Non-viral Lesions Formed in Non-inoculated Upper Leaves of Local Lesion Hosts Following Inoculation of the Lower Leaves with Tobacco Mosaic Virus

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**SUMMARY**

When the lower leaves of *Nicotiana glutinosa* or Samsun NN tobacco, local lesion hosts for tobacco mosaic virus (TMV), were inoculated with TMV and kept at 20 °C in continuous light, non-viral lesions began to appear on non-inoculated upper leaves about 8 days later. It is postulated that these non-viral lesions might be induced by substances that move through stem tissues from the inoculated leaves bearing local lesions.

When *Nicotiana glutinosa* or *Nicotiana tabacum* cv. Xanthi-nc were inoculated with tobacco mosaic virus (TMV) on their lower leaves and kept at high temperature (30 °C), TMV multiplied and moved from inoculated leaves to other parts of the plants and the plants became systemically infected, developing mosaic symptoms on the upper leaves (Shimomura, 1972). When the plants were kept at low temperature (22 °C) after inoculation, however, local lesions were formed in inoculated leaves on the second day after inoculation and TMV did not move from inoculated leaves to upper leaves (Weintraub, Kemp & Ragetli, 1963; Shimomura, 1972). This paper will describe non-viral lesions formed on non-inoculated upper leaves of *N. glutinosa* or tobacco plants kept at 20 °C for 8 days after inoculation.

*Nicotiana glutinosa* and *N. tabacum* cv. Samsun NN, both local lesion hosts for TMV, and *N. tabacum* cv. Samsun, a systemic host for TMV, were used. Plants were raised in a greenhouse, but after inoculation they were kept in a growth chamber maintained at 20 °C and illuminated continuously by fluorescent lamps (about 3000 lux). Inocula, containing a Japanese isolate of the common strain of TMV (Nozu & Okada, 1968), were applied with a cotton pad to leaf surfaces previously dusted with carborundum, and then immediately rinsed with tap water.

Each of six lower leaves of *Nicotiana glutinosa* or Samsun NN tobacco plants (2.5 to 3 months old) were inoculated with TMV (10 to 50 µg/ml for *N. glutinosa*, 5 to 10 µg/ml for Samsun NN tobacco) and the plants were placed in a growth chamber. About 8 days after inoculation, necrotic lesions began to appear on non-inoculated upper leaves (accompanied by leaf twist), as shown in Fig. 1a and b. Usually 2 to 3 lesions first appeared at the edge of the 3rd or 4th leaf above the topmost inoculated leaf. Later they developed all over the leaf, so that there were sometimes 400 to 500 lesions per leaf at 2 weeks after inoculation, and they also developed on leaves younger or older than the 3rd or 4th leaf. Such lesions did not appear on the upper leaves of control plants after their lower leaves were rubbed with distilled water. The lesions appearing on the upper non-inoculated leaves enlarged less and did not become as brown as local lesions formed in inoculated leaves. The non-inoculated leaves bearing these lesions were ground with 0.05 M-phosphate buffer, pH 7.0, and infectivity was assayed by inoculating carborundum-dusted leaves of Samsun NN tobacco plants. No TMV was detected, although it was detected in abundance in the
Fig. 1. Non-viral lesions formed in leaves of *Nicotiana glutinosa*, Samsun NN or Samsun tobacco, photographed about 2 weeks after inoculation with TMV. (a) Local lesions formed in TMV-inoculated lower leaf of *N. glutinosa* (left). Non-viral lesions formed in non-inoculated upper leaves of *N. glutinosa* (middle and right). (b) Local lesions formed in TMV-inoculated lower leaf of Samsun NN tobacco (left). Non-viral lesions formed in non-inoculated upper leaves of Samsun NN tobacco (right). (c) Non-viral systemic necrosis of non-inoculated upper leaves (lateral shoot) of Samsun NN tobacco. (d) Small non-viral lesions formed in the green area between the large viral local lesions that developed in a TMV-inoculated leaf of Samsun NN tobacco. (e) and (f) Non-viral lesions formed in a leaf of Samsun NN tobacco (scion) grafted on Samsun NN tobacco (stock), which was inoculated with TMV. In (f) a few non-viral lesions are seen in addition to necrosis of veins. (g) Non-viral lesions formed in leaf of Samsun tobacco (scion) grafted on Samsun NN tobacco (stock), which was inoculated with TMV.
Fig. 2. Systemic resistance induced in upper leaf of Samsun NN tobacco. (a) Inoculated with TMV on lower leaves and challenge inoculated 11 days later with TMV on upper leaf. (b) Rubbed with distilled water on lower leaves and challenge inoculated with TMV on upper leaves (control plant). Photographs were taken 4 days after challenge inoculation.

