out, each sample is compared at each leaf position on the assay plants. An example of an experimental outline of this kind is shown in **Fig. 3C**, comparing samples A–D.

In each of these three cases, replication can be achieved by duplication of the sets of plants.

2. The dilutions at which the samples are compared are important for a number of reasons. Local lesion curves relating dilution to number of lesions are generally sigmoidal in shape. At very high concentrations of virus, a change in concentration has very little change in the number of lesions. This may be because of aggregation of virus particles or inhibitors present in the inoculum; at very low concentrations of virus, changes in concentration similarly have little overall effect on the number of lesions present, because of the low efficiency of the mechanical inoculation procedure ***(2)***. The slope of the curve may also vary, depending on the number of particles required to cause infection. However, some virus–host combinations may not respond to give the predicted single- or multiple-hit curves ***(3)***. To make meaningful comparisons, it is necessary to always compare samples within the middle range of the curve, where a change in concentration is accompanied by an equivalent change in lesion number. An example of a local lesion dilution curve, comparing a sample to a standard, is shown in **Fig. 2**. It may be necessary to carry out preliminary experiments to reach the desired number of lesions per leaf, followed by an experiment in which the dilutions can be closer together, around the range that gave the desired number of lesions. For leaves the size of cowpea, for example, leaf counts in the range of 10–200 local lesions would give useful estimates. It is worth bearing in mind that crude extracts from some plants contain inhibitors of infection, and it may be necessary to dilute the inoculum significantly to obtain adequate lesion numbers.
3. As an alternative to pipeting a standard volume of inoculum onto the leaf and spreading it with a Parafilm-coated finger, a small pad of muslin can be soaked in inoculum and rubbed over the leaf surface. In any event, it is important to ensure that the leaf surface is completely wetted with inoculum.
4. It is only necessary to rub the inoculum gently over the leaf surface once or twice. Repeated rubbing is unnecessary and will lead to unwanted leaf damage.
5. If a standard (for example, purified virus) is included in each local lesion assay, the data can be normalized to it and the data from successive experiments can then be compared. To normalize the data, use the following equation, setting the standard lesion number to 100 in each experiment.

   Number of lesions produced by undiluted / Number of lesions produced by the undiluted standard sample × 100 = Normalized lesion number

   If aliquots of the same standard are used in each experiment, and if multiple freeze–thaw cycles are avoided, data from successive experiments can be compared by using the normalized data.
6. **Table 1** lists the local lesion hosts appropriate for the type members of all the genera; if the virus–host combination you require is not in this list, the best