

Some Properties of Potato Mop-Top Virus and its Serological Relationship to Tobacco Mosaic Virus

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SUMMARY

Potato mop-top (PMTV) and tobacco mosaic (TMV) viruses are serologically related, but only slightly. An antiserum with a titre of 1600 against TMV had a titre of only 5 against PMTV. Similarly, an antiserum with a titre of 2048 against PMTV had a titre of only 8 against TMV; when diluted in saline this antiserum did not precipitate TMV, but did when diluted in 0.1 M EDTA pH 7.7. Particles of PMTV were of many lengths, with peaks in the distribution of lengths at 250 to 300 nm. and 100 to 150 nm. Only the longest particles were infective. They had the same width and pitch of the protein helix as TMV particles. Sap from infected leaves contained only few particles, many of which were defective; the main defect was the uncoiling of the protein from one end. Plants infected with TMV are partially protected against infection by PMTV.

INTRODUCTION

Harrison & Jones (1970) found that sap extracted from leaves infected with potato mop-top virus (PMTV) contained only few particles, which differed in length, but were about 20 nm. wide. The coat protein helix at the end of many particles was partly uncoiled. Although buffer extracts had little infectivity, they found no evidence that the virus occurred as free RNA. Attempts at purification were hindered by the virus sedimenting at 8000g, when most of the infectivity was in the pellet. They suggested that in some ways PMTV resembled the defective strains of tobacco mosaic virus (TMV) isolated by Kassanis & Woods (1969). Because PMTV resembles TMV in appearance, we investigated some of the properties of PMTV and its serological relationship to TMV.

METHODS

Infectivity. An isolate of PMTV was kindly provided by Dr I. Macfarlane and was propagated in *Nicotiana tabacum* cv. Xanthi-nc, which was also used for assaying infectivity. The number of lesions obtained depended greatly on the age of the plants and the environment in which they were kept; also top leaves were more susceptible than bottom ones. Tender plants were used when they had 7 fully developed leaves; they were trimmed to four top leaves, which were dusted with carborundum before they were inoculated. When inoculating a large batch of plants, fresh inoculum was used for every 20 plants because the inoculum quickly lost infectivity. In tobacco cv. Xanthi the virus produced necrotic concentric rings (Fig. 1) or solid whitish lesions, depending on the environment and the age of the plant and leaf.

Serology. The antiserum to PMTV was produced by injecting a rabbit three times with 1 ml. of partially purified preparations (see below) containing about 0.3 mg. of virus (estimated by analytical ultracentrifugation). The first two injections, at an interval of 2 months,

were intravenous and the third, 3 months later, intramuscular. Two weeks after the second injection the antiserum had a titre of 128, and 4 weeks after the intramuscular injection the titre had risen to 2048. The antiserum to TMV was prepared by a prolonged immunization procedure in an attempt to enhance its cross-reactivity with related antigens. Three intramuscular injections were given at intervals of 6 months, each of 1 ml. containing 10 mg. of purified virus. The titre was 1280 2 weeks after the first injection and 6400 2 weeks after the second, but had fallen to 1600 2 weeks after the third injection. For the intramuscular injections the virus was emulsified in Freund's complete adjuvant for the first injection, and

