Statistical Analysis of Some Dilution Assays of Maize Dwarf Mosaic Virus

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ABSTRACT

An established statistical model, based upon the assumption that the number of effective virus particles in an aliquant of infective sap follows a Poisson distribution, is applied to previously published dilution assays of maize dwarf mosaic virus. The model gives good results for the number of virus particles when sap from infected plants is diluted with sap from healthy plants, but not after dilution with water or phosphate buffer unless data from higher concentrations are omitted. This is apparently due to the presence of inhibitors in the original infective sap, which become so dilute as to be insignificant at dilutions of 100 times or more. The inhibitors are partly, but not totally, destroyed when the plant sap used for dilution is heated or clarified.

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In the absence of a local lesion host, it is necessary to assay virus infectivity by observing the proportion of plants developing systemic symptoms after inoculation by aliquants of infective material from a dilution series. Brakke (2) has pointed out that, contrary to common opinion, suitable statistical techniques are available to estimate the actual number of virus particles per aliquant in the original infective material, under fairly realistic model assumptions. The statistical methodology involved will not be presented, but computational details can be found in Finney (3), and the method is discussed in relation to virology in Kleczkowski (5) and Brakke (2). The computations involved are lengthy, but are

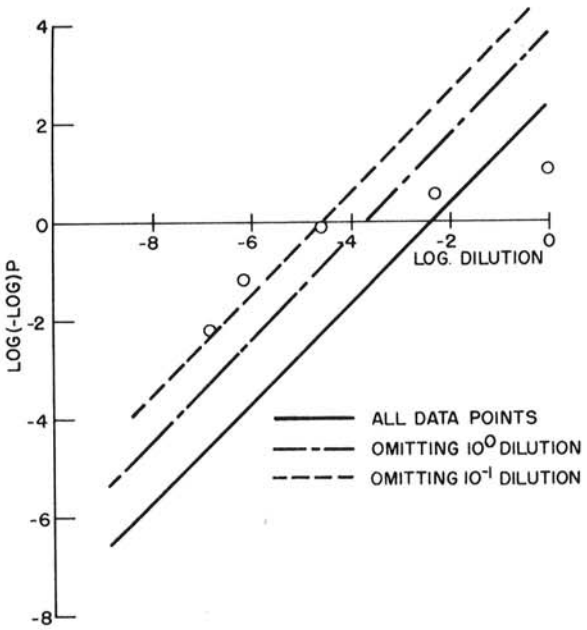

Fig. 1. Proportion of plants uninfected (P) by MDMV, and dilution of infective sap (C) related by the model $\log_e(-\log_e)P = \log_e n + \log_e C$, where n is the number of virus particles in undiluted sap. Infective sap from plants grown in complete nutrient solution.

readily programmed for high-speed computers, and in many cases, considerable success may be achieved by suitable data plots and freehand straight-line fitting.

The statistical model depends on the assumption that the number of effective virus particles in an aliquant of infective material, as defined by Brakke (1), will follow a Poisson distribution. From this it can be deduced that a plot of log (-log p_i) against log dilution should trace a line of unit slope, where p_i is the proportion of plants uninfected at the i-th dilution, and the logs are usually expressed to the base e.

MATERIALS AND METHODS, RESULTS.—Tu & Ford (7, 8) presented data on several dilution assays of maize dwarf mosaic virus (MDMV), and we re-examined this data under the above statistical model. Utilizing all the dilutions of infective sap, we found that the model does not fit the data. A portion of their data (7) is shown in Fig. 1, representing the four replications termed "control", where about 80 maize plants/dilution were inoculated with aliquants of successive dilutions of sap expressed from infected maize plants grown in complete Hoagland's solution. Dilution was with phosphate buffer.

The plot of log (-log p_i) against dilution does not follow a straight line, and similar results were obtained from all the other treatments in the experiment. Frequently, the iterative procedure used to fit the line failed to converge at all, although in the case presented, convergence did occur, and the lack of fit was indicated by a very large value of chi-square. The problem is that too large a proportion of plants remain uninfected when undiluted or 10 times diluted sap is used. Examining only the 100 times dilution, 33 out of 80 plants remained uninfected, indicating an average of 0.9 effective virus particles/aliquant at this dilution, and leading to estimates of 9 and 90 particles/aliquant in the 10 times and undiluted sap, respectively. With such mean numbers of virus particles, it seems unlikely that any plants would escape infection, yet 12 and 5 did escape at these dilutions.

