of the virus from inoculated leaves of resistant inbreds.** In the first experiment, in which leaf samples were collected 7 days after inoculation, the dilution end points in both the ELISA and the bioassay tests were 1:1,000 in Pa405, Bsq, and Ma5125 (Table 3). In the second experiment, leaf samples were collected 12 days after inoculation and, in all three inbreds, the dilution end points in ELISA were 1:10,000, whereas the dilution end points of virus infectivity were 1:100 (Table 3).

**Resistance to initial infection.** In all experiments, the numbers of infection loci continued to increase up to 36–56 hrs after inoculation (Fig. 1). Bsq consistently exhibited more infection loci than Pa405 and Ma5125, whereas the numbers of infection loci in Pa405 and Ma5125 were different in some experiments but not in others.

**Resistance to cell-to-cell spread.** Linear regression analysis of the data indicated that in the first two experiments, which were performed during winter, the rates of cell-to-cell spread were higher in Bsq and Pa405 than in Ma5125 (Fig. 2A). The estimated slopes of Bsq, Pa405, and Ma5125 were 0.034, 0.030, and 0.023, respectively ( $R = 0.87$ , based on the pooled data of first and second experiments). The LSD ( $P = 0.05$ ) of the slope was 0.007. In the third experiment, which was performed during spring, the rates of cell-to-cell spread were not significantly different (Fig. 2B). The estimated slopes of Bsq, Pa405, and Ma5125 were 0.015, 0.017, and 0.013, respectively ( $R = 0.81$ ).

**Resistance to long-distance spread.** In Ma5125 discrete secondary infection loci began to appear on the proximal side of the inoculation strips 6 days after inoculation (Fig. 3). No secondary loci were detected on days 2 and 4. The secondary loci were present in the same interveinal areas as, and were apparently derived from, corresponding primary infection loci in the area of inoculation, but such loci were not connected through a series of infected cells until several days after their appearance. The secondary loci became numerous and many of them fused into continuous lines of infected cells by day 9. There were few discrete secondary infection loci on the distal side of the inoculation strips. Secondary loci, which were not in the same interveinal areas as, and therefore could not be traced to, any primary locus, were observed on day 12. Most of the proximal spread of virus reached the edge of the leaf section 6 days after inoculation.

In Pa405 and Bsq, occasional secondary loci began to appear not earlier than 9 days after inoculation (Fig. 3). In these inbreds, the spread of the virus appeared in most cases to be continuous. The front edge of the virus spread was usually pointed.

The average distances of total (proximal plus distal) spread of the virus in inoculated leaves are given in Figure 4. Standard deviations are indicated by error bars. According to Duncan's multiple range test of the data ( $P = 0.05$ ), the rate of long-distance spread of the virus was generally higher in Ma5125 than in Pa405 and Bsq, except that at days 12 and 14 the average distances were not significantly different due to large standard deviations (Fig. 4). The rate of virus spread in Pa405 was lower than in Bsq in two experiments performed during November and December (Fig. 4A), but virus spread in the two varieties was not different in one experiment performed during March and April (Fig. 4B).

The distances of proximal (toward leaf base) spread of the virus were longer than those of distal spread in Ma5125 (analyzed by the procedure of least square means, total  $P = 0.05$ ). In contrast, the distances of virus spread in the proximal or distal directions were not different in Pa405 and Bsq (Fig. 5).

**Tests for acquired immunity.** Seven days after challenge inoculation, Pa405 plants, previously inoculated with sap from virus-infected plants, produced higher virus titer ( $0.75 \pm 0.09$ ) than plants previously inoculated with healthy sap ( $0.24 \pm 0.09$ ). In the

TABLE 2. Incidence and disease severity of maize dwarf mosaic symptoms in inoculated plants of resistant and susceptible corn inbreds in the field

Inbred or F <sub>1</sub>	Time of experiment		
	1982		1983
	1 inoculation <sup>w</sup>	4 inoculations <sup>w</sup>	2 inoculations <sup>w</sup>
Pa <sup>x</sup>	0 <sup>y</sup> /0 <sup>z</sup>	0/0	0/0
Bsq	0/0	0/0	0/0
Pa × Ma	0/0	0/0	0/0
Ma × Bsq	72/1.31	80/1.35	6/0.012
Ma	100/4.0	100/4.0	100/4.0

<sup>w</sup>Number of inoculations at weekly intervals started at five-leaf stage.