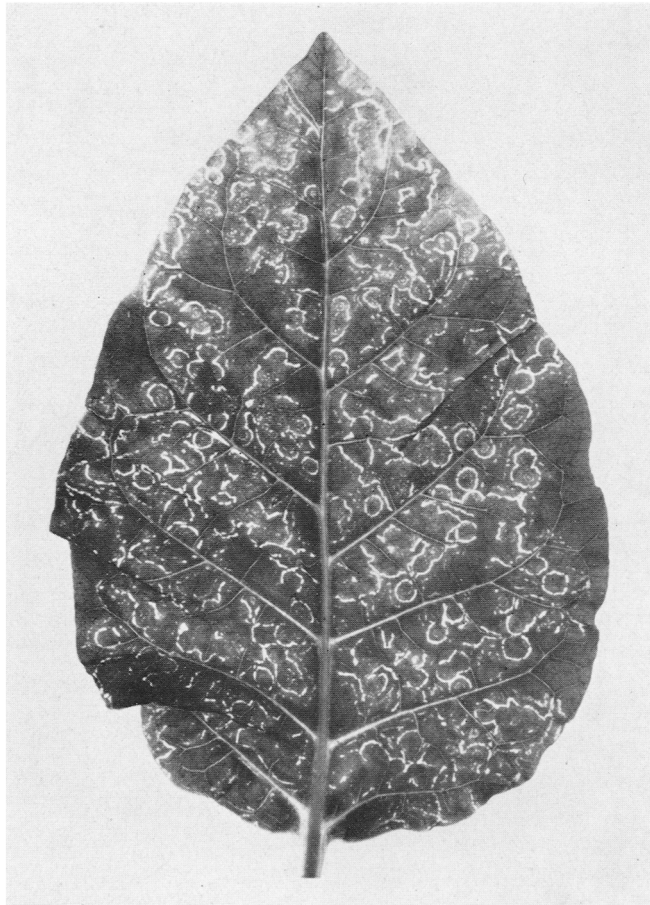


Fig. 1. A tobacco leaf cv. Xanthi infected with PMTV.

with incomplete adjuvant for the others. The precipitation tests were made in narrow tubes using 0.4 ml. of the antiserum dilution and an equal volume of antigen. Unless otherwise mentioned, the antiserum was diluted in 0.85 % saline. The PMTV antigen for the precipitation tests was obtained as follows. Sap from infected leaves was centrifuged for 10 min. at 9000g and the pellet extracted with a volume of 0.5 M-borate buffer pH 7.5 equal to half the volume of the sap. The extract was centrifuged at 9000g, heated at 50° for 10 min. and centrifuged again. Such preparations were satisfactory for serological precipitation tests if, when examined in the Siemens IA electron microscope at a magnification of $\times 40,000$, 5 to

10 particles were seen in each field. Sap from healthy plants treated similarly was used as control. The antisera did not react with healthy plant antigens. PMTV purified by the ether-carbon tetrachloride method was unsuitable for precipitation tests because such preparations often precipitated non-specifically. Purified TMV was used as test antigen. Antiserum titres are quoted as the reciprocals of the dilution end-points.

Electron microscopy. Virus preparations were either mixed with an equal volume of 2% sodium phosphotungstate and sprayed on to carbon-coated, seven-hole mounts, or stained with phosphotungstate or uranyl formate and mounted using the gross-drop technique.

Density-gradient centrifugation. Density gradients ranging from 10 to 40% (w/v) sucrose in borate buffer pH 7.5 were mechanically made in 30 ml. centrifuge tubes. One to 2 ml. of partially purified PMTV was overlaid and the tubes centrifuged for 2 hr at 50,000g. Fractions of 1.6 ml. each were removed using an ISCO density-gradient fractionator.

Sedimentation analysis. This was made in a Spinco Model E analytical ultracentrifuge using schlieren optics.

RESULTS

Infectivity

Extracts of plants infected with strains of TMV such as PM_2 that occur in plants as free nucleic acid are more infective when made with phenol than with buffer. In contrast, phenol extracts of leaves infected with PMTV had only one-twentieth of the infectivity of buffer extracts, showing that the leaves contained little or no free virus nucleic acid. For example, when 0.5 g. of leaf tissue infected with PMTV was extracted either in a mixture of 2 ml. of 0.066 M-phosphate buffer pH 7 with 2 ml. water-saturated phenol and 0.6 ml. of 3.8% bentonite in water or in 2.6 ml. of buffer alone, the average numbers of lesions per half leaf were 5 and 105, respectively.