Figure 1 also shows the successive improvement in fit after the elimination first of the undiluted sap only, and then both the undiluted and 10 times diluted sap. This improvement was apparent in all the data examined, and in fact after the elimination of the two lowest dilutions, the lack of fit chi-square test ceased to be significant for all except this control treatment, where 4 times as many plants were used at each dilution than for other treatments in the experiment, and consequently the test had greater power. We attribute the failure to fit the model to the presence of virus inhibitors in the plant sap, which become comparatively ineffective when diluted 100 times by phosphate buffer.

In a second series of experiments, Tu & Ford (8) presented data where a variety of diluents, including crude sap, were used. Data for the three crude sap series are presented in Table 1, and graphically in Fig. 2. In this series, the fit to the model is excellent, as indicated by the nonsignificant chi-square values. However, the values of three to five effective virus particles/aliquant of undiluted sap obtained from these experiments are much smaller than the value of 93 from the control series first described, when the

TABLE 1. Estimated numbers of effective virus particles per aliquant of undiluted sap (n) when the infective sap is diluted with crude sap from three corn varieties

Variety	$\log_e n$	SE[a]	n	Chi-square df = 8
Field corn, Ill-A	1.41	0.22	4.1	4.4
Sweet corn, Seneca Chief	1.15	0.21	3.2	6.0
Field corn, Ohio W-49	1.63	0.23	5.1	7.7

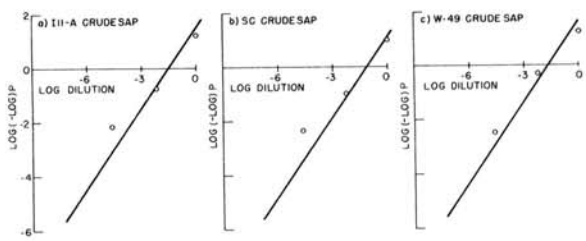

Fig. 2. Proportion of plants uninfected (P) by MDMV, and dilution of infective sap (C), related by the model $\log_e(-\log_e)P = \log_e n + \log_e C$, using crude sap from three corn varieties as diluent, where n is the number of particles in undiluted sap.

TABLE 2. Estimated numbers of effective virus particles (n) of maize dwarf mosaic virus per aliquant of undiluted sap, in assays using different diluents

Diluent	Omitting undiluted and 10 times diluted sap			
	$\log_e n$	SE[b]	Chi-square df = 6	n
Water	4.86	0.15	3.1	129
Phosphate buffer	4.57	0.16	10.1	97
Clarified 3-lf sap[a]	3.64	0.24	4.9	38
Clarified 4-lf sap	3.43	0.26	7.0	31
Clarified 5-lf sap	3.57	0.24	5.9	36
Clarified 6-lf sap	3.01	0.32	2.0	20
Heated field corn, Ill-A sap	3.53	0.25	4.4	34
Heated sweet corn, Seneca Chief sap	3.54	0.25	4.1	35
Heated field corn Ohio W-48 sap	3.40	0.26	4.3	30

[a] Numbers indicate position of the leaf from which the sap was obtained for diluent; 3 = third leaf from bottom of plant (oldest); 6 = sixth leaf from bottom of plant (youngest).

high concentrations were eliminated. This is to be expected if inhibitors are involved.

Where diluents other than crude sap were used, elimination of the two highest concentrations was again necessary to produce a good fit to the statistical model. The analysis of this data is presented in Table 2. It can be seen that, contrary to the original report of Tu & Ford (8), this analysis does not support the thesis that there is a difference between infectivity when dilution is with water, compared with phosphate buffer. However, the values of the number of effective virus particles obtained when dilution is with clarified or heated plant sap are consistently lower than when dilution is with water or phosphate buffer, although not so low as when dilution is with crude sap. This indicates that the inhibitor is only partially destroyed by the process of clarification or heating. The ratio of estimates of numbers of virus particles in phosphate buffer dilution (eliminating the higher concentrations) and in crude sap dilution vary from 25:1 to 41:1, rather less than the reported value of 100:1 for inhibition of cucumber mosaic virus (4).

DISCUSSION.– The significance of these results lies in the indication that, under certain circumstances, caution may be necessary in applying the Poisson statistical model to plant virus data, in spite of its considerable attraction in providing actual estimates of the number of virus particles. The model may be more successfully applied, and more meaningfully interpreted, after the elimination of the higher concentrations of inoculant. In this series of experiments, the fit of the statistical model was good, but not perfect, using dilutions of 100 times or more. However, it is not clear that the remaining lack of fit is necessarily due to residual inhibitory activity. Shortley & Wilkins (6) discussed the failure of data to fit models of the kind used here because of host heterogeneity for susceptibility. That this cannot be the prime cause of the failure of the model to fit this data is clear from the crude sap dilution series, but it could be a secondary cause of deviation from the model after the elimination of higher concentrations. The data is probably not adequate to test this. In all other respects, this analysis confirms previously presented interpretations (7, 8).

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