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Destruction of TMV and PVX Caused by Sap from the Halo Zones Surrounding Homologous Virus-Induced Local Lesions in Leaves of Host Plants

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When tobacco mosaic virus (TMV) and potato virus X (PVX) preparations were mixed with the sap from the halo zone (HZ) tissues surrounding homologous virus-induced local lesions in *Datura stramonium* and *Gomphrena globosa* leaves, respectively, and the mixtures were incubated for 18 h at 37 °C, the virus particles underwent destruction. Under the electron microscope abnormal (swollen and "thin") virions were observed in the incubated virus preparations negatively stained with phosphotungstic acid. Sometimes the TMV particles were "cut" across into fragments. Treatment of the virus preparations with the sap from the healthy leaves or HZ surrounding heterologous virus-induced local lesions may cause certain destructive changes of virus particles but to a far lesser extent than treatment with the sap from the homologous virus-induced HZ. Possible mechanisms of destruction of the virus particles are discussed.

Keywords: Tobacco mosaic virus, potato virus X, viral local lesions.

There is evidence that host plants have the ability to cause inactivation and destruction of viruses. Such ability can be realized intracellularly (Reunov and Lapshina, 1984; Roggero and Pennazio, 1984; Ismail et al., 1987; Salomon, 1989b; Kolesnik, 1996; Reunov et al., 1996) or when viruses contact with the apoplast (Kassanis and Kenten, 1978; Golinowski et al., 1981, 1986; Kassanis, 1981; Varfolomeeva et al., 1985; Shchetinin, 1989; Malinovsky, 1998). For some viruses, it was shown that homogenization of the infected tissues as well as purification and storage of virus preparations are accompanied with destructive changes in the viral coat protein (Koenig et al., 1970, 1978; Tung and Knight, 1972; Cech et al., 1977; Pozdena and Cech, 1983; Salomon, 1989a).

According to existing data, the above inactivation and destruction of viruses could be caused by the action of plant hydrolases. Our results suggest that a decrease (after reaching a maximum) of both infectivity and amount of virus coat protein in the TMV-induced local lesions in *Datura stramonium* leaves is due to RNase and protease action, respectively (Reunov et al., 1996). This type of enzymes assumed earlier to be the cause of degradation of PVX in the intercellular spaces of potato leaves (Golinowski et al., 1981). Destruction of the virus coat proteins in the process of isolation procedures and storage of virus preparations resulted from the action of plant proteases (Koenig et al., 1970, 1978; Tung and Knight, 1972; Cech et al., 1977; Pozdena and Cech, 1983; Salomon, 1989a).

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Causing virus destruction, the plant hydrolases probably can prevent virus infection. It was recently reported (Malinovsky, 1998) that the development of TMV-induced systemic acquired resistance in *Nicotiana tabacum* cv. Xanthi-nc was accompanied by stimulation of hydrolase-mediated degradation processes of virus particles injected into the host leaves. Therefore, it was of interest to study the ability of the halo zone (HZ), tissues surrounding virus-induced local lesions and exhibiting local acquired resistance (Yarwood, 1960; Ross, 1961), to cause virus destruction. This paper cites data on the action of sap from the HZ surrounding TMV- and PVX-induced local lesions in host plant leaves upon the homologous and heterologous virus particles.

Materials and Methods

Three-week-old plants of *Datura stramonium* L. and *Gomphrena globosa* L. grown in a greenhouse were used in the work. Almost fully expanded *D. stramonium* leaves were dusted with Carborundum and inoculated with sap from healthy or affected with TMV ordinary strain leaves of *Nicotiana tabacum* L. var. Samsun. Similarly, the developed *G. globosa* leaves were inoculated with sap from healthy or affected with PVX severe strain (Reifman and Kolesnikova, 1973) leaves of *D. stramonium* L. Purificated preparations of TMV and PVX were obtained according to Otsuki et al. (1977) and Otsuki et al. (1974).