However, PMTV did resemble PM_2 in the way that most of the infectivity seemed to be associated with a large cell component and sediments readily. Thus, whereas uncentrifuged sap produced an average of 22 lesions per half leaf, the supernatant after 15 min. at 9000g produced only 1 and the pellet resuspended in a volume of water equal to the sap produced 55. In contrast to type TMV (Kassanis & Woods, 1969), the infectivity of PMTV was completely lost when the sap was extracted in buffer containing 3.8% bentonite, probably because bentonite adsorbs the cellular component to which the virus is attached. Harrison & Jones (1970) suggested that PMTV sediments easily because the virus particles aggregate side to side and end to end to form large aggregates. However, aggregation cannot explain the total removal of the infectivity because electron microscopy shows sap to contain mostly single particles and some aggregates too small to sediment at 9000g.

The infectivity of sap extracted in 0.5 M-borate buffer pH 7.5 dropped considerably on heating for 10 min. at 70° and the virus was completely inactivated at 80°. In these tests, the saps were dialysed against water before inoculation because borate buffer injures leaves. Sap that produced an average of 127 lesions per half leaf before heating produced 66 after heating at 50°, 36 after heating at 60° and 3 after heating at 70°. The only change in the appearance of the particles after heating was that some had aggregated end to end.

It will be shown that PMTV particles have two modal lengths. However, the dilution curve of the infectivity is more similar to a theoretical one-hit than two-hit curve. By contrast, using a strain of cowpea mosaic virus, which has a two-component system, we obtained a dilution curve similar to a theoretical two-hit curve.

Appearance and length distribution of virus particles

The virus particles resembled those of TMV and, as is found with most defective strains of TMV, sap contained very few particles, of which many were shorter than 300 nm. and showed defects along the surface (Kassanis & Woods, 1969). The most common and obvious

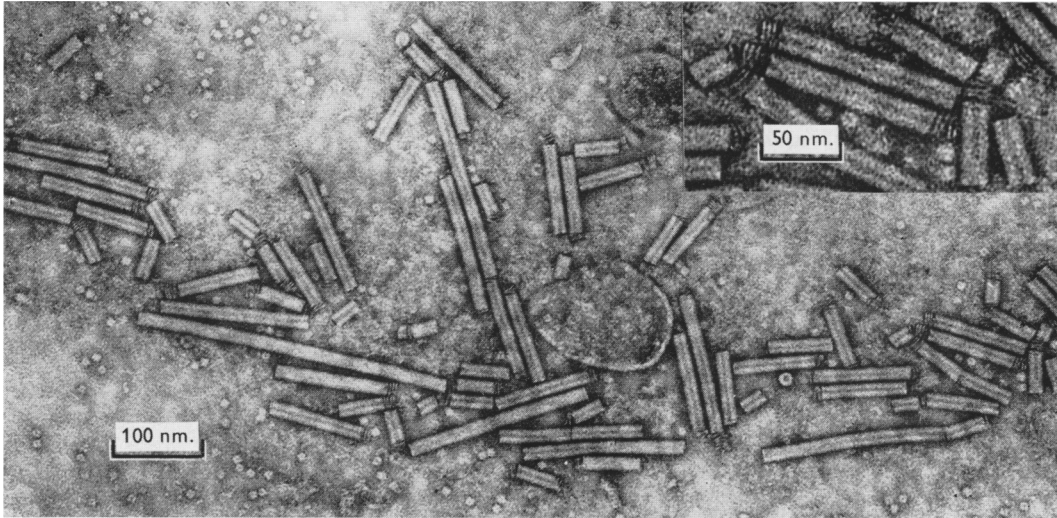


Fig. 2. Partially purified preparation of PMTV; inset shows the virus particle defect.

