Effects of Temperature on the Maintenance of Resistance to Soybean Mosaic Virus in Soybean

L. M. Mansky, D. P. Durand, and J. H. Hill

Departments of Plant Pathology and Microbiology, Iowa State University, Ames 50011.
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Address reprint requests to J. H. Hill.
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ABSTRACT

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The effects of temperature on naturally occurring disease resistance to soybean mosaic virus (SMV), strain G2, were studied by using resistant soybean lines PI 96983, L78-379, and Davis. When plants were shifted from 20 C to 10 C for 10 days, coat protein of SMV-G2 accumulated in trifoliolate leaves of resistant plants inoculated with SMV, but did not at the higher temperatures tested. Infectious SMV was recovered from these leaves by a local-lesion assay. Temperature had no apparent effect on the accumulation of coat protein of SMV-G2 in trifoliolate leaves of inoculated plants of the susceptible cultivar Williams '82. Moreover, temperature did not influence accumulation of coat protein in susceptible and resistant lines inoculated with a resistance-breaking strain, SMV-G7.

Additional keywords: potyvirus, systemic spread.

Temperature is a significant factor that can influence the disease process in virus-infected plants (17). However, studies that specifically address the effects of temperature on virus disease resistance, known to be conditioned by one or a few genes, are limited. The most notable examples are illustrated by the *Tm* genes in tomato. The *Tm*-1 gene completely suppresses symptoms induced by tobacco mosaic virus (TMV), strain 0, when plants are grown at constant temperatures from 20 to 35 C; high temperatures severely reduce virus multiplication (10). In contrast, TMV-1 causes more severe symptoms at higher temperatures; multiplication of strain 1 is thought to be inhibited at 25 C, but not at 33 C (10). Tomato plants that have the *Tm*-2 or *Tm*-2^2 gene for resistance to TMV are symptomless at normal temperatures after inoculation with strain 0, but severe systemic necrosis occurs at elevated temperatures (8,20,23). Similar effects of high temperature on resistance to TMV have also been reported with the N and N′ genes in tobacco (9,24) and with transgenic tobacco expressing the coat protein of TMV (19).

Disease resistance to soybean mosaic virus (SMV) in soybean provides an appropriate model to study disease resistance. First, there are a large number of known virus strains that differ in pathogenicity. Second, several lines of soybean have been identified with known single, dominant genes conferring resistance to SMV. Third, SMV is a member of the largest group of plant viral pathogens (the potyvirus group) of economic importance.

Isolates of SMV have been placed into virulence groups (G1–G7, G7a, and C14) based on the phenotypic response of differential soybean lines to virus inoculation (3,6,7,22). In addition, several soybean lines contain a single dominant allele that confers resistance (i.e., host immunity for purposes of this study) to strains of SMV (1,2,14,21,22). The line PI 96983 contains the single dominant allele, *Rsv*, which conditions resistance to members of all strain groups except G7 and G7a (5,22). L78-379, a resistant line derived from a cross of the susceptible soybean cultivar Williams Union with PI 96983 containing *Rsv* (R. Bernard, *personal communication*), is also resistant to all virulence groups except G7 and G7a (5,22). The soybean cultivars Davis, Dorman, Ware, and York are susceptible to strains SMV-G4, -G5, or -G6, as well as -G7 and -G7a (5,22). These lines are thought to possess a single, dominant resistance gene from PI 96983 that is allelic to *Rsv* (22).

The objective of this research was to develop experimental parameters to manipulate disease resistance by testing the effects of temperature on resistance. The data indicated that low temperatures overcame or repressed disease resistance and induced systemic spread of SMV in resistant soybean lines.

MATERIALS AND METHODS

Plants and viruses. The soybean lines and virus strains used in this study are described in Table 1. Plants were maintained in growth chambers operating at constant temperatures of 10, 15, 20, 25, 30, or 35 C with an 18-hr day and an irradiance of 50 W/m^2. The two fully expanded primary leaves of soybean seedlings (5–10 cm tall) grown at 20 C were mechanically inoculated with infectious sap prepared in 50 mM sodium phosphate buffer, pH 7.0. Leaves were rinsed with running water immediately after inoculation.