Seven days after infection, 50 disks containing the HZ surrounding TMV- and PVX-induced local lesions were punched out from *D. stramonium* and *G. globosa* leaves, respectively. Previously, the local lesions were took away. Similar 50 disks were prepared from *D. stramonium* and *G. globosa* leaves rubbed with sap from healthy *N. tabacum* and *D. stramonium* leaves. The disks were homogenized in 5 ml of 0.1 M phosphate buffer, pH 5.7 and the sap samples obtained were clarified by centrifugaton at 5 500 g. In the experiments with sap from the HZ surrounding homologous virus-induced local lesions, 2 ml of each supernatant sample were placed in two-test tubes, one of which was heated up for 10 min in the boiling water bath. Then, 1 ml of TMV or PVX in concentration of 1 mg/ml was mixed with 1 ml of the prepared samples of sap and the mixtures were held for 18 h at 37 °C. In parallel the virus preparations untreated with the sap were similarly incubated.

The virus preparations were placed on formvar-coated grids, desiccated and stained with 2% phosphotungstic acid (PTA), pH 7.0. The samples prepared were investigated under an JEM 7A electron microscope.

Results

Treatment of TMV preparation with the sap from the HZ surrounding TMV-induced local lesions in *D. stramonium* leaves (TMV-HZ) resulted in destruction of the virions. Masses of virus particles were found in examining under the electron microscope of the initial virus preparation incubated for 18 h at 37 ºC and stained with PTA *(Fig. 1),* whereas

only separate virions or small of their groups were revealed after incubation of the virus preparation with the sap from the TMV-HZ. Different destructive changes of TMV particles treated with the TMV-HZ sap were observed *(Figs 2–6).* In many cases the virions had a significantly less diameter than the normal TMV particles *(Figs 2–5).* Often "thin" virions appeared to be connected end-to-end and formed long rods *(Figs 2, 3, 5).* Another conspicuous abnormal sign of the TMV particles was their swelling *(Figs 4–6).* Sometimes it was possible to observe the virions "cut" across into fragments *(Fig. 6).*

When the TMV preparation was treated with the sap from healthy *D. stramonium* leaves (rubbed with the sap from healthy *N. tabacum* leaves) or HZ surrounding PVX-induced local lesions in *G. globosa* leaves (PVX-HZ), certain destructive changes of the virus were also observed but those were lesser noticeable than in the case of treatment with sap from the TMV-HZ.

In the TMV preparation treated with the TMV-HZ sap heated up previously in a boiling water bath, the virions had, as a rule, normal diameter and their quantity did not decrease appreciably *(Fig. 7).*

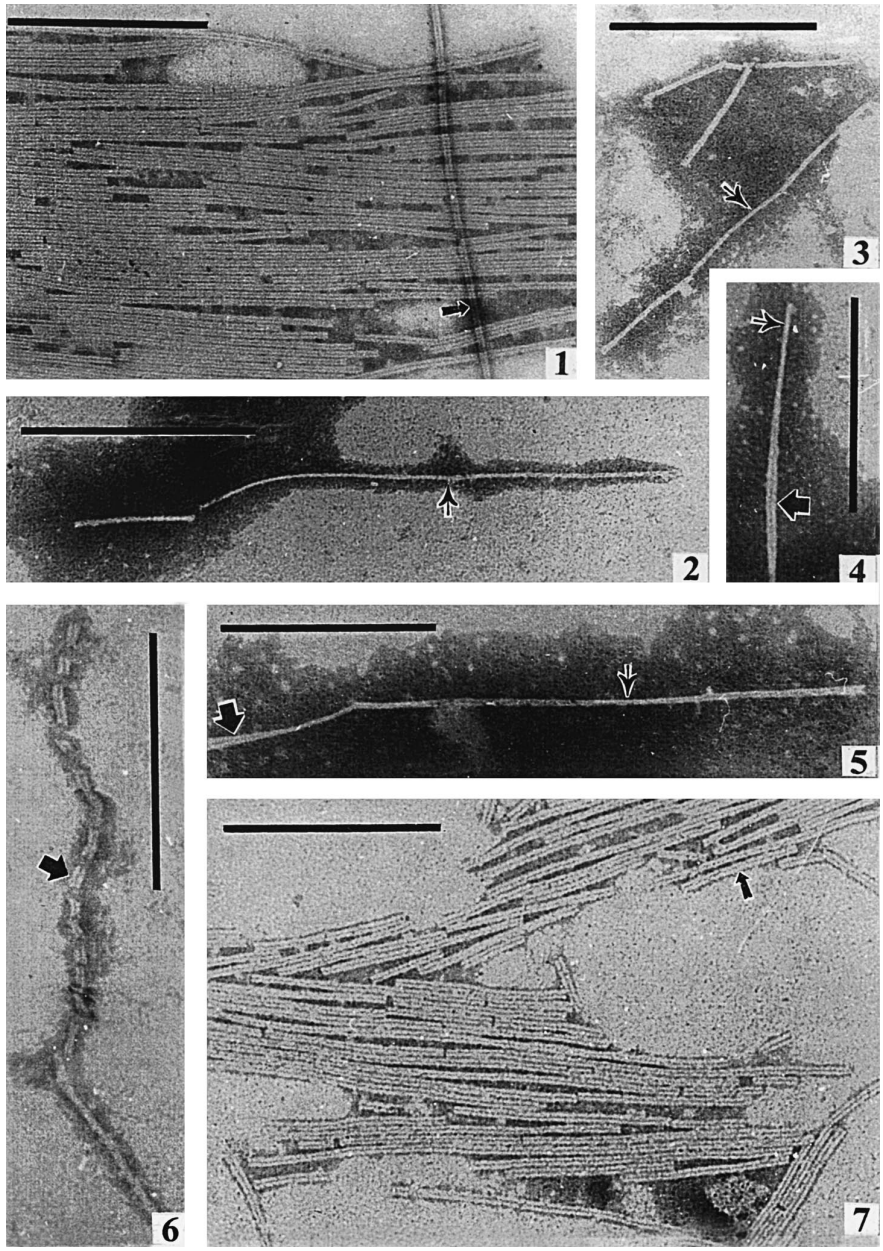
Similar results were obtained in studying influence of the sap treatments on PVX particles. Incubation of the initial PVX preparation *(Fig. 8)* with the sap from the PVX-HZ caused considerable destruction of the virus particles. As a result, a number of virions in the preparation was largely decreased. The remnant virions often appeared swollen or decreased in diameter *(Fig. 9).*