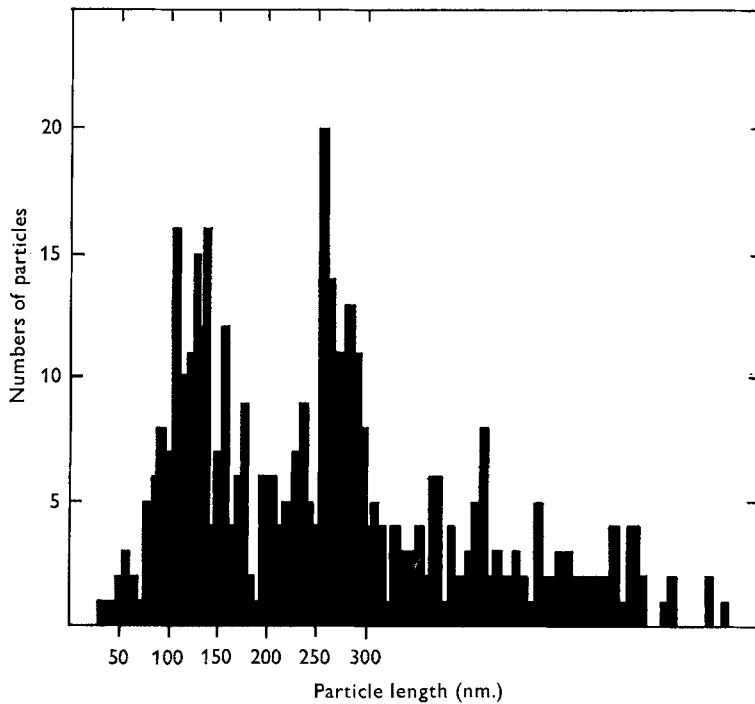


Fig. 3. Length distribution of PMTV particles after minimum treatment (see text).

defect with PMTV was the uncoiling of the protein helix usually at one end of the particles, so that they became shorter during extraction and purification (Fig. 2). The particles were 17 nm. wide, the same as TMV, and when stained in 1% uranyl formate showed cross-banding with a periodicity very near that of TMV. Dr J. T. Finch, of the Molecular Biology Laboratory, Cambridge, measured the periodicity by optical diffraction analysis and found the pitch of the helix to be 2.4 nm. Within the margin of error, this is not distinguishable from the value of 2.3 nm. reported for TMV.

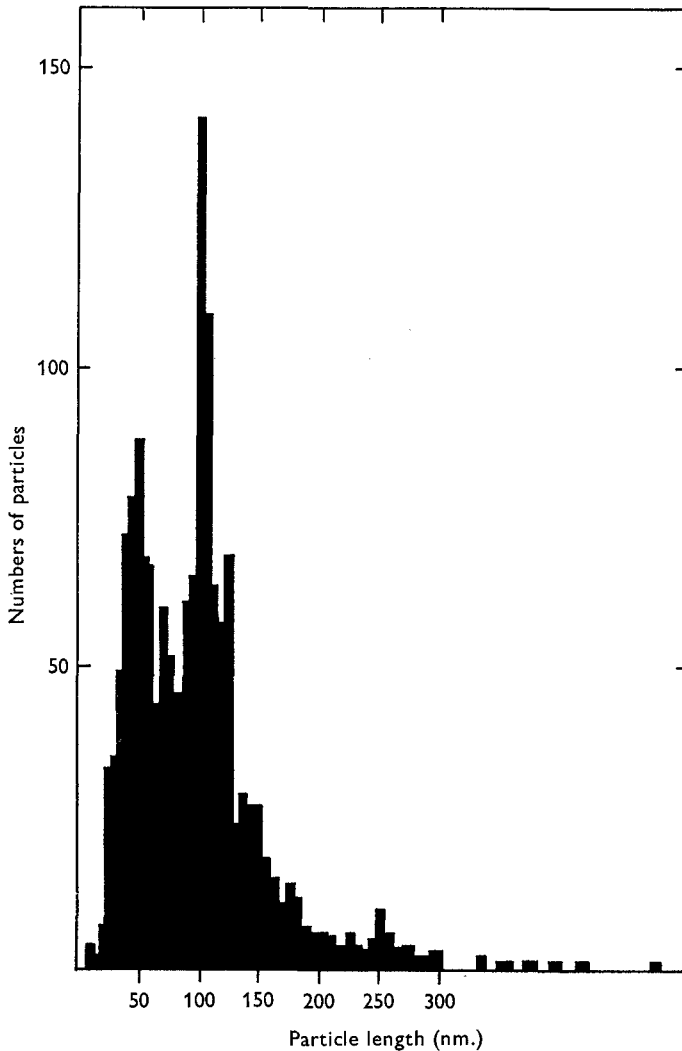


Fig. 4. Length distribution of PMTV in a partially purified preparation (see text).

