Virus-induced RNA Polymerase and Synthesis of Bromegrass Mosaic Virus in Barley

(Accepted 22 January 1970)

A virus-induced RNA polymerase activity (VPA) resistant to actinomycin D has been characterized in cell-free extracts of barley leaves infected with bromegrass mosaic virus (BMV), but not in extracts of leaves infected with barley stripe mosaic virus (BSMV) (Semal & Hamilton, 1968a, b; Semal, 1970). In order to investigate the significance of VPA for the synthesis of BMV, we compared the kinetics of VPA development in extracts of BMV-infected barley leaves with the in vitro synthesis of the virus. As with some plant viruses VPA was detectable in vitro only at definite stages of infection (Semal, 1969), we also looked for VPA in extracts of BSMV-infected barley leaves at intervals after inoculation.

Barley seedlings were grown in a greenhouse and inoculated at the one-leaf stage with the juice of barley leaves infected with BMV or with BSMV. Control plants gave identical results when either not inoculated, or rubbed with water and Carborundum only. After inoculation, the plants were placed under continuous light at 22° to 24°. Systemically infected second leaves were removed at intervals and the leaf homogenates (250 mg. fresh leaf tissue per assay) fractionated by sedimentation between 10000 g and 10,000 g (Semal & Hamilton, 1968a).

VPA was estimated by measuring the incorporation of [3H]UTP into RNA after 20 min. incubation of leaf fractions in the presence of actinomycin D and requirements for RNA synthesis in vitro. After deproteinization with phenol and detergent, RNA was precipitated by adding two volumes of 96 % ethanol, and the pellets thus obtained were resuspended in water or in ×2 Standard Saline Citrate (0.15M-NaCl, 0.015M-sodium citrate, pH 7.0: SSC).

Resistance of the labelled product to the action of pancreatic ribonuclease was determined by incubating for 30 min. at 37° with 5 μg./ml. of ribonuclease A (Sigma), controls being incubated without addition of enzyme. The acid-insoluble radioactivity associated with DNA was measured by scintillation counting, after addition of carrier protein and Millipore filtration.

The virus content of BMV-infected leaves was determined (Proll, 1967) and expressed as mg. of virus per g. of fresh leaf tissue: a difference of 1 between the $E_{260}^{1cm}$ and $E_{290}^{1cm}$ of the product indicated a virus concentration of 0.24 mg./ml.

Resistance to actinomycin D of UTP incorporation by extracts or the second leaf of barley, and resistance of the labelled product to ribonuclease, were unchanged following infection 4 to 10 days after inoculation of the first leaf with BSMV. This confirms a previous report (Semal & Hamilton, 1968b), but contrasts with results for a fraction prepared from BMV-infected barley.

The detection of VPA in extracts of the whole second leaf of BMV-infected barley was made as early as 54 hr after inoculation of the first leaf (Fig. 1), and a maximum level was reached at 3 days in three independent experiments. At these stages of infection, no symptoms were observed and little or no virus was detected in the leaves. Accumulation of BMV was active from 3 to 5 days, while VPA decreased during this period.

Our results are generally comparable with those published for animal and bacterial virus systems (Horton et al. 1964; Polatnick & Arlinghaus, 1967; Watanabe & August, 1967).
Short communications

The rapid increase of VPA that precedes the accumulation of BMV in leaf 2 of barley suggests that the virus-induced enzyme-template complex associated with the leaf fraction is functional in the *in vivo* multiplication of virus, and is a precursor in the synthesis of viral RNA. It is not known, however, whether the enzyme-template system operates identically *in vitro* and in the cell.

![Graph showing activity of virus-induced RNA polymerase](image)

Fig. 1. Activity of virus-induced RNA polymerase (○) in extracts of barley leaves infected with bromegrass mosaic virus. Virus content (●) of the leaves expressed as mg. of virus per g. of fresh leaf tissue. Symptoms appeared at 4 days after inoculation.

In our conditions (Proll, 1967) the upper part of the second leaf of barley did not show symptoms when the first leaf was inoculated with BMV. In subsequent experiments, the terminal third of leaf 2 was discarded, and the basal two-thirds used to prepare cell-free extracts. The results (in terms of actinomycin resistance of UTP incorporation: Fig. 2) are essentially similar to those for whole leaf extracts (Fig. 1). The deproteinized product of VPA in extracts of BMV-infected leaves, shown to be partially duplex RNA from 7 to 10 days after inoculation (Semal & Hamilton, 1968a; Semal, 1970), was also largely resistant to ribonuclease in ×2: SSC at earlier stages of infection (Fig. 2). The labelled product was entirely acid-soluble after incubation with ribonuclease in water.

Total *in vitro* incorporation of UTP 3 days after inoculation was increased by infection, as shown also for *in vivo* incorporation of uridine (Kummert & Semal, 1969). This was largely due to the rapid increase of actinomycin-resistant synthesis of duplex RNA, the DNA-dependent synthesis of single-stranded RNA being drastically reduced at 3 days and partially restored at later stages of infection. The virus-induced inhibition of the synthesis of host cell single-stranded RNA from 3 to 5 days after inoculation (Fig. 2) was accompanied by a reduction in the weight and length of fresh leaves.
Fig. 2. Activity of RNA polymerase in cell-free extracts of barley leaves, either healthy or infected with bromegrass mosaic virus. Results are expressed as acid-insoluble radioactivity. Incorporation of [3H]UTP (●); incorporation of [3H]UTP in the presence of 20 μg/ml of actinomycin D (○); incorporation of [3H]UTP in the presence of 20 μg/ml of actinomycin D: residual acid-insoluble radioactivity after treatment of the RNA product with ribonuclease in × 2: SSC (■).

This work was supported by the ‘Fonds National de la Recherche Scientifique’, Brussels. We are grateful to Dr W. Fiers for reviewing the manuscript, to Anne-Marie Pollard and Paulette Janssens for able technical assistance and to E. Francois and A. Riga for radioactivity measurements. Actinomycin D was a gift from Merck, Sharp and Dohme Research Laboratories, Rahway, N.J.

Laboratoire de Pathologie végétale
Faculté des Sciences agronomiques
Gembloux, Belgium

J. Semal
J. Kummert

REFERENCES
Short communications


(Received 8 December 1969)