Some decrease in the number of PVX particles as well as their destructive changes were also found in the virus preparation treated with the sap from the TMV-HZ or healthy *G. globosa* leaves (rubbed with the sap from healthy *D. stramonium* leaves). However, such changes were expressed on a much lower extent than in the case of treatment of the virus preparation with the sap from the PVX-HZ.

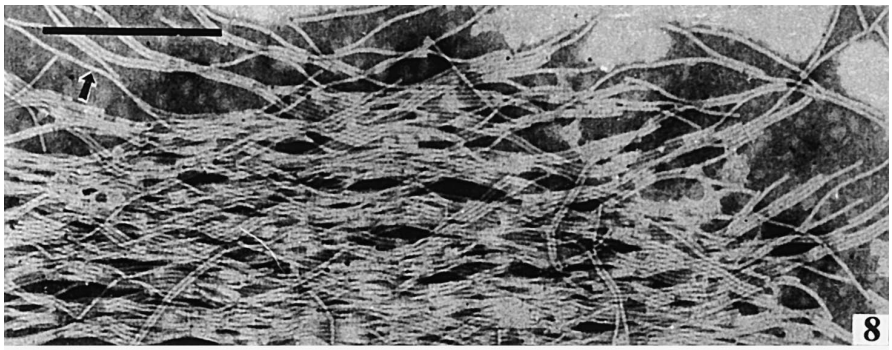
The number and morphology of PVX particles treated with the PVX-HZ sap previously heated up in a boiling water bath did not change substantially *(Fig. 10).*