<sup>x</sup>Pa = cultivar Pa405, Bsq = an inbred developed from B68 × Silver Queen, and Ma = cultivar Ma5125.

<sup>y</sup>Percentage of symptomatic plants 4 wk after first inoculation. Each treatment contained 20 plants.

<sup>z</sup>Average disease severity index, scale of 0–4, 0 = no symptoms, 1 = mosaic confined to narrow bands on the upper one or two leaves, 2 = mosaic covering entire surface of one or two upper leaves, 3 = mosaic covering the entire surface of three to six of the upper leaves, 4 = mosaic covering entire surface of most of the leaves.

TABLE 3. Comparison of virus infectivity and ELISA values in resistant and susceptible corn genotypes inoculated with maize dwarf mosaic virus strain B

Inbred or F <sub>1</sub>	Days after inoculation	Sap dilution			
		10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-3</sup>	10 <sup>-4</sup>
Pa <sup>x</sup>	7	1.67 <sup>y</sup> (21) <sup>z</sup>	0.62 (3)	0.12 (1)	0.01 (0)
Bsq	7	1.71 (22)	0.71 (8)	0.18 (1)	0.01 (0)
Pa × Ma	7	1.85 (23)	0.72 (12)	0.20 (3)	0.02 (0)
Ma × Bsq	7	1.59 (25)	0.56 (18)	0.12 (1)	0.00 (0)
Ma	7	1.87 (25)	1.00 (14)	0.32 (1)	0.02 (0)
Pa	12	2.01 (21)	1.72 (3)	0.44 (0)	0.10 (0)
Bsq	12	1.99 (24)	1.96 (10)	0.81 (0)	0.24 (0)
Pa × Ma	12	2.00 (20)	1.90 (6)	0.56 (0)	0.24 (0)
Ma × Bsq	12	1.99 (24)	1.97 (1)	1.08 (0)	0.22 (0)
Ma	12	2.00 (20)	1.99 (3)	1.05 (0)	0.29 (0)

<sup>x</sup>Pa = cultivar Pa405, Bsq = an inbred developed from B68 × Silver Queen, and Ma = cultivar Ma5125.

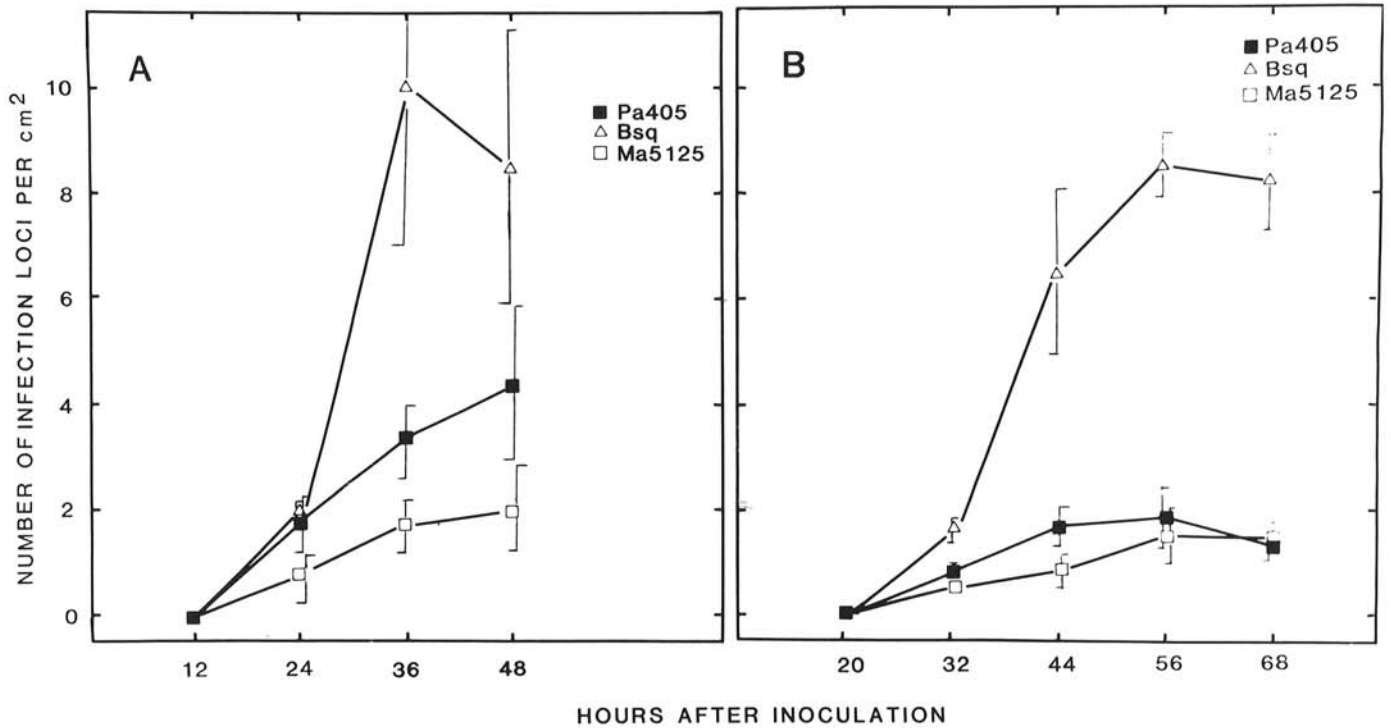
<sup>y</sup> $A_{405nm}$  of 20 leaf sections pooled from 10 plants. Healthy sap gave average  $A_{405nm}$  values of 0.02.