Temperature shift of inoculated plants. Seedlings grown in pots

TABLE 1. Symptoms induced in soybean lines inoculated with two strains of soybean mosaic virus

Soybean line	Resistance gene	Phenotypic response of soybean lines inoculated with virus isolate[a]	
		G2[b]	G7
Williams '82	...	S	S
PI 96983	Rsv	O	N
L78-379	Rsv	O	N[c]
Davis	Rsv?	O	S

[a] O = no reaction; N = systemic necrosis; S = systemic mottling; data from Roane et al (22).
[b] Isolate Ia 75-16-1 (11,12).
[c] Determined in this study. Primary leaves of soybean seedlings (5–10 cm tall) grown at 20 C were mechanically inoculated with strain G7. Plants were observed for symptom development.

at 20 C (approximately 5–8 plants per pot) were inoculated with either SMV-G2, -G7, or were mock-inoculated (50 mM sodium phosphate buffer, pH 7.0), placed at a constant 10, 15, 20, 25, 30, or 35 C for 10 days, and then returned to 20 C for an additional 10 days. Plant height was measured at inoculation and at 10 and 20 days postinoculation.

Press blotting. On the 10th day after returning to 20 C, secondary or tertiary trifoliolate leaves from plants were sampled for the presence of virus antigen by press blotting as previously described (16) with monoclonal antibody S10 to the coat protein of SMV (13).

Enzyme-linked immunosorbent assay (ELISA). Trifoliolate leaf samples (0.25 g) were ground in 1 ml of 50 mM sodium phosphate buffer, pH 7.0, and the sap was centrifuged at 12,000 × *g* for 1 min. The supernatant was then assayed for SMV coat protein by ELISA with Immulon 1 microtiter plates as described (4). Reactions were regarded as positive when the response was greater than the mean response of the control (containing no virus antigen) plus two standard deviations; samples with response values below this value were considered negative.

Local-lesion assay. Recovery of infectious SMV from trifoliolate leaves of soybean lines was tested by local-lesion assay. Primary, secondary, or tertiary trifoliolate leaves (approximately 0.25 g of leaf tissue), collected 10 days after plants were returned to 20 C, were ground in 1 ml of 50 mM sodium phosphate buffer, pH 7.0. Assays were done in triplicate for three plants of each inoculated soybean line grown at defined temperatures. The expressed sap was used to inoculate detached leaves of *Phaseolus vulgaris* 'Top Crop' as described (18).

RESULTS

Plant growth measurements. Growth of virus-inoculated soybean lines, as measured by plant height, was generally directly correlated with temperature (Fig. 1). Growth of "mock-inoculated" soybean lines was similar to that of virus-inoculated plants (data not shown). Because trifoliolate leaves did not develop on plants grown at 10 C, plants were transferred to the original growth temperature (i.e., 20 C) for an additional 10 days. To compare results obtained in this experiment with those from plants grown at other temperatures, all plants were returned to the original growth temperature at the same time.

Press-blotting analyses of virus location in upper leaves. Press blotting of trifoliolate leaves maintained at 10 C for 10 days after inoculation showed that SMV coat protein accumulated in all virus-inoculated plants (i.e., Williams '82, PI 96983, L78-379, and Davis). Because of the variation in staining of press blots, comparison of results from leaves within individual panels was found appropriate. However, direct comparison of leaves in different panels was inappropriate. In each panel, dark-stained press blots represented virus-infected leaf tissue and relatively light-colored press blots represented uninfected leaf tissue. Coat protein was absent in mock-inoculated plants (Fig. 2). SMV-G7 coat protein appeared at all temperatures. Only in Williams '82 did SMV-G2 coat protein accumulate at all temperatures. In resistant lines, strain G2 coat protein was found with plants shifted to 10 C for 10 days, but not with plants shifted to 15, 20, 25, 30, or 35 C for 10 days. Presence of SMV coat protein in trifoliolate leaves was also confirmed by ELISA (Table 2).

