Synthesis and Movement of Southern Bean Mosaic Virus in Cowpea Hosts with Virus-Induced Necrotic Local Lesions

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ABSTRACT

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Synthesis and movement of the cowpea strain of southern bean mosaic virus (SBMV-CS) were studied in three cowpea lines. At temperatures between 21 and 28 C, Early Ramshorn was systemically invaded without necrosis, Clay developed necrotic local lesions and no systemic symptoms, and plant introduction (P.I.) 399419 developed necrotic local lesions and systemic necrosis followed by death. No lesions developed at 32 C on Clay or P.I. 399419 and plant death in the latter host did not occur at this temperature. To prevent lesion formation, incubation of inoculated plants at 24 C could not be longer than 50-60 hours before transfer to 32 C. The length of the photoperiod after inoculation had little effect on number of lesions. Virus was synthesized readily, and to a similar degree, in Early Ramshorn and P.I. 399419 in both inoculated primary and systemically-infected trifoliolate leaves at both 24 and 32 C. In Clay, synthesis was slow but detectable in both inoculated and noninoculated leaves. Although more SBMV-CS was produced in Clay at 32 than at 24 C, the total nucleoprotein was less than 5% as much as in the other two hosts. Direct extraction and assay of RNA from host tissue indicated that there were not large quantities of free viral RNA in plants incubated at 32 C. Host necrosis caused by SBMV-CS did not inhibit virus synthesis or prevent virus movement to noninoculated portions of cowpea plants.

Additional key words: hypersensitivity, virus localization.

When mechanical inoculation of a plant virus induces necrotic local lesions, the host response usually is referred to as a hypersensitive one. Within 24-96 hours after inoculation, three phenomena are generally recognized: (i) virus synthesis is initiated and new virions are produced, (ii) cell necrosis occurs at or near the site of infection, and (iii) the virus is localized and remains within or close to the necrotic area. Evidence is accumulating to indicate that these three reactions are independent events.

Necrosis, for example, does not necessarily inhibit virus synthesis nor is it required to localize the virus. Beachy and Murakishi (2) found that 90% of tobacco mosaic virus (TMV) synthesis in tobacco callus tissue occurred after necrotic lesions were evident. Synthesis of TMV was stopped in starch lesions in cucumber cotyledons, and the virus was localized without necrosis occurring (5). Furthermore, spreading chlorotic lesions (peanut mottle virus on Bountiful bean) (10) or infection without symptoms (TMV in cotton) (4, 11) can occur in an inoculated leaf without virus movement to noninoculated leaves.

In a preliminary study (1), we found that the cowpea strain of southern bean mosaic virus (SBMV-CS) did not cause necrotic local lesions on so-called hypersensitive cowpea when inoculated plants were maintained at 32 C. This effect of high temperature appeared to be similar to that on TMV infection in hypersensitive tobacco as reported by Samuel (13). However, more detailed studies, reported herein, on virus synthesis and movement showed distinct differences from the TMV-tobacco virus-host combination.

MATERIALS AND METHODS

The cowpea strain of southern bean mosaic virus was maintained in *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'Early Ramshorn.' Leaf tissue ground in 0.01 M neutral phosphate buffer was used for inoculation. The reactions of three cowpea lines, Early Ramshorn, Clay, and plant introduction (P.I.) 399419, were studied. Each responded differently to SBMV-CS.

In most studies, the cowpea plants were grown in the greenhouse (21 to 32 C) until the time of inoculation, 7-10 days after seeding. Plants were then transferred to growth chambers with a photoperiod of 16 hours, an illumination of about 10,000 lx, and specific temperatures from 21 to 32 C depending on the nature of the experiment. Necrotic lesions were counted and measured with a dial caliper 7 days after inoculation.

To determine viral nucleoprotein concentration, three replications, each with 10 primary leaves or 10 trifoliolate leaflets, were harvested for each treatment. The leaves were homogenized in chloroform, *n*-butanol, and 0.05 M neutral phosphate buffer (1:1:1:1, w/v). Following clarification by freezing and low-speed centrifugation (10,000 *g*, 10 minutes), the virus was subjected to two cycles of differential ultracentrifugation. Nucleoprotein concentrations were determined spectrophotometrically (6.0 A_{260nm} = 1 mg/ml). When virus preparations had low optical density readings, the concentration was determined by measuring the virus peak area produced on

chart paper of a fractionator following density-gradient centrifugation.

