Multiplication of Vesicular Stomatitis Virus in the Leafhopper
Peregrinus maidis (Ashm.), a Vector of a Plant Rhabdovirus

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SUMMARY

Vesicular stomatitis virus (VSV) was found to multiply efficiently in whole
Peregrinus maidis (Ashm.), the leafhopper vector of maize mosaic virus (MMV),
a plant rhabdovirus. Insects were inoculated with VSV by means of a micro-
syringe, collected at 1-day intervals and tested individually for the presence of virus.
Exponential virus multiplication occurred within the first 4 days, reaching titres
of $10^6$ p.f.u. per insect in days 5 to 10 after inoculation. These observations show
that a common host is available to study the multiplication of a plant and an animal
rhabdovirus.

Viruses belonging to the rhabdovirus group appear to be ubiquitous in nature, being
found infecting plants, vertebrates and insects. An interesting feature is that most of the
plant and some of the animal rhabdoviruses are able to multiply in insects. This has led to
some speculations on the possible role of insects as the origin of both plant and animal
rhabdoviruses (Johnson, Tesh & Peralta, 1969).

Vesicular stomatitis virus (VSV), the prototype of the rhabdovirus group, is a virus which
is able to multiply in a variety of insects and insect tissue cultures, including Aedes aegypti
(Mussgay & Suárez, 1962; Bergold, Suárez & Munz, 1968) Aedes albopictus (Schloemer &
Wagner, 1975), Drosophila melanogaster (Printz, 1973) and the moth Antheraea eucalypti
(Yang, Stoltz & Prevec, 1969). The induction of CO2 sensitivity in D. melanogaster infected
with VSV demonstrates that the relationship of Sigma virus and VSV is not only morpho-
logical but physiological as well (Printz, 1973).

Few attempts have been made to compare biologically a plant and an animal rhabdovirus.
Hackett et al. (1968) were unable to demonstrate any serological relationship between sow-
thistle yellow vein virus (SYVV) and VSV. They also failed to obtain multiplication of VSV
in Hyperomyzus lactucae (L), the aphid vector of SYVV. We now report the multiplication
of VSV. Indiana strain, in whole Peregrinus maidis (Ashm.), the leafhopper vector of maize
mosaic virus (MMV). This plant rhabdovirus is able to replicate in the leaf-hopper vector
(Herold & Munz, 1965).

Virus of the Indiana serotype grown in a suckling mouse brain was passed twice in Vero
cells and then inoculated into P. maidis after clarification by low-speed centrifugation.
Infection was carried out as follows: a virus suspension, containing $1 \times 10^8$ p.f.u./ml was
diluted 1:10 in phosphate buffered saline (PBS) containing 100 units/ml of penicillin,
100 µg/ml of streptomycin and 2.5 µg/ml of amphotericin B. The virus suspension was
injected into the insects by means of a Hamilton microsyringe. Each P. maidis received
0.1 µl of virus suspension, containing approx. 1000 p.f.u. Inoculated insects were kept on
healthy corn plants at a temperature of approx. 22 °C. At 1-day intervals after inoculation,
groups of ten insects were removed and stored at −90 °C. After thawing, the insects were
ground in glass homogenizers with 1 ml of PBS containing antibiotics. The suspension
obtained was then centrifuged for 10 min at 2500 g and the supernatant fluid used for
infectivity assays. Virus infectivity was tested in Vero cells by a plaque method utilizing a serum-free agarose overlay (Bergold & Mazzali, 1968).

Preliminary experiments gave unequivocal indication of VSV multiplication in P. maidis. However, when insects were tested in pools, the kinetics of virus multiplication were confused with inexplicable variations in virus titres. When individual insects were titrated, it resulted in a clearer picture of the phenomenon. Fig. 1 shows the data of such an experiment, in which the titres obtained with individual insects are depicted.
Virus titres increased rapidly, reaching a peak of about $1 \times 10^6$ p.f.u./insect on day 5 after inoculation. Titres were maintained, up to day 10, at which time titres dropped. *P. maidis*, however, can remain infected for at least 21 days after inoculation (data not shown).

The confused results obtained in our earlier experiments could be a consequence of the inability of all *P. maidis* to show detectable titres of VSV at different times after infection. Only 33% of the inoculated insects yielded positive results when examined from day 2 to 14 after inoculation. However, the great majority of the positive insects have titres which fitted the proposed curve of virus multiplication kinetics. Non- and low-producer insects are the result of technical difficulties, since carefully controlled inoculations resulted in essentially 100% successful infection.

It is interesting to note that some plant rhabdoviruses transmitted by leafhoppers are able to multiply very rapidly in the vector when they are injected, reaching maximum titres 7 days after inoculation (Paliwal, 1968; Sinha & Chiykowski, 1969). This is comparable with the kinetics of VSV multiplication in *P. maidis*.

Our study is, as far as we are aware, the first demonstration of a common host for the multiplication of a plant and an animal rhabdovirus.

The morphogenesis of MMV, as with most of the plant rhabdoviruses, appears to be associated with nuclei. On the other hand, animal rhabdoviruses mature in plasma or intracytoplasmic membranes (Francki, 1973). It would be of interest to study the site of replication of VSV and MMV in the common insect cell, since previous comparisons have been made with data obtained from different hosts. Electron microscopy and tissue culture techniques are being used to investigate this system further.

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**REFERENCES**


Short communications


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