Inoculated lower leaves. In further trials, detached leaves bearing these lesions were placed on wet filter paper in Petri dishes, kept at 30 °C for 3 days in continuous light, and then tested for infectivity. If there is a very small amount of virus in the lesions, this will increase sufficiently during this period at high temperature to be detectable. The results indicated no TMV was detectable in these leaves. Thus, the lesions which appeared in the non-inoculated upper leaves were apparently not caused by infection of these leaves with TMV from the inoculated lower leaves. They are therefore called non-viral lesions. In Samsun NN tobacco, lesions resembling non-viral lesions also formed in the green area between virus local lesions in inoculated leaves after 10 to 14 days (Fig. 1d). No TMV could be detected, by infectivity assay, in the lesions formed in the green area between virus local lesions. When decapitated plants of *N. glutinosa* or Samsun NN tobacco were used, non-viral systemic necrosis appeared in some lateral shoots 10 days after inoculation of the lower leaves (Fig. 1c). Non-viral lesions began to appear in upper leaves about 8 days after inoculation and increased in number with time as described above. However, the increase in number stopped when the inoculated leaves grew older and wilted or were detached from the plant. These findings suggest that non-viral lesions in non-inoculated leaves may be induced by some substances that move through stem tissues from the inoculated leaves bearing local lesions. Treatments of lower leaves of *N. glutinosa* or Samsun NN tobacco with tenuazonic acid (Mikami et al. 1971), a toxin produced by *Alternaria longipes*, induced necrotic lesions in the treated leaves but not in the upper leaves. Nor were any lesions induced in untreated upper leaves by chemical injury to lower carborundum-dusted leaves rubbed with trisodium phosphate, perchloric acid or mercuric chloride solutions. These results indicate that necrosis caused by agents other than TMV failed to induce lesions in the upper non-treated leaves. Further experiments to determine whether
non-viral lesions would form following the development of necrosis caused by viruses other than TMV are being made.

Experiments were next made to ascertain whether non-viral lesions would appear on leaves of scions of systemic or local lesion hosts grafted on a local lesion host rootstock 1 week before the leaves of the stock were inoculated with TMV. When *Nicotiana glutinosa* or Samsun NN tobacco scions were grafted on Samsun NN tobacco rootstocks, non-viral lesions appeared on leaves of the scions 8 to 10 days after inoculation (Fig. 1e). Sometimes the veins also became necrotic (Fig. 1f). Similarly, when scions of the systemic host, Samsun tobacco were grafted on Samsun NN tobacco rootstocks, non-viral lesions appeared on leaves of the scion about 10 days after inoculation (Fig. 1g). Non-viral lesions did not appear in the upper leaves of scions grafted to rootstocks that were rubbed with distilled water.

Ross (1961, 1966) reported that when lower leaves of Samsun NN tobacco were inoculated with TMV, systemic resistance was induced in the upper leaves. To ascertain whether systemic resistance had been induced in upper leaves of Samsun NN tobacco kept at 20 °C in continuous light and bearing a few non-viral lesions, the following experiments were made. Each of six lower leaves of non-decapitated plants were inoculated with TMV (5 μg/ml) and the corresponding leaves of control plants were rubbed with distilled water. Eleven days later, the upper leaves of the TMV-inoculated plants had developed a few non-viral lesions. Four well expanded upper leaves of plants in each lot were then challenge inoculated with TMV (50 μg/ml). As shown in Fig. 2a and b, TMV infection of the lower leaves induced much resistance in the upper leaves. Four days after challenge inoculation, the diam. of lesions produced in the previously TMV-inoculated plants was much smaller (0.1 to 0.4 mm) than of those (0.5 to 0.8 mm) in control plants, although no reduction in lesion numbers was observed (TMV-inoculated plants, 45/50 mm²; control plants 16/50 mm²).

It seems that virus-induced non-viral lesions have never been observed under ordinary greenhouse conditions (18 to 30 °C) because the inoculated leaves bearing many local lesions became senescent and wilted within 10 days after inoculation in these conditions. The character of the substances that give rise to the formation of non-viral lesions in upper leaves of TMV-inoculated plants, and the relation between those substances and the systemic acquired resistance reported by Ross (1961, 1966), remain to be resolved by future investigations.

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*I nstitute for Plant Virus Research*  
959 Aobacho, Chiba, Japan  

T. SHIMOMURA  
YUKO OHASHI
Short communications

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