Viral RNA was extracted from leaf tissue as described previously (6). Similar infectivity results were obtained when the extractions were made with a solution containing water-saturated phenol, sodium dodecyl sulfate (1%), bentonite (1%), and ethylenediaminetetraacetic acid (0.01 M).

Bioassays for estimates of relative infectivity of virus or viral RNA and for indexing for the presence of SBMV-CS were conducted with P.I. 399419.

RESULTS

Host reactions to the cowpea strain of southern bean mosaic virus.—The cowpea lines reacted differentially to SBMV-CS when young plants, 7-10 days old, were inoculated and maintained in the greenhouse or at continuous specific temperatures from 21-28 C. Early Ramshorn was clearly susceptible; chlorotic spots developed on the inoculated primary leaves and systemic symptoms included mosaic, leaf distortion, and stunting. Clay appeared to be a hypersensitive host; necrotic local lesions appeared on the inoculated leaves 2-3 days after inoculation and no systemic symptoms were observed. Plants of line P.I. 399419 reacted in an intermediate manner; necrotic local lesions, similar to those on Clay, developed, but the new leaf growth on all plants became necrotic and most of the plants died 10-15 days after inoculation.

TABLE 1. Effect of incubation temperature on southern bean mosaic virus local lesion development in two cowpea lines[a,b]

Temperature (C)	Clay		P.I. 399419	
	Lesion no.[c]	Lesion size[d] (mm)	Lesion no.[c]	Lesion size[d] (mm)
24	124	0.51	205	0.46
28	146	0.91	170	0.99
30	3	...	14	...
32	0	...	0	...

[a]Plants were grown in the greenhouse and transferred to the incubation temperature immediately after inoculation.
[b]Counts and measurements were made 7 days after inoculation.
[c]Average number of lesions per leaf on six plants.
[d]Average of 100 lesions, 10 per plant.

TABLE 2. Effect of pre-inoculation temperature on southern bean mosaic virus local lesion development in two cowpea lines

Temperature (C)		No. of lesions/leaf[b]	
Before inoculation[a]	After inoculation	Clay	P.I. 399419
24	24	107	112
32	24	337	585
32	32	0	0

[a]Plants were started in the greenhouse, then transferred to this temperature 4 days before inoculation. All plants received the same inoculum.
[b]Average number of lesions for 10 leaves, each on a different plant.

Effect of temperature on local lesion develpment.—The incubation temperature after inoculation with SBMV-CS greatly affected local lesion development on Clay and P.I. 399419. At 24 and 28 C, numerous lesions developed (Table 1); their size was two-fold greater at 28 C than at 24 C. Very few lesions were observed at 30 C, and even though none occurred at 32 C, local chlorotic areas sometimes appeared.

Necrotic local lesions did not develop at 32 C if plants were exposed continuously after inoculation. Distinct lesions formed when plants were held at 32 C before inoculation and then transferred to an incubation temperature of 24 C (Table 2). In fact, more lesions developed with the high pre-inoculation temperature than with a continuous exposure of 24 C.

Length of incubation period at different temperatures.—When inoculated plants were first incubated at 24 C and then transferred to 32 C, lesions did not develop unless the initial, low temperature period was longer than 50-60 hours (Table 3). Lesions appeared about 10 hours earlier on P.I. 399419 than on Clay. On the other hand, a high initial incubation temperature (32 C) did not prevent lesion formation if a transfer to 24 C was made within 96 hours after inoculation. The longer the initial incubation period at 32 C, the larger the lesions were on both cowpea lines (Fig. 1). In fact, following initial incubation at 32 C for 48-96 hours, the lesions on P.I. 399419 coalesced and most of the leaf tissue

TABLE 3. Effect of length of incubation period at 24 C before transfer to 32 C on southern bean mosaic virus local lesion development in two cowpea lines

Incubation time at 24 C[a] (hours)	No. of plants with lesions	
	Clay	P.I. 399419
0-32	0 of 12	0 of 12
50	0 of 12	3 of 12 (25)[b]
60	2 of 12 (73)[b]	12 of 12 (390)[b]
84	12 of 12 (250)[b]	12 of 12 (375)[b]
96	12 of 12 (240)[b]	12 of 12 (330)[b]

[a]Plants were incubated initially at 24 C, then transferred to 32 C.
[b]Number of lesions per plant in parentheses.

