Sugarcane Mosaic Virus: Shape of the Inoculum-Infection Curve Near the Origin

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ABSTRACT

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A dilution series of 1/160,000, 1/80,000, 1/40,000, and 1/20,000 was prepared from freeze-dried crude extracts of sorghum tissue infected with sugarcane mosaic virus. Each dilution was assayed on 525 sorghum seedlings; the experiment was repeated seven times. Infection of plants that had received the highest concentration of virus ranged from 4.5 to 9.0%. Analysis of variance showed that 99.7% of the variation among the means of the four dilutions was attributable to linear regression. The data were compatible with the conclusion that in the range of dilutions used, the dilution curve is a straight line passing through the origin. The linearity of the curve provides evidence that the genome of sugarcane mosaic virus is contained in a single particle.

The relationship between inoculum concentration and the amount of infection (the inoculum-infection curve) is relevant to problems involving plant disease epidemiology (11), viral bioassay (10), and the number of particles comprising a particular viral genome (7). Fulton (7) pointed out that some viral genomes may be divided among particles physically inseparable by current laboratory methods. If this condition exists in a particular virus, an examination of its inoculum-infection curve might be the only method capable of detecting it.

The literature of plant pathology contains conflicting assertions about the shape of inoculum-infection curves at low levels of inoculum. Parris (9) lists as a principle of plant pathology that, "the amount of disease varies with the logarithm of the inoculum potential." Virology textbooks note that viral dilution curves are often S-shaped when both inoculum and infection are plotted on arithmetic scales (2,8). The use of the probit transformation in the interpretation of viral bioassay data is based on the assumption that the dilution curve is one type of S-shaped curve (6). Van der Plank (11), allowing exceptions only for viruses with divided genomes, states that, "near the origin, disease/inoculum curves follow two rules. *One*, the curve starts at the origin. *Two*, the curve is for all practical purposes, a straight line." It should be emphasized that the disagreement is about the shape of the curve at low levels of inoculum. At high levels, various interactions preclude any universal rule.

Obviously there is no logical necessity for all diseases to fit one rule, but van der Plank (11) maintains that they do with the exceptions already mentioned. Lack of consensus on this point indicates the need for more data on more diseases. Van der Plank remarks that few viral data are available in the low inoculum concentration range pertinent to this issue. The purpose of this paper is to report such data for sugarcane mosaic virus (SCMV).

MATERIALS AND METHODS

The isolate of SCMV used in this work was recovered originally from *Stenotaphrum secundatum* in 1966 near Canal Point, Florida, and was maintained in that host in the greenhouse until September, 1972, when it was transmitted to *Sorghum bicolor* (L.) Moench 'Mn 1056.' At that time, a single batch of liquid, crude-sap inoculum prepared from infected sorghum leaves in phosphate-cysteine buffer at pH 7 (0.01 M potassium phosphate buffer containing 0.025 M cysteine) was divided among several dozen vials, freeze-dried, and stored in a deep freeze. Inoculum used in these tests remained in storage until July - August of 1975, the period when these tests were conducted. This isolate was identified as SCMV strain E on sugarcane differential cultivars (1), and produced local lesions on sorghum cultivars Atlas and CK-60-MS (3, 4). It is maintained by the American Type Culture Collection as PV-115.

In each of seven runs of the experiment, dilutions of 1/160,000, 1/80,000, 1/40,000, and 1/20,000 were prepared in phosphate-cysteine buffer at pH 7 (0.01 M potassium phosphate buffer containing 0.025 M cysteine) from a single vial of freeze-dried inoculum. This series will be referred to hereafter as relative virus concentrations of 1, 2, 4, and 8. In each of the seven runs, each concentration was assayed on 21 pots each containing 25 Mn 1056 sorghum seedlings inoculated by a procedure described previously (5). Data collected were numbers of systemically infected seedlings per pot. In the strict sense, "infection," in the context of the inoculum-infection curve, refers not to the number of systemically infected plants, but to the number of infected sites. Data on systemic infection can be transformed to give an estimate of the number of infected sites (11), but at the infection levels obtained in