<sup>z</sup>Number of symptomatic plants of a total of 25 inoculated seedlings.

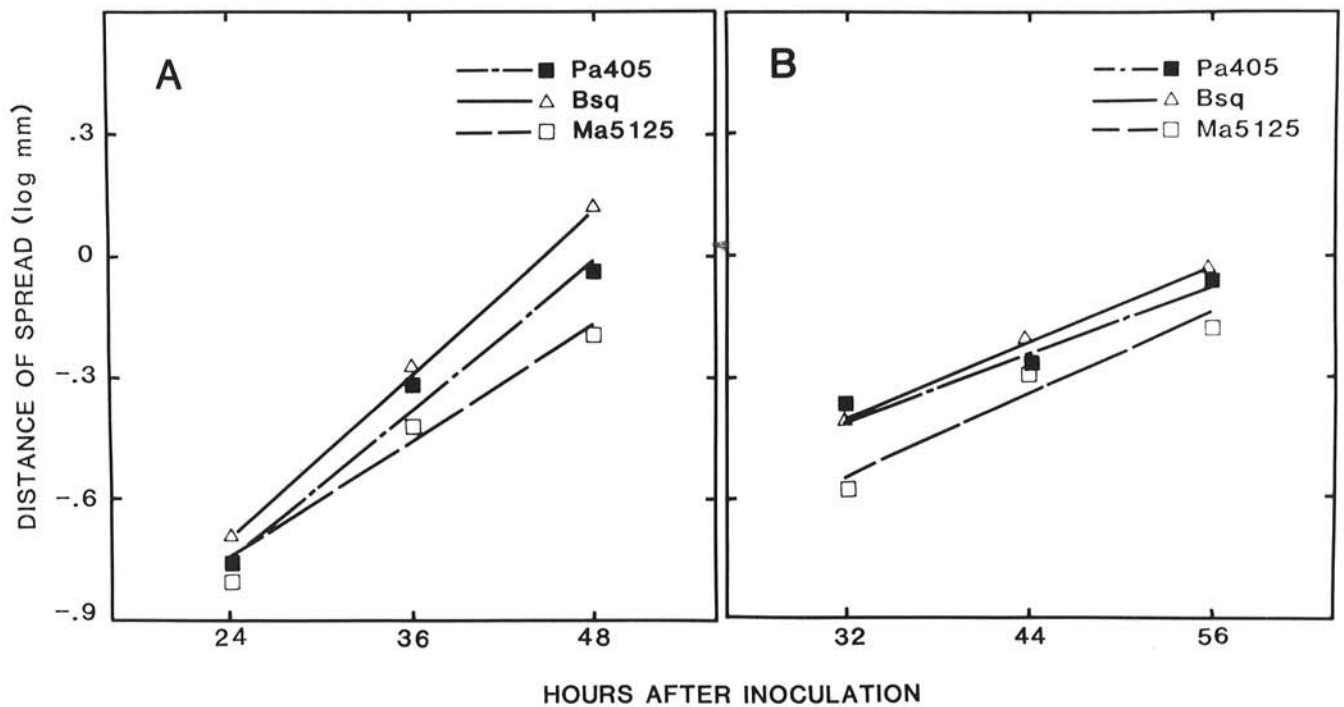


experiment in which plants not inoculated previously were used as control, on challenge-inoculation of the new leaves, the new leaves of previously inoculated Pa405 and Bsqr plants produced virus titer that was similar to or higher than, respectively, that of the previously not-inoculated plants. In two experiments, in which

some plants inoculated with MDMV-B were not challenge inoculated in order to determine the extent of spread of the virus from the first inoculation in relation to virus spread following challenge inoculation, leaf sections immediately outside the presumed front of virus spread (as indicated by the virus spread in



**Fig. 1.** Numbers of maize dwarf mosaic virus strain B (MDMV-B) infection loci in leaves of a susceptible (Ma5125) and two resistant (Pa405 and Bsqr) corn inbreds mechanically inoculated with MDMV-B. **A**, Average of two experiments performed during December and January. **B**, One experiment performed during March and April. In all experiments, the number of infection loci in Bsqr was higher than that in Pa405 and Ma5125, whereas it was not significantly different between Pa405 and Ma5125. Error bars indicate standard deviation.



**Fig. 2.** Rate of cell-to-cell spread of maize dwarf mosaic virus strain B in mechanically inoculated leaves of a susceptible (Ma5125) and two resistant (Pa405 and Bsqr) corn inbreds. **A**, Average of two experiments performed during December and January; the rates of cell-to-cell spread of the virus were higher in Bsqr and Pa405 than in Ma5125. **B**, One experiment performed during March and April; the rates of cell-to-cell spread in the three inbreds were not significantly different.

unchallenged plants) also produced high virus titer after challenge inoculation (Table 4). None of the resistant inbreds showed any symptoms up to 30 days after the last inoculation.

### DISCUSSION

Neither of the resistant inbreds used in this work was found to be immune. MDMV-B replicated to high titer in the inoculated leaves

of all genotypes (Table 1), but the virus movement remained localized in the resistant inbreds and only plants of the susceptible inbred became systemically infected. This result confirms the results reported by Tu and Ford (18). The disease severity rating of the five cultivars in the field (Table 2) correlated well with the rates of MDMV-B spread in the inoculated leaves (Table 1, 9 days). Ma5125 × Bsq appeared intermediately resistant (Table 2), whereas Pa405 × Ma5125 remained highly resistant. This