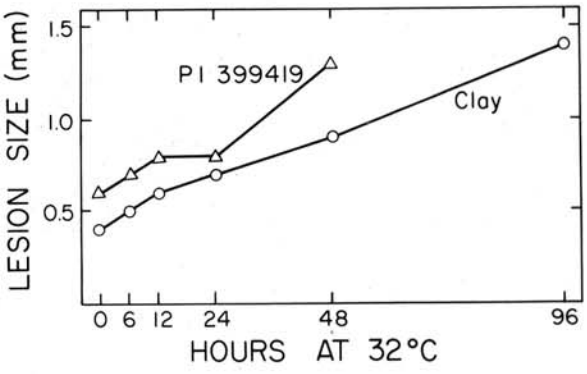

Fig. 1. Effect of the incubation time at 32 C on southern bean mosaic virus local lesion development in two cowpea lines. Plants were transferred to 24 C after the 32 C incubation period. Measurements were made 7 days after inoculation.

collapsed. Lesions on Clay were smaller than on the P.I. line.

If Clay plants were transferred back to 24 C after a 7- to 14-day incubation period at 32 C, discrete lesions developed on both inoculated and noninoculated leaves. Under the same circumstances, P.I. 399419 plants became necrotic and died within 3-5 days.

Effect of photoperiod.—Usually, the control environmental conditions that were used to cause lesions to develop were a continuous temperature of 24 C and a 16-hour photoperiod. When temperature and photoperiod were varied, it was obvious that temperature, and not photoperiod, was the primary environmental factor affecting SBMV-CS lesion production. Lesions developed in continuous light, and varying the length of the temperature period influenced lesion development more than varying the photoperiod. A high temperature (32 C) during the light period caused lesion size to be increased about 1.8 times more than the control.

Effect of temperature on virus synthesis.—In Early Ramshorn and P.I. 399419, SBMV-CS was synthesized readily in both inoculated primary and systemically-infected trifoliolate leaves at both 24 and 32 C (Table 4). Virus synthesis was slightly less at the higher temperature, but not enough to suggest a major difference in rate of synthesis. At 24 C, P.I. 399419 plants became necrotic and died 10-15 days after inoculation. At 32 C, necrotic symptoms did not develop, and a relatively mild mosaic was observed in both Early Ramshorn and P.I. 399419.

Synthesis of SBMV-CS was slow and greatly reduced in Clay cowpea at both low (21 and 24 C) and high (32 C) temperatures. However, measurable quantities of virus were purified from both inoculated primary leaves and systemically-infected trifoliolates at both 24 and 32 C (Table 4). At 24 C, only 1-3% as much virus was detected in Clay as in Early Ramshorn and P.I. 399419. In contrast to the latter two hosts, more virus was produced in Clay at 32 C than at 24 C.

Extraction of ribonucleic acid from leaves.—With some virus-host combinations, infectious RNA without a protein coat has been produced at relatively high temperatures (9). Since SBMV-CS nucleoprotein particles were produced in small quantities in Clay cowpea, we extracted leaf tissue, both inoculated primary and trifoliolate, by two phenol methods and inoculated the test host P.I. 399419. These RNA preparations from both inoculated and systemically-infected leaves of Clay caused less than 5% as many lesions as similar RNA preparations from the infected susceptible host, Early Ramshorn. Therefore, it appears that large quantities of nonencapsidated viral RNA are not present in Clay cowpea after continous exposure to 32 C.

Virus movement.—Although SBMV-CS caused small discrete local lesions on Clay cowpea, virus did move from the lesion areas of the inoculated primary leaves to noninoculated trifoliolate leaves. The movement and subsequent synthesis occurred at both 24 and 32 C. The virus was detected in individual plants by sap inoculation (90% at 24 C and 100% at 32 C) and by using the virus zone from density-gradient columns as inoculum. Approximately 2.5 times as much virus was produced in Clay trifoliolate leaves at 32 C as at 24 C (Table 4).