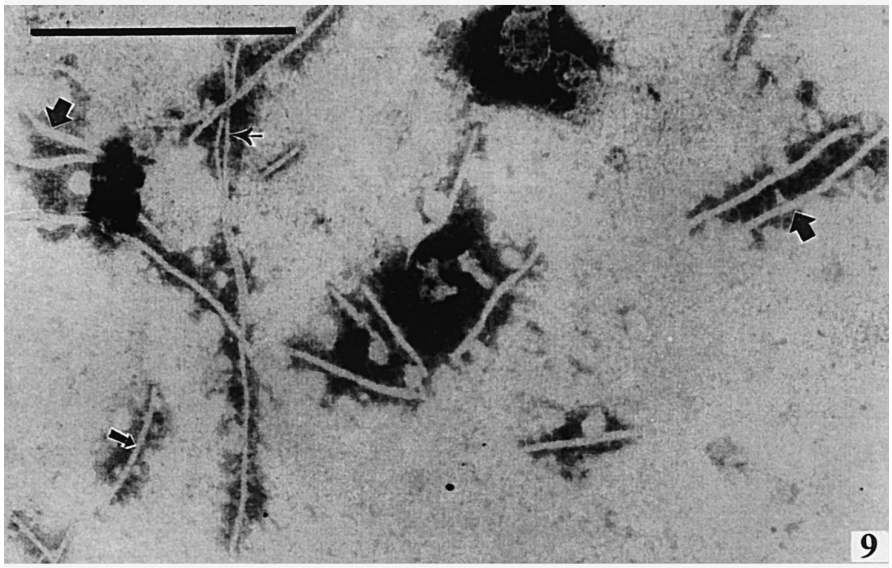
Discussion

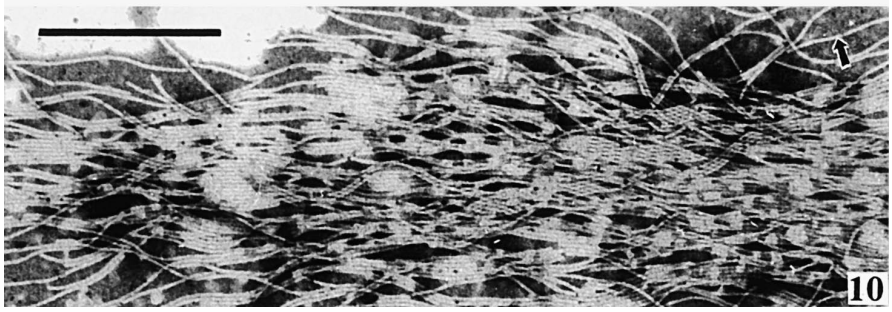
We demonstrate here that TMV and PVX particles undergo destruction when the virus preparations are mixed with the sap from the TMV-HZ and PVX-HZ, respectively, and incubated at 37 ºC. In our view, this may occur as follows: Changed environmental conditions (for example pH) cause the virion structure to become alterated in such a way as to make the partial loosening and untwineing of coat protein. As a consequence, the virus particles swell that is seen in the PTA-stained preparations. The observed "cutting" of TMV virions into fragments seems to be connected to breakdown in the capsid stability and availability of the viral RNA for RNase attack. The untwined parts of the coat protein can be subjected to proteolysis. An appearance of thin virions may be associated with this fact. Everitt et al. (1988) explained the destruction of proteins of adenovirus 2, similarly. It was shown that adenovirus 2 hexons changed their conformation at low pH in such a way that hexon parts sensitive to protease action exposed outside and underwent proteolysis (Everitt et al., 1988).



Figs 1–7. Particles of TMV preparation untreated *(Fig. 1)* and treated by sap from the HZ surrounding TMV-induced local lesions in *D. stramonium* leaves *(Figs 2–7).* The particles are incubated for 18 h at 37 ºC and stained with PTA. *Fig. 7.* The preparation treated by the TMV-HZ sap previously heated up in a boiling water bath. The middle, thick and thin arrows indicate the normal, swollen and thin virions, respectively. Bar = 500 nm







Figs 8–10. Preparation of PVX untreated *(Fig. 8)* and treated with the sap from the HZ surrounding PVX-induced local lesions in *G. globosa* leaves *(Figs 9 and 10).* The virus particles were incubated for 18 h at 37 ºC and stained with PTA. *Fig. 10.* The preparation treated with the PVX-HZ sap previously heated up in a boiling water bath. The middle, thick and thin arrows indicate the normal, swollen and thin virions, respectively. Bar = 500 nm

The results of this study are in accordance with the data of other authors showing degradation of the coat protein of some plant viruses during isolation procedures and storage of virus preparations (Koenig et al., 1970, 1978; Tung and Knight, 1972; Cech et al., 1977; Pozdena and Cech, 1983; Salomon, 1989a). In our experiments, the virus particles did not show visible destruction when mixed with the HZ sap previously heated up in a boiling water bath. This may be due to inactivation of the hydrolases.

It should be noted that the sap from healthy leaves or HZ induced by a heterologous virus affected destructively the virus particles but to a lesser extent than the sap from the HZ induced by a homologous virus. This shows certain specificity in the action of hydrolases present in the homologous virus-induced HZ. However, the mechanism of such specificity remains to be studied.

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