The length of the particles seemed to be unaffected by centrifuging extracted sap for 20 min. at 9000g and resuspension of the pellet in a small volume of water. A histogram of particle-length distribution of virus treated in this manner showed two peaks, one at 250 to 300 nm. and the other at 100 to 150 nm. (Fig. 3). The type strain of TMV in extracted sap gave one peak at 300 nm., whereas defective strains of TMV gave length distributions similar

to that of PMTV (Kassanis & Woods, 1969; Kassanis & Bastow, 1971). Francki (1966) published a histogram of particle lengths said to be of the type strain of TMV, with three peaks at about 75, 150 and 300 nm., but as so many particles are shorter than 300 nm. it seems possible that this isolate is also a defective strain.

After further purification PMTV particles were shorter, at least after staining with phosphotungstate. A histogram of length of particles in a partially purified preparation of PMTV still has two peaks, but these are at shorter lengths (40 and 80 nm.) than those found with unpurified preparations (Fig. 4).

Partial purification

The method we adopted took advantage of the fact that most of the virus sediments at 9000g. The virus was eluted from the pellet with 0.5 M-borate buffer pH 7.5 using half the original volume of sap during winter and an equal volume during summer. Attempts to concentrate at this stage by more than a factor of two often failed to resuspend the virus. After resuspension the preparations were examined in the electron microscope to ensure that they contained most of the virus. The extract was clarified at 9000g, emulsified with an equal volume of ether by a gentle shaking, and then centrifuged to break the emulsion. The aqueous phase was similarly treated with an equal volume of carbon tetrachloride, separated from the emulsion and then centrifuged for 2 hr at 100,000g. The greenish pellets were suspended in a small volume of water and left for 24 hr at 4° before being clarified at 9000g. These partially purified preparations were slightly coloured but lost colour when emulsified with an equal volume of ethylene dichloride and centrifuged to break up the emulsion. The purification was much less satisfactory during the summer as the final preparations were coloured and had less virus. Heating the preparations at 60° left the preparations unchanged.

Table 1. *Electron microscopy and infectivity of the different fractions obtained from a sucrose-gradient centrifugation*

Fraction no.	Electron microscopy	No. of lesions per half-leaf
1 to 3	No particles were seen	0
4	Short particles, less than 1/field*	0
5	Short particles, 5/field	0
6	Short particles, 1/field	1
7	Long particles, 6-8/field	17
8	Long particles, 2/field	37
5+7		5

* At $\times 40,000$ in a Siemens Elmiskop IA electron microscope.

Partially purified preparations gave three peaks in the analytical ultra-centrifuge with sedimentation coefficients $S_{20,w}$ of 239, 171 and 126 s; the middle value is near that of TMV, which had a sedimentation coefficient of 187 s. Possibly the 239 s component represented dimers, and the 126 s peak corresponded to the short particle mode in the histogram of Fig. 3. There seems to be a discrepancy between the results obtained in the analytical ultracentrifuge and the particle-length distribution of partially purified preparations. The sedimentation coefficients showed that the particles were not as broken as suggested by their appearance in the electron microscope (Fig. 4). Possibly, purification causes defects that render the virus particles unstable in phosphotungstate stain. This explanation is probably correct, because when the virus particles in purified preparations were shadow cast with metal they were as long as in untreated preparations that have been stained.