TABLE 1. Summary of data from seven tests comparing infectivities on sorghum of four concentrations of sugarcane mosaic virus in each test

Test	Sums (Y) and Y^2 of infected plants following inoculation with relative virus concentrations[b] of:							
	1		2		4		8	
	ΣY[a]	ΣY^2	ΣY	ΣY^2	ΣY	ΣY^2	ΣY	ΣY^2
1	4	4	9	11	19	33	47	135
2	0	0	3	3	7	9	24	42
3	5	5	12	24	33	83	54	206
4	8	10	11	13	22	40	28	56
5	4	4	9	11	20	30	38	90
6	3	3	7	9	16	24	30	60
7	2	2	5	5	11	13	20	32

[a]The sum of the numbers of infected plants per pot = ΣY. Twenty-five plants per pot, and 21 pots per virus concentration were inoculated in each test. Pots were arranged in a completely random design.

[b]Relative virus concentrations of 1, 2, 4, and 8 correspond to dilutions of 1/160,000, 1/80,000, 1/40,000, and 1/20,000, respectively.

these tests, the effect of the transformation is negligible (5, 11), and it was not applied.

RESULTS AND DISCUSSION

Table 1 supplies data needed for calculation of the statistics discussed in this paper or for calculation of others that may be of interest to the reader. Fig. 1 shows lines and data points for linear regression of mean number of infected plants per pot on inoculum concentration. A line is shown for each test separately and for all tests combined.

In the combined analysis of all tests (Table 2), a comparison of the sum of squares for variation among means with the sum of squares for linear regression on means shows that practically all (99.7%) of the variation among means is accounted for by linear regression. Departure from linearity did not approach significance.

Also for each test separately it was shown that linear regression accounted for most of the variation among the four concentration means; r^2 values ranged from 0.918 to 0.998, and regression was significant at $P = 0.001$ for every test.

Each test was analyzed individually for deviation of the regression line from passage through the origin. Two of the seven lines deviated significantly at $P = 0.05$, but one of these (Test 2) had a positive intercept and the other (Test 4) had a negative intercept. This suggests random variation rather than a trend in the data. If the seven lines are regarded as a sample from a population of lines, the average intercept (−0.192 with a standard error of 0.0169) does not differ significantly from zero.

Significance among tests in Table 2 indicates only that the mean percentage of infection (averaged over all virus concentrations) differed among tests. Significance of tests × linear trends shows that there were differences among slopes of the seven regression lines. This is a condition commonly found when essentially similar viral bioassays are repeated in time (10). The infectivity of the preserved inoculum used in these tests remained constant over the 3 yr preceeding the tests and for at least 1 yr after the tests; the sampling error involved in removal from storage and reconstitution of the freeze-dried inoculum was not significant (J. L. Dean, *unpublished*). Because inoculum activity was constant, and inoculation procedures were standardized, differences in slope among regression lines probably are attributable to week-to-week differences in test plant resistance to infection.