Virus infectivity in trifoliolate leaves. To correlate the presence of viral coat protein in trifoliolate leaves of Williams '82, PI 96983, L78-379, and Davis (as determined by press blotting) with viral infectivity, other leaf samples from the same plants were used for the local-lesion assay (Table 2). Samples that contained infectious virus, as indicated by local lesion formation, correlated with the detection of viral coat protein in other leaf samples from

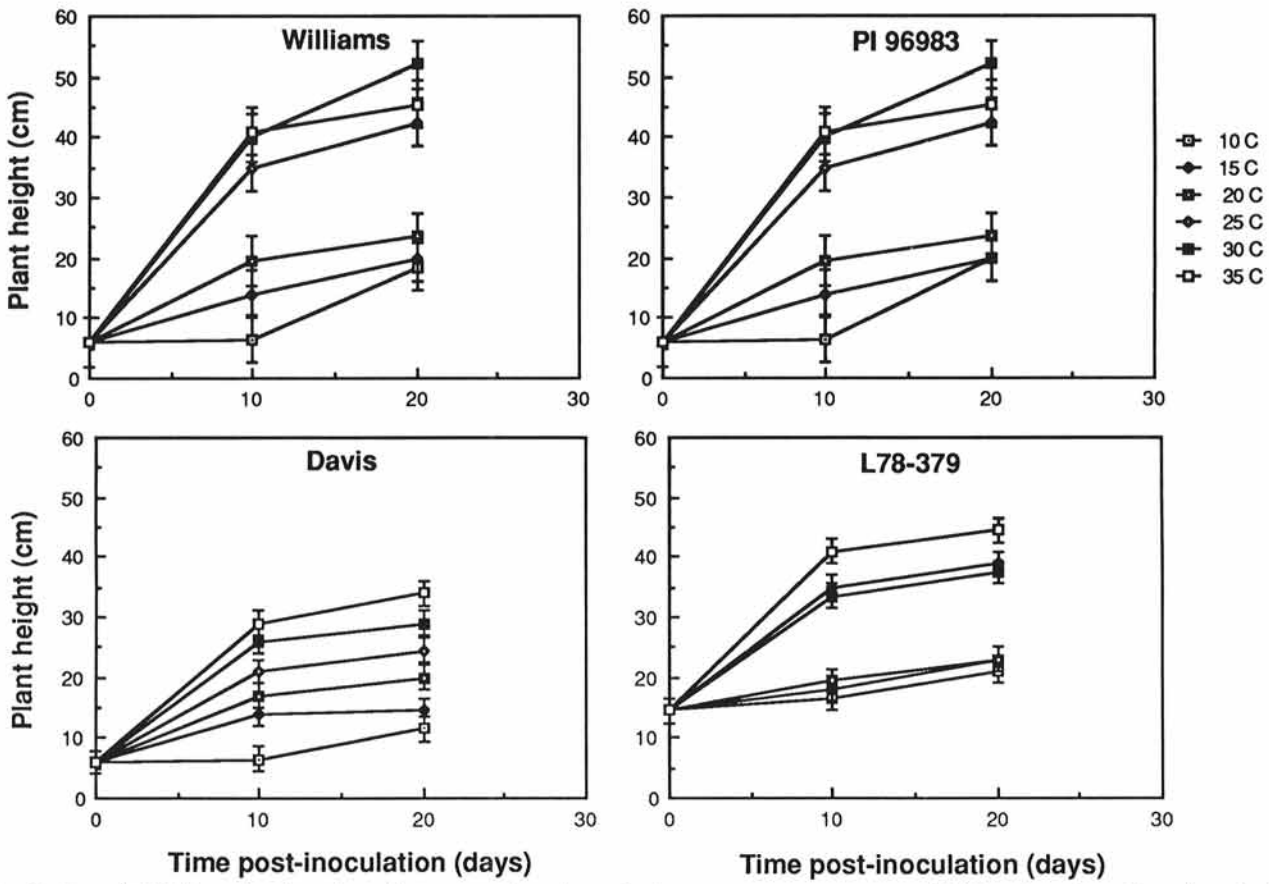

Fig. 1. Height of SMV-inoculated soybean lines at various times during temperature treatments. Height of soybean lines inoculated with SMV-G2 or -G7 was recorded at inoculation (day 0), at temperature shift (day 10), and when leaves were sampled for analysis by press blotting and local-lesion assay (day 20). Data are the mean and standard deviation (vertical bars) of combined data for the height of inoculated plants of each soybean line transferred to 10, 15, 20, 25, 30, or 35 C.

the same plants (Fig. 2). Variation in the presence of local lesions occurred in different samples from the same plant; however, at least two of the three samplings always contained infectious virus.