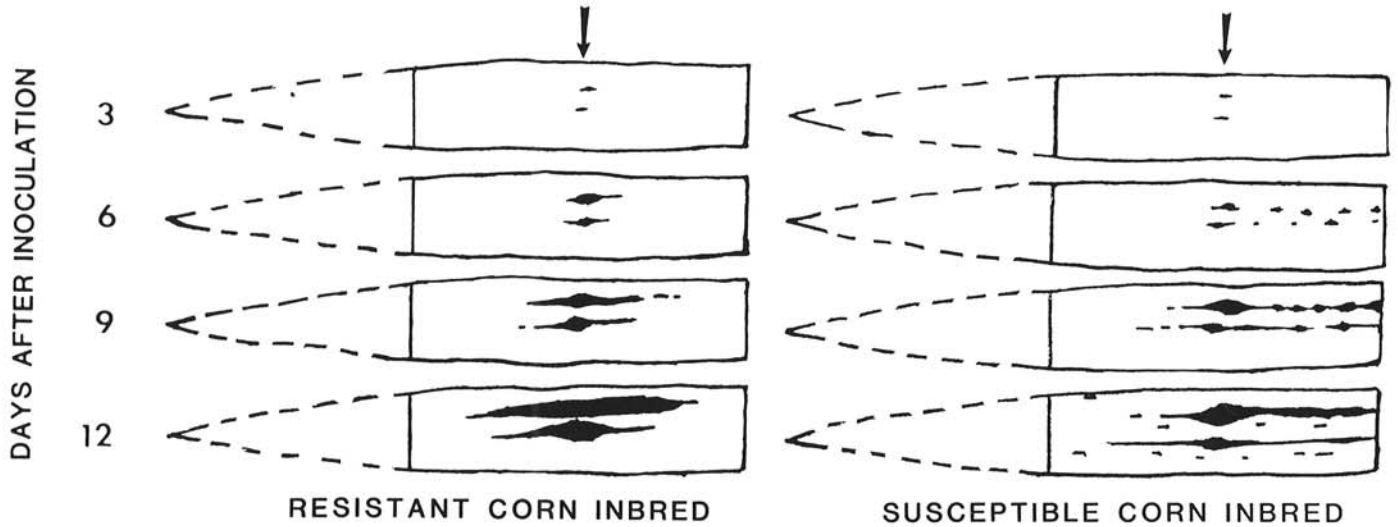


Fig. 3. Schematic representation of spread of maize dwarf mosaic virus strain B in inoculated leaves of a susceptible (Ma5125) and two resistant (Pa405 and Bsq) corn inbreds. Discrete secondary infection loci occurred on the proximal side of primary infection loci (arrow) in the susceptible inbred but not in the resistant ones 6 days after inoculation. Spread of virus in the resistant inbreds was mostly continuous. Discrete secondary infection loci could occasionally be found in resistant inbreds near the front of the continuous primary spread 9 days after inoculation. Secondary infection loci occurred randomly in susceptible leaves 12 days after inoculation. (Arrow: 2-mm inoculation strip.)

