Radioautographic Studies on the Photosynthetic CO_2 Fixation in Virus-Infected Leaves

N. Doke and T. Hirai

Plant Pathology Laboratory, Faculty of Agriculture, Nagoya University, Nagoya, Japan 46400.
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ABSTRACT

Radioautograms of virus-inoculated leaves of systemically infected hosts which were exposed to $^{14}CO_2$ in light showed lesions having localized and enhanced radioactivity. Tobacco mosaic virus (TMV), tobacco etch virus (TEV), potato virus X (PVX), and cucumber mosaic virus-Y (CMV-Y) strain were studied. The number of TMV lesions increased in proportion to the concentrations of the virus inoculated. Firstly, lesions were small and compact, then each lesion enlarged into a zone with high radioactivity on the periphery and low radioactivity in the center. As lesions enlarged, the radioactivity at the periphery disappeared. Noninoculated but systemically infected leaves produced almost the same radioactive lesions as inoculated leaves, but a low radioactive center was not detected and the radioactivity at the periphery did not disappear but remained apparent. Leaves with visible mosaic symptoms of TMV showed high radioactivity localized in the yellow-green area. Radioautograms of leaves inoculated with TEV, PVX, and CMV-Y showed almost the same radioactive lesions as those inoculated with TMV.

Leaves of local-lesion infection hosts produced radioactive lesions before the appearance of visible local lesions, but produced no additional radioactive lesions after visible lesions developed. Chasing of locally infected leaves which had been exposed in $^{14}CO_2$ caused an accumulation of radioactive substances around the visible lesions, but chasing of systemically infected leaves caused no accumulation. Phytopathology 60:988-991.

Effects of virus infection on the photosynthesis of green leaves have been investigated (2, 3, 9). In most of the virus-host combinations there were no differences in the rate of photosynthesis between infected and noninfected leaves until symptoms appeared. We found that the photosynthetic $^{14}CO_2$ fixation in tobacco leaves was not affected by tobacco mosaic virus (TMV) infection, except that a slight activation occurred in young leaves 1 day after inoculation (*unpublished data*). This report concerns the radioautography of $^{14}CO_2$ fixed leaves which are infected systemically or locally with viruses.

MATERIALS AND METHODS.—*Plants.*—Detached leaves of *Nicotiana tabacum* L. 'Bright Yellow' and 'Samsun', *N. glutinosa* L., *Datura stramonium* L., and *Phaseolus vulgaris* L. grown in a greenhouse were used.

Virus and inoculation.—The ordinary strain (TMV-O) and the bean strain (TMV-B) of TMV, tobacco etch virus (TEV), potato virus X (PVX), and the Y strain of cucumber mosaic virus (CMV-Y) were used. The upper surfaces of the detached leaves were immediately inoculated with TMV-O and TMV-B purified according to the routine method consisting of ammonium sulfate and isoelectric point precipitations, or with extracts with phosphate buffer (pH 7.0) from tobacco leaves systemically infected with TEV, PVX, and CMV-Y using Carborundum as an abrasive. Inoculated leaves were incubated on wet filter papers in a petri dish at 26 C under continuous illumination (ca. 7,000 lux) from daylight fluorescent lamps for 1, 2, 3, 4, and 5 days after inoculation until they were exposed to $^{14}CO_2$.

Photosynthetic $^{14}CO_2$ fixation.—The leaves were transferred at definite intervals 1, 2, 3, 4, and 5 days after inoculation onto wet filter papers in a hermetically sealed, transparent plastic chamber in which the air was exchanged with air containing 0.05% $^{14}CO_2$ (0.05 $\mu C/cm^3$ air). The leaves were exposed in $^{14}CO_2$ for 30 min and then incubated, when chased, in flowing air for 23 hr after the evacuation of $^{14}CO_2$, under light condition mentioned above.

Radioautography.—The leaves exposed in $^{14}CO_2$ were held between several sheets of filter paper and dried at 60 C with a pressure of about 0.55 g/cm^2. The dried leaves were put on X-ray film (Fuji X-ray film No. 100, nonscreen type, made in Japan by Fuji Photo Film Co., Ltd.). A sheet of cellophane was inserted between leaf and film. They were incubated at room temp for 3-4 days.

RESULTS.—Leaves of Bright Yellow tobacco infected

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