DISCUSSION

The detection of SMV coat protein in trifoliolate leaves of plants inoculated with SMV was used as a criterion for susceptibility or resistance; these data were confirmed by local-lesion assays and ELISA (Table 2). Because a concentration of 1 mg of virus per milliliter of 50mM sodium phosphate buffer is needed to produce local lesions with SMV (18), local lesion formation suggested that a relatively high concentration of virus was present in the trifoliolate leaves sampled.

Breaking of disease resistance in plants by low temperature has not been previously reported. Most studies addressing the effects of temperature on resistance to viruses have examined only elevated temperatures (9,10,24) and not low temperatures. However, the breaking of disease resistance reported here may not be unique to the SMV-soybean system and could exist in other systems.

Resistance in the line L78-379 was derived from soybean line PI 96983; thus, the response of L78-379 was expected to be similar to that of PI 96983 (21,22). In contrast, resistance in the cultivar Davis is derived from an allelic gene (6,22); in addition, the reaction of Davis to SMV strains G4, G5, and G6 is also different from PI 96983 and L78-379 (5,7,22). However, resistance in Davis was also altered by low temperature; this may suggest a similar resistance mechanism to the G2 strain in these lines.

It may be important to follow and quantitate viral multiplication during and after the low-temperature shift. Trifoliolate leaves on soybeans do not develop at 10 C during a 10-day period; therefore, only primary leaves could be used for analysis at 10 C. If primary leaves are inoculated on only one-half of the leaf with virus, the other side of the leaf could be used to determine virus spread. Analysis of the effects of low temperature on viral multiplication in protoplasts may also yield significant results.

The mechanism responsible for disease resistance is unknown. The results of this study suggest that disease resistance may be repressed at low temperatures, even after plants are returned to higher temperatures. Alternatively, resistance may be overcome at low temperatures, thus allowing for viral multiplication. But, resistance may eventually be restored to inhibit viral multiplication when plants are returned to a higher temperature.

In our initial studies, we manipulated temperature to induce susceptibility of several soybean lines resistant to a strain of SMV. The development of an experimental procedure to alter resistance may be a useful tool to study disease resistance.