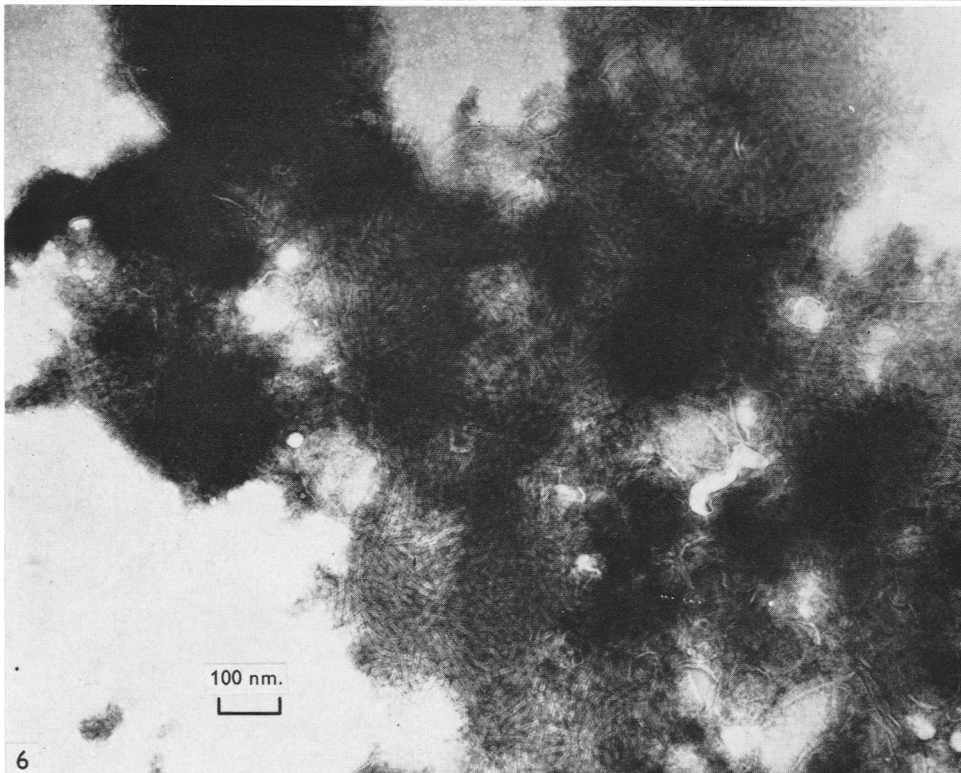
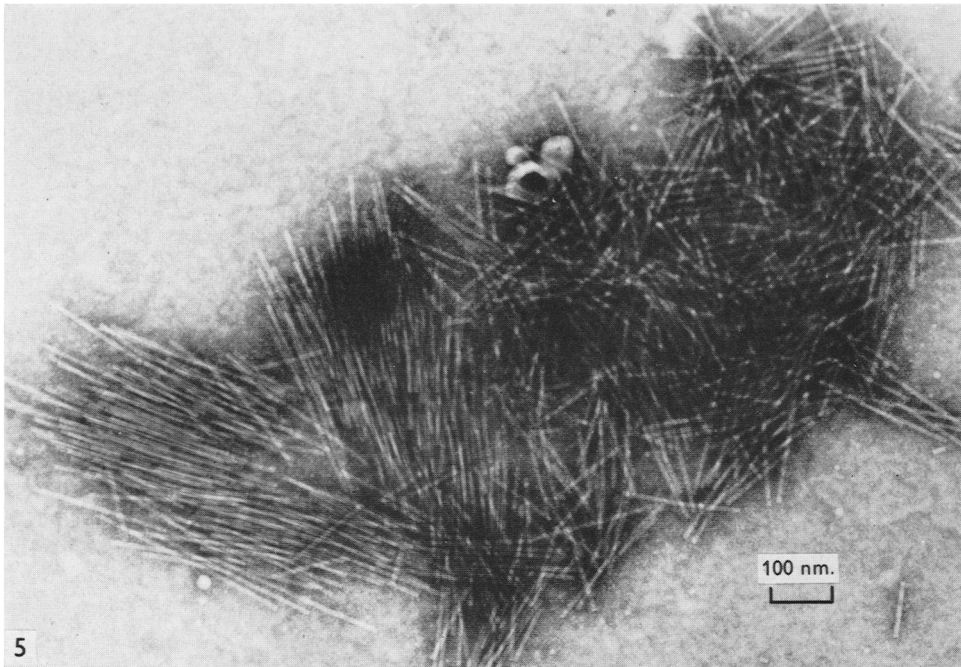