DISCUSSION

The SBMV-CS reaction in cowpea lines Clay and P.I. 399419 during the first 3-4 days after inoculation appeared to be one of hypersensitivity. Discrete necrotic local lesions formed which were in relative proportion to the inoculum concentration. It quickly became obvious, however, that the virus was not localized in P.I. 399419. Systemic necrosis occurred on all plants which were kept in the greenhouse or at continous temperatures of 21 or 24 C. De Zeeuw and Ballard (7) described a similar reaction for tobacco ringspot virus in cowpea, and they considered the reaction to be a susceptible one. Systemic infections, often necrotic, have been noted in hypersensitive hosts, but they occur infrequently and can sometimes be related to exposure to temperatures of 29 C or higher for some interval of the infection period (8). Systemic necrosis caused by SBMV-CS was noted previously (3) on all of

TABLE 4. Effect of temperature on synthesis of southern bean mosaic virus in susceptible and resistant cowpea lines[a,b]

Cultivar	Days after inoculation	mg of virus per gram of fresh leaf tissue			
		24 C		32 C	
		Primary	Trifoliolate	Primary	Trifoliolate
Early Ramshorn	7	0.813	...[c]	0.546	...[c]
P.I. 399419	7	0.449	...[c]	0.380	...[c]
Clay	7	0.014	...[c]	0.081	...[c]
Early Ramshorn	14	1.143	1.297	0.854	0.613
P.I. 399419	14	...[d]	...[d]	0.538	0.973
Clay	14	0.026	0.009	0.045	0.023
Early Ramshorn	28	...[e]	1.321	...[e]	0.964
P.I. 399419	28	...[d]	...[c]	...[e]	0.828
Clay	28	...[e]	0.004	...[e]	0.010

[a]Average of three experiments.
[b]Plants were grown in the greenhouse until the primary leaves were inoculated at 9 days, then transferred to 24 C or 32 C.
[c]Leaves too small for harvest.
[d]Plants died about 10 days after inoculation because of the severe necrotic reaction.
[e]Primary leaves had dropped from plants.

the F_1 plants of a Topset cowpea (susceptibility similar to Early Ramshorn) cross with Clay. However, the researchers were unable to relate the systemic necrosis reaction to the genetic data in the study.

With SBMV-CS in Clay, no systemic symptoms were observed, and necrosis appeared to have restricted the location of the virus. However, SBMV-CS was found in noninoculated, trifoliolate leaves by indexing and purification studies. The systemic movement in Clay occurred in the greenhouse and at 21 and 24 C.

At 32 C, SBMV-CS necrotic local lesions were prevented in both Clay and P.I. 399419, and the latter plants were not killed by necrosis. This reaction appeared to be similar to one observed by Samuel (13) in which the hypersensitivity to TMV did not occur in tobacco at temperatures over 30 C: TMV was no longer localized and a systemic mottle occurred. With SBMV-CS in Clay, systemic movement occurred at lower temperatures, and no systemic symptoms were observed at either high or low temperatures.

Although SBMV-CS necrotic lesions are produced on both Clay and P.I. 399419, the amount of virus produced in each host was very different. In Clay, viral nucleoprotein accumulated slowly (less than 5% as much as in Early Ramshorn) in both inoculated and systemically-infected leaves at both 24 and 32 C. Synthesis of SBMV-CS was rapid and similar in P.I. 399419, a host with a necrotic reaction, and Early Ramshorn, a susceptible host with no necrosis. Otsuki et al. (12) demonstrated that protoplasts isolated from hypersensitive tobacco synthesize TMV equally as well as protoplasts from susceptible tobacco. Obviously, individual cells of necrotic hosts P.I. 399419 and tobacco have the ability to synthesize SBMV-CS and TMV, respectively, similar to susceptible, but non-necrotic, hosts. The differences between Clay and P.I. 399419 in virus replicating capacity and necrosis lead us to believe that the two processes are independent and controlled by different host genes. Specific cowpea crosses are being made to test this genetic independence.

The mechanism of virus localization [reviewed by Loebenstein (11)] is poorly understood, but the SBMV-CS-cowpea system appears to be different from the TMV-tobacco system. With the latter system, both host necrosis and virus localization occurred at temperatures below 30 C, and both processes were abolished at higher temperatures. With SBMV-CS in cowpea, host necrosis at 21 and 24 C did not restrict virus movement. It is possible that different physiological mechanisms are operative with different virus-host combinations and generalizations on individual studies should be avoided.

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