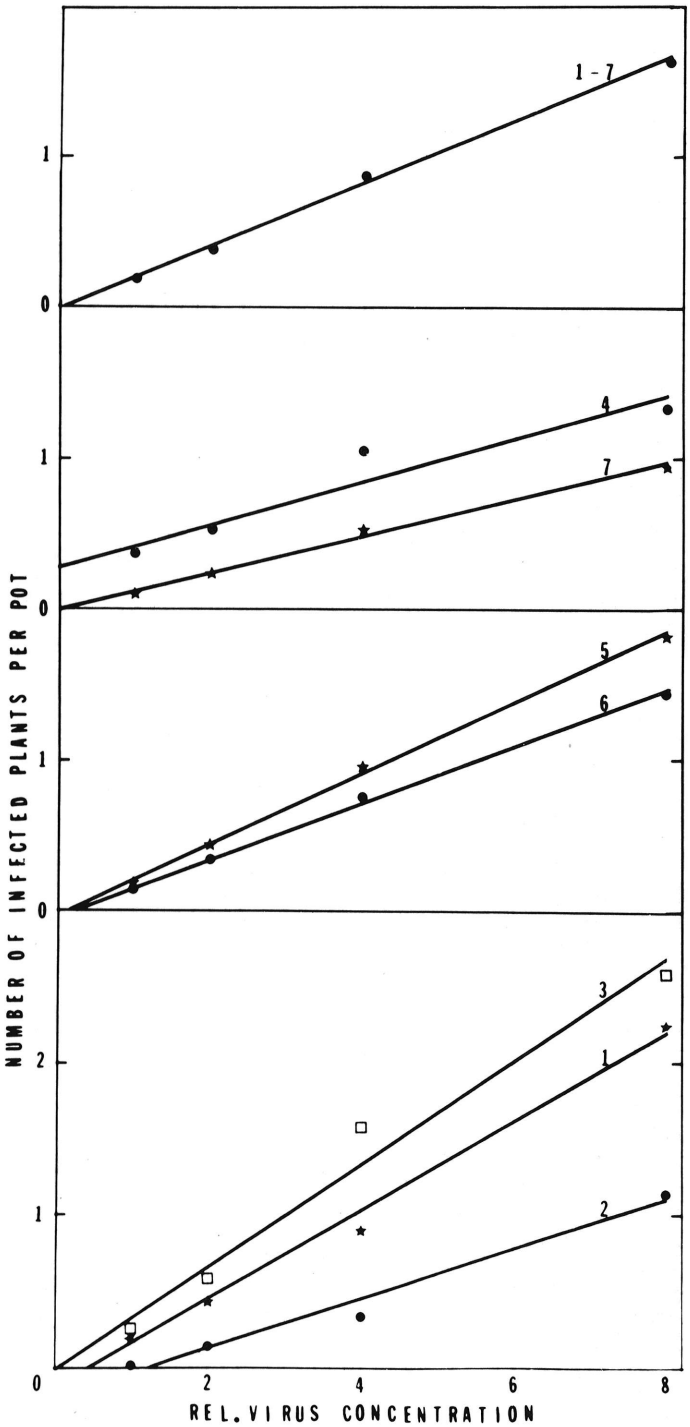

Fig. 1. Linear regression of infection with sugarcane mosaic virus, strain E, on concentration for Tests 1–7 separately and for Tests 1–7 combined. In curves for separate tests, each data point represents the mean of 21 pots each containing 25 test plants per pot. In the curve for all tests combined, each point is based on 147 pots. Relative virus concentrations of 1, 2, 4, and 8 correspond to dilutions of 1/160,000, 1/80,000, 1/40,000, and 1/20,000, respectively.

TABLE 2. Analysis of variance of data from seven tests comparing infectivities on sorghum of four concentrations of sugarcane mosaic virus in each test

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-ratio[a]
Among tests	6	42.12	7.02	12.53 **
Among concentration means	3	186.58	...	...
Linear regression on means	(1)	(186.06)	186.06	332.25 **
Departure from linearity	(2)	(0.52)	0.26	...
Tests × concentrations	18	68.80	...	...
Tests × linear trends	(6)	(61.32)	10.22	18.25 **
Tests × nonlinear trends	(12)	(7.48)	0.62	1.90 NS
Error	560	313.59	0.56	
Total	587	611.08		

[a]The asterisks (**) indicate statistical significance, $P = 0.01$, and the abbreviation NS = not significant.

The data presented are compatible with the conclusion that, at low levels of inoculum, the inoculum-infection curve for this host-virus system is a straight line passing through the origin. Since multiple-hit curves are nonlinear, lack of a significant nonlinear component in these data is evidence that the genome of SCMV is undivided.

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