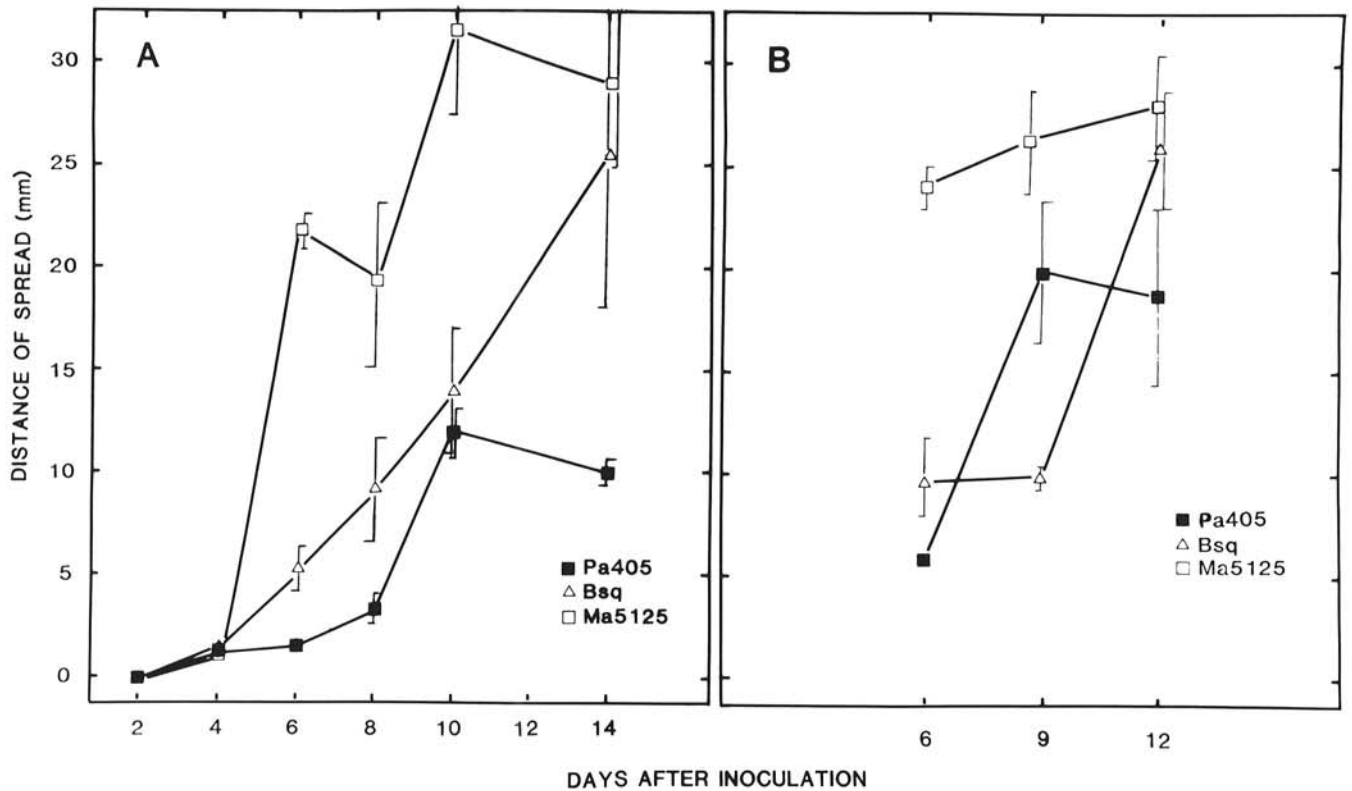


Fig. 4. Long-distance spread of maize dwarf mosaic virus strain B (MDMV-B) in mechanically inoculated leaves of a susceptible (Ma5125) and two resistant (Pa405 and Bsq) corn inbreds. **A**, Average of two experiments performed during November and December. **B**, One experiment performed during March and April. The rate of MDMV-B spread in Ma5125 was significantly higher than that in Pa405 and Bsq. The rate of virus spread in Bsq was higher than that in Pa405 during November and December (**A**), whereas the two were not significantly different during March and April (**B**). Error bars indicate standard deviation.

phenomenon suggests that the resistance genes of Pa405 and Bsq are not identical. It is interesting to note that the resistance of Ma5125 × Bsq was greatly reduced by predisposition through cooler summer weather (Table 2).

Possible hypotheses that may explain virus localization in the inoculated leaves in the absence of structural barriers include: The virus was rapidly inactivated in the resistant tissue; new leaves of inoculated plants became resistant to the virus after initial replication of the virus in older leaves; and the virus failed to spread into or via the vascular system and, therefore, did not travel for a long distance.

The virus from resistant plants was just as infectious as that from susceptible ones (same dilution end point) and the infectivity correlated well with virus concentration. There was a drop of virus infectivity with time but the decrease was equivalent in both susceptible and resistant genotypes. The drop of MDMV-B infectivity in susceptible genotypes was also reported by Tu and Ford (17). Thus, no virus-inactivation mechanism specifically related to resistance was observed.

The new leaves of the inoculated plants supported high virus replication when they were challenged (Table 4). Thus, no acquired resistance was expressed by the new leaves. When areas just outside the edge of virus spread were challenged, however, they also produced high concentration of virus (Table 4). These data suggest that no activity of translocatable antiviral substance was present in inoculated resistant plants. Additional evidence that supported this supposition was the fact that virus spread in resistant varieties was still increasing (Fig. 4) when virus was first detected in the new leaves of susceptible plants (3–6 days after inoculation).

