The Susceptibility of *Rhoeo discolor* to Infection by Tobacco Mosaic Virus

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Gordon and Smith (1) reported that leaves of *Rhoeo discolor*, although unsusceptible to infection by whole tobacco mosaic virus, became infected when inoculated with the virus ribonucleic acid. If established, this would be perhaps the strongest possible circumstantial evidence that infection by tobacco mosaic virus necessarily entails the ribonucleic acid separating from the protein and that some plants resist infection because they do not contain systems able to disrupt the virus particles. The result of the experiment described by Gordon and Smith, however, was not fully conclusive, because the inoculum with which they demonstrated infection contained 20 mg per liter, whereas the apparently ineffective virus inoculum was 0.5 mg per liter, which is only 0.00125 as much nucleic acid. Infection of such hosts as tobacco, in which the virus readily spreads from cell to cell and reaches a high concentration, is easily demonstrated with dilute inocula, but this is not necessarily so with a host in which spread is limited and the virus content per cell is small. Detecting virus multiplication in such plants will depend more on the number of initial infection sites, which in turn will depend on the concentration of virus in the inoculum.

**RESULTS**

My first experiment with concentrated inoculum containing "Celite" (a diatomaceous earth that increases the number of infections in all hosts previously studied) left no doubt that tobacco mosaic virus can infect and multiply in *R. discolor*. Table I compares the infectivities of extracts made from leaves at different intervals after they were inoculated on both surfaces with virus at 5 mg per ml. The inoculated leaves were washed in running water, each was cut transversely into four pieces of equal length, and the pieces were put in dishes containing water, which were kept in a glasshouse at a mean temperature of 18°. The initial extract was infective, as was to be expected after using such a concentrated inoculum, and the infectivity of successive extracts (Assay 1) followed the course usual in such circumstances with other hosts, first falling and then rising. Assay 2, with variously diluted extracts, suggests that the virus concentration increased by at least 20 times between both the 2nd and 4th and the 4th and 8th days after inoculation. This contrasts with the results of a parallel experiment made with *Phaseolus vulgaris* var. Prince, which seems not to be a host, sap extracted from bean leaves 7 hours after inoculation produced a mean of 46 lesions per *Nicotiana glutinosa* leaf, and sap extracted 1, 2, 4, and 8 days afterwards produced 3, 0.3, 0, and 0.

The results shown in the table were obtained in July, when days were long and the light bright. Other experiments in similar conditions showed: (a) unequivocal multiplication in leaves inoculated with virus at 1 mg per ml, but not with virus at 0.1 mg per ml, whether or not Celite was used; (b) that inoculating only the upper leaf surface led to almost the same virus content in sap extracted 8 days later as did inoculating both upper and lower surfaces; and (c) that sap extracted 8 days after inoculation was less infective when the original inoculum was RNA than when it was a solution of intact virus containing the same amount of RNA; the apparent multiplication factor was greater, however, because sap extracted from leaves immediately after inoculation with RNA was not infective, whereas sap from leaves rubbed with intact virus contained residual virus.

These results seemed to show that Gordon and Smith had failed to demonstrate infection with intact virus simply because they had used too dilute inocula, but when I suggested this to Dr. M. P. Gordon, he told me they had used virus at 10 mg per ml without success. However, the results which he kindly sent of his tests on leaves inoculated with 1 mg per ml of virus did not exclude the possibility of multiplication, for although sap extracted 8 days after inoculation produced fewer lesions (64 per half leaf of *Nicotiana tabacum* var. Xanthi) than sap extracted immediately after inoculation (111 lesions per half leaf), it was infective and the virus in it might have been newly produced virus and not residue from the inoculum. Obviously, though, the multiplication, if any, was less than in my experiments, and there was need to find an explanation for the discrepancy. Further work suggests that the light intensity in which inoculated *R. discolor* leaves are kept is important in determining the extent to which virus multiplies. Gordon and Smith kept their leaves in artificial light of low intensity, and in the Rothamsted glasshouses results during autumn and winter came to resemble theirs, with sap extracted 6 to 8 days after inoculation sometimes being less infective and rarely much more infective than sap extracted from leaves immediately after inoculation and washing. Sampling at daily intervals after inoculation usually produced evidence of multiplication even when the 8-day sample was less infective than the immediate one, for the infectivity of successive samples usually fell and then rose, but this was not always so; in some tests it remained more or less constant and in others it fluctuated.

One experiment in the short days and dull light of December will serve to contrast with the one given in the table and to show how virus multiplication depends on the conditions in which the *R. discolor* leaves are kept. Pieces of leaves inoculated with 5 mg per ml of virus were kept in the glasshouse, one lot in day-light, one in darkness, and a third in daylight supplemented by continuous light from a 1000-watt incandescent lamp at a distance of 25 cm; sap extracted immediately after inoculation...
produced 35 lesions; the 3-day samples produced no lesions; the 6-day samples from the three conditions produced 8, 1, and 254 lesions, respectively, and the 9-day ones, 16, 3, and 790 lesions. The water in the dish under the lamp was 27°, 8° more than in the other two dishes, which may also have influenced virus multiplication. However, the mean temperature of the water containing the other leaf pieces was approximately the same as in the unheated house in July, so temperature differences cannot explain the much greater multiplication of virus in leaves kept in daylight during July.

Tobacco mosaic virus also multiplies less extensively in detached tobacco leaves in darkness than in light, and it multiplies more, both in the light and dark, when leaves are in solutions of sucrose and Ca(H$_2$PO$_4$)$_2$ than when in water (2). Floating inoculated leaves of *R. discolor* in nutrient solution, however, gave no higher virus content than floating in water, whether or not the leaves were illuminated.

The results in repeat experiments with *R. discolor* leaves have varied considerably even when leaves were treated similarly after inoculation, which suggests that their physiological state at the time of inoculation is also important in determining susceptibility to infection. The variations occurred both in the amount of virus ultimately produced and in the interval between inoculation and when multiplication became detectable. Neither the reason for the erratic behavior nor for the effect of light on virus multiplication is known, but various possibilities, not mutually exclusive, can be suggested: (a) leaves in different physiological states may have different numbers of potential infection sites, or different amounts of virus may be required to infect their sites; (b) the initially infected cells in different leaves may support multiplication to different extents; (c) the virus may spread to different numbers of cells from each initial site; and (d) the time taken either to initiate infection or to move to adjacent cells may differ.

That *R. discolor* can be infected by intact tobacco mosaic virus seems certain, but, equally obvious is the fact that it is a poor host even in the most favorable conditions for virus multiplication yet found.

**SUMMARY**

*Rhoeo discolor* is susceptible to infection by tobacco mosaic virus, but it is a poor host in which virus multiplication becomes readily demonstrable only when inoculated leaves are brightly illuminated. Multiplication is more readily demonstrated with inocula of nucleic acid than of intact virus because results are not complicated by residual infectivity of the inoculum.

**REFERENCES**


**TABLE I**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Time of sampling</th>
<th>Dilution of sap</th>
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<tr>
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