Fig. 5. A clump of TMV formed after incubation with PMTV antiserum.
Fig. 6. A clump of PMTV formed after incubation with TMV antiserum.

Density-gradient centrifugation

The corresponding fractions from a density-gradient centrifugation of three tubes were pooled and ultracentrifuged. The pellets were resuspended in a small volume of water, examined in the electron microscope, and tested for infectivity. The infectivity of the samples increased with increasing length of the virus particles they contained (Table 1). Fraction 8 contained the longest particles and was more infective than fraction 7, although it contained fewer particles. A mixture of fraction 5 (short particles) and fraction 7 (long particles) was less infective than fraction 7 alone. In another test similar to that reported in Table 1, fractions 5, 6, 7 and 8 gave 3, 60, 109 and 148 lesions per half leaf. The short particles seem to be unnecessary for infection, and when a mixture of PMTV and TMV was centrifuged in a sucrose gradient most of the infectivity of each occurred in the same fraction.

Serological cross-reaction

The antiserum to PMTV that first precipitated TMV was obtained two weeks after the intramuscular injection (third and final injection). The homologous titre of this antiserum was 512, and its titre against TMV was 5. Although in later bleedings (4 and 6 weeks after the final injection) the homologous titre increased to 2048, the titre against TMV did not increase. Antisera to PMTV precipitated TMV when diluted in 0.1 M-EDTA pH 7.7 but not when diluted in 0.85 % saline. Tests to seek the effect of EDTA on the precipitation reaction showed that whereas it increased the homologous titre of TMV antisera it decreased the homologous titre of PMTV antisera. Thus EDTA seems to help the precipitation of TMV but not of PMTV. It is unexpected that a chelating agent such as EDTA should help the precipitation of TMV because precipitation is usually aided by heavy metals.

The antiserum to TMV obtained 2 weeks after the second intramuscular injection, although it had the highest homologous titre of 6400, did not precipitate PMTV. The antiserum to TMV that first precipitated PMTV was obtained 4 weeks after the final intramuscular injection, when the homologous titre was 1600. It precipitated PMTV when it was diluted 1/8 in saline or when diluted 1/4 in EDTA. It did not cause precipitation when mixed with similar preparations from healthy plants.

The reacting mixtures of virus and antiserum were centrifuged at 9000g, the pellets suspended in a little water and examined in the electron microscope. Large clumps of virus particles were found in each mixture of antiserum with the heterologous virus (Fig. 5, 6).

Plant-protection tests

As strains of many different viruses interfere with one another in plants, and unrelated viruses usually do not, the ability of TMV to protect plants from infection with PMTV was tested. Opposite half leaves of tobacco cv. White Burley were inoculated with water and with TMV at 2.5 mg./ml., and washed. TMV does not produce local lesions in these plants while PMTV forms necrotic rings. Five days later the whole leaf surfaces were dusted with carborundum and inoculated with PMTV. PMTV produced on average 164 lesions on the half leaves inoculated with water and 99 on the half leaves inoculated with TMV. The difference was greater than average on the bottom leaves, which gave on average 9 lesions for the half inoculated with TMV and 52 with water. In another test, the opposite half leaves were inoculated with PMTV in water and in 1.25 mg./ml. TMV. TMV interfered with the appearance of the PMTV lesions (Fig. 7).