The fluorescent antibody staining technique revealed that the numbers of infection loci in the always-asymptomatic resistant inbreds were either higher or equal to those in the susceptible inbred (Fig. 1). It is clear, therefore, that the mechanism of resistance is not due to resistance to initial infection. The rates of cell-to-cell spread of virus in resistant inbreds were higher than or equal to those in the susceptible inbred (Fig. 2). This strongly suggests that the resistance mechanism could not be due to inhibition of cell-to-cell spread of the virus.

Numerous discrete secondary infection centers, which formed along veins and could be traced back to primary infection loci present along the same interveinal areas in the inoculated part of the leaf, marked the more rapid spread of the virus and the appearance of systemic symptoms in the susceptible inbred (Fig. 3). Such secondary infection centers were rare in resistant inbreds (Fig. 3) and, when they did occur, they appeared only near the front of the continuous spread of the virus. It is quite possible, however, that in these rare instances MDMV-B actually traveled through parenchyma cells in the deeper layers of the leaf, which were not detected by the immunofluorescent staining method. The rates of long-distance spread of virus in inoculated leaves were higher in the susceptible than in the resistant inbreds (Fig. 4). The more rapid spread in the susceptible inbred, however, was due to movement via the vascular system. The spread of virus in the susceptible inbred accelerated 6 days after inoculation at the same time when secondary infection loci began to appear (Fig. 3). In the susceptible inbred, proximal spread of virus was greater than distal spread, whereas this type of directional spread was not observed in the resistant inbreds (Fig. 5). Because metabolite flow in a mature leaf is mostly basipetal, virus spread correlated with metabolite flow in the susceptible inbred but not in the resistant inbreds. These data suggest that, in resistant genotypes, there is little or no spread of virus via the vascular system.