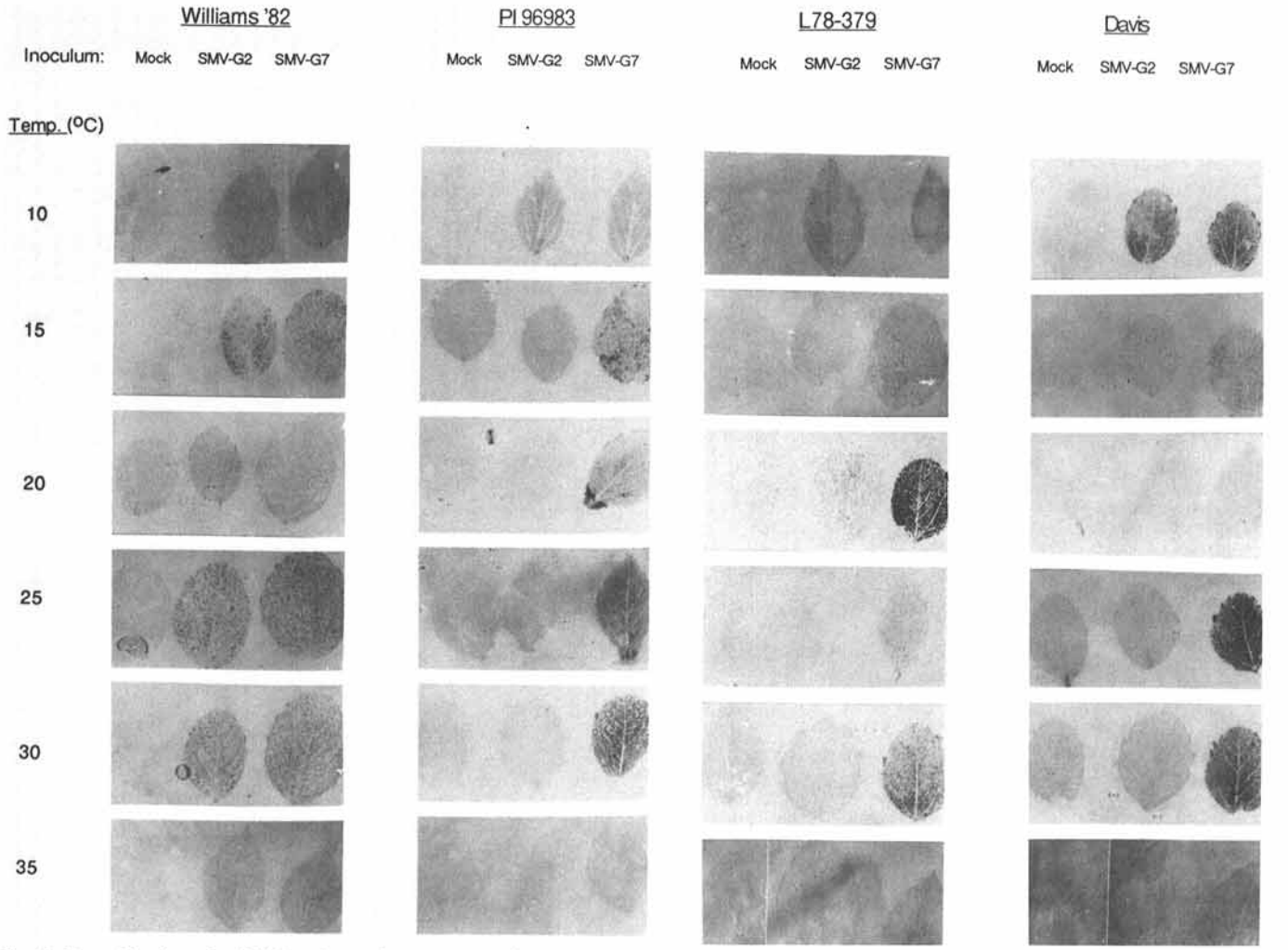

Fig. 2. Press blotting of trifoliolate leaves from soybean lines grown at various temperatures after "mock" or mechanical inoculation with either SMV-G2 or -G7. Soybean lines "mock-inoculated" or inoculated with SMV-G2 or -G7 were shifted to 10, 15, 20, 25, 30, or 35 C. Plants were then returned to 20 C for 10 days. Secondary or tertiary trifoliolate leaves were collected and analyzed by press blotting. Comparison of results from leaves within individual press-blot panels is appropriate; however, because of variability in the staining of blots, direct comparison of leaves in different press-blot panels is inappropriate. Dark-stained press blots represent virus-infected leaf tissue; light-colored press blots represent uninfected leaf tissue.

TABLE 2. Results of local-lesion and enzyme-linked immunosorbent (ELISA) assays of soybean lines inoculated with soybean mosaic virus strains G2 or G7, or "mock-inoculated" with phosphate buffer

Soybean line	Shift temperature[a] (C)	Local-lesion assay and ELISA[b]		
		Mock	SMV-G2	SMV-G7
Williams '82	10	−(−)	+(+)	+(+)
	15	−(−)	+(+)	+(+)
	20	−(−)	+(+)	+(+)
	25	−(−)	+(+)	+(+)
	30	−(−)	+(+)	+(+)
	35	−(−)	+(+)	+(+)
PI 96983	10	−(−)	+(+)	+(+)
	15	−(−)	−(−)	+(+)
	20	−(−)	−(−)	+(+)
	25	−(−)	−(−)	+(+)
	30	−(−)	−(−)	+(+)
	35	−(−)	−(−)	+(+)
L78-379	10	−(−)	+(+)	+(+)
	15	−(−)	−(−)	+(+)
	20	−(−)	−(−)	+(+)
	25	−(−)	−(−)	+(+)
	30	−(−)	−(−)	+(+)
	35	−(−)	−(−)	+(+)
Davis	10	−(−)	+(+)	+(+)
	15	−(−)	−(−)	+(+)
	20	−(−)	−(−)	+(+)
	25	−(−)	−(−)	+(+)
	30	−(−)	−(−)	+(+)
	35	−(−)	−(−)	+(+)

[a]Inoculated plants were placed at a constant 10, 15, 20, 25, 30, or 35 C for 10 days and then returned to 20 C for an additional 10 days.
[b]Three samples of trifoliolate leaves were assayed from three different plants of each soybean line inoculated on primary leaves and subjected to temperature treatment. + = Presence of local lesions and positive ELISA (in parentheses); − = absence of local lesions and negative ELISA.

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