The molecular weight of the coat protein

Dr J. Carpenter compared for us the size of the protein sub-units in PMTV, type TMV and the cowpea strain of TMV. The virus proteins were analysed in 10 % polyacrylamide gels in the presence of sodium dodecyl sulphate using the method of Shapiro, Vinuela & Maizel (1967). The molecular weights were estimated from a calibration curve determined with standards. The molecular weight of the type strain was 17,700 to 19,400, of the cowpea strain 17,000 to 18,500 and of PMTV 18,500 to 20,000. Although the molecular weight of PMTV is about 1000 greater than that of the type strain, the difference does not exclude PMTV being a strain of TMV because about the same difference was found between cowpea and type strain.

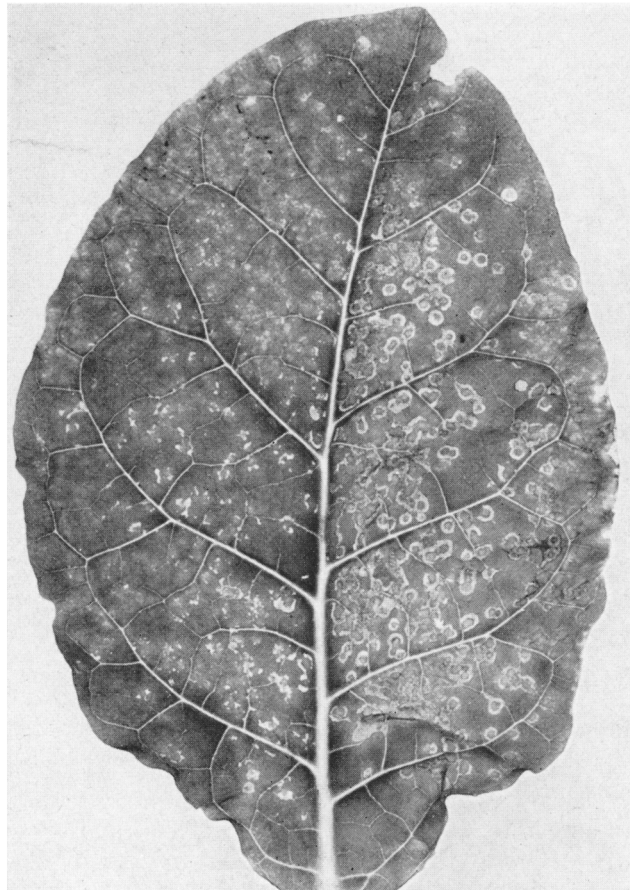


Fig. 7. Cross-protection test between PMTV and TMV: right half of the leaf inoculated with PMTV alone, left half with the mixture of PMTV and TMV.

DISCUSSION

Our results all indicate that it is reasonable to regard PMTV as an aberrant strain of TMV, having virus particles defective in a particular way, i.e. the protein helix uncoils at the end of the particles. The width of the particles and the pitch of the protein helix are indis-

tinguishable from TMV. The two peaks in the particle-length distribution places PMTV with the defective strains of TMV. The reason for the low number of infective particles (300 nm. long) in the peak of the long particles is probably because they uncoil at the extreme end and break. The second peak may arise in the same way. Often, particles have their helix extended or shown fissures in other parts along their length giving rise to a weak place that breaks during extraction. The infectivity tests with fractions obtained from sucrose gradients and the comparison with TMV have shown that the infective length is 300 nm. There is no evidence that the short particles play any part in infection. Also, the dilution curve resembles more a theoretical one-hit than a two-hit curve.

PMTV differs from the other strains of TMV in the type of symptoms it produces in tobacco cv. Xanthi. Most strains of TMV produce solid necrotic local lesions, differing in size between strains, with or without a brown halo. By contrast, PMTV often produces necrotic rings which, when few, spread to form necrotic line patterns. During winter, occasionally the virus spreads to uninoculated leaves, in contrast to TMV.

We are indebted to Dr D. A. Govier for preparing the antisera.

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