It is not known why MDMV-B does not move into and through the vascular system of resistant genotypes. It could be due to some kind of physical blockage of the vessels. Another possibility, as suggested by Siegel et al (16), would involve some kind of recognition signal on the virus, such as its coat protein, in the vascular transport of viruses. Shalla et al (15) suggested that vascular transport of virus may involve prior virus-induced modification of plasmodesmata. In that case, the inability of a virus to modify the host tissue could contribute to a resistant reaction by the host.

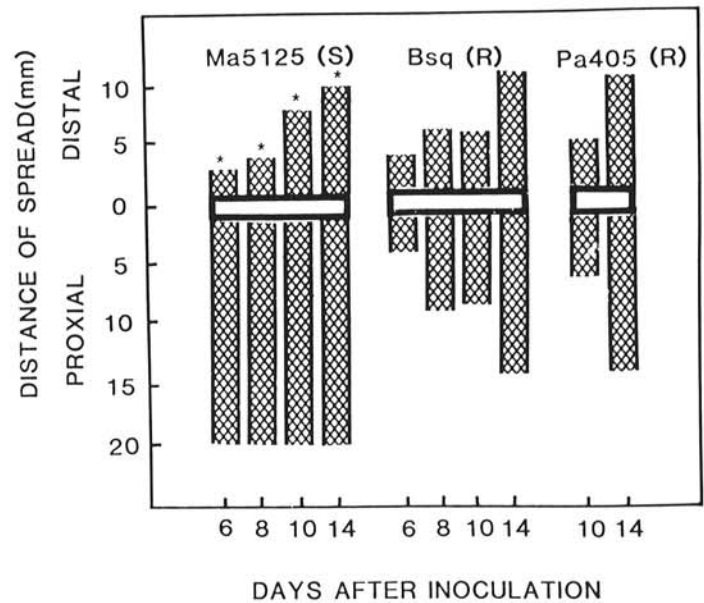


Fig. 5. Proximal and distal spread of maize dwarf mosaic virus strain B in mechanically inoculated leaves of a susceptible (Ma5125) and two resistant (Pa405 and Bsq) corn inbreds. Asterisk denotes that proximal spread was different ( $P = 0.05$ ) from respective distal spread.

TABLE 4. ELISA values of maize dwarf mosaic virus strain B titer in leaves of two resistant inbreds after challenge-inoculation of previously inoculated plants<sup>a</sup>

Inbred	Inoculation		Absorbance (405 nm) of leaf sections					
	1st	2nd	Inoc. <sup>b</sup>	0–4 cm <sup>c</sup>	4–8 cm	8–12 cm	NL1 <sup>c</sup>	NL2
Bsq <sup>d</sup>	Virus	Virus	.50* <sup>f</sup>	.43*	.39*	.36*	.48*	.50*
	Virus	None	.59*	.53*	.27*	.09	.0	.0
	Virus	Virus	.57*	.52*	.42*	.36*	.49*	.49*
Pa405	Virus	None	.62*	.45*	.00	.00	.00	.02

<sup>a</sup> From one of two similar experiments with eight plants per treatment. The challenge inoculation was made both on new leaves and on leaf sections adjacent to the area of first inoculation.

<sup>b</sup> Leaf sections of first inoculation.

<sup>c</sup> 0–4 cm, 4–8 cm, 8–12 cm from the inoculated leaf section. NL1, NL2: new leaves 1 and 2.

<sup>d</sup> Bsq = an inbred developed from B68 × Silver Queen.

<sup>e</sup> ELISA values at  $A_{405nm}$ .

<sup>f</sup> \* = leaf sections that yielded an ELISA value more than twice that of the uninoculated control (average ELISA value 0.03) and greater than 0.1 were considered to contain a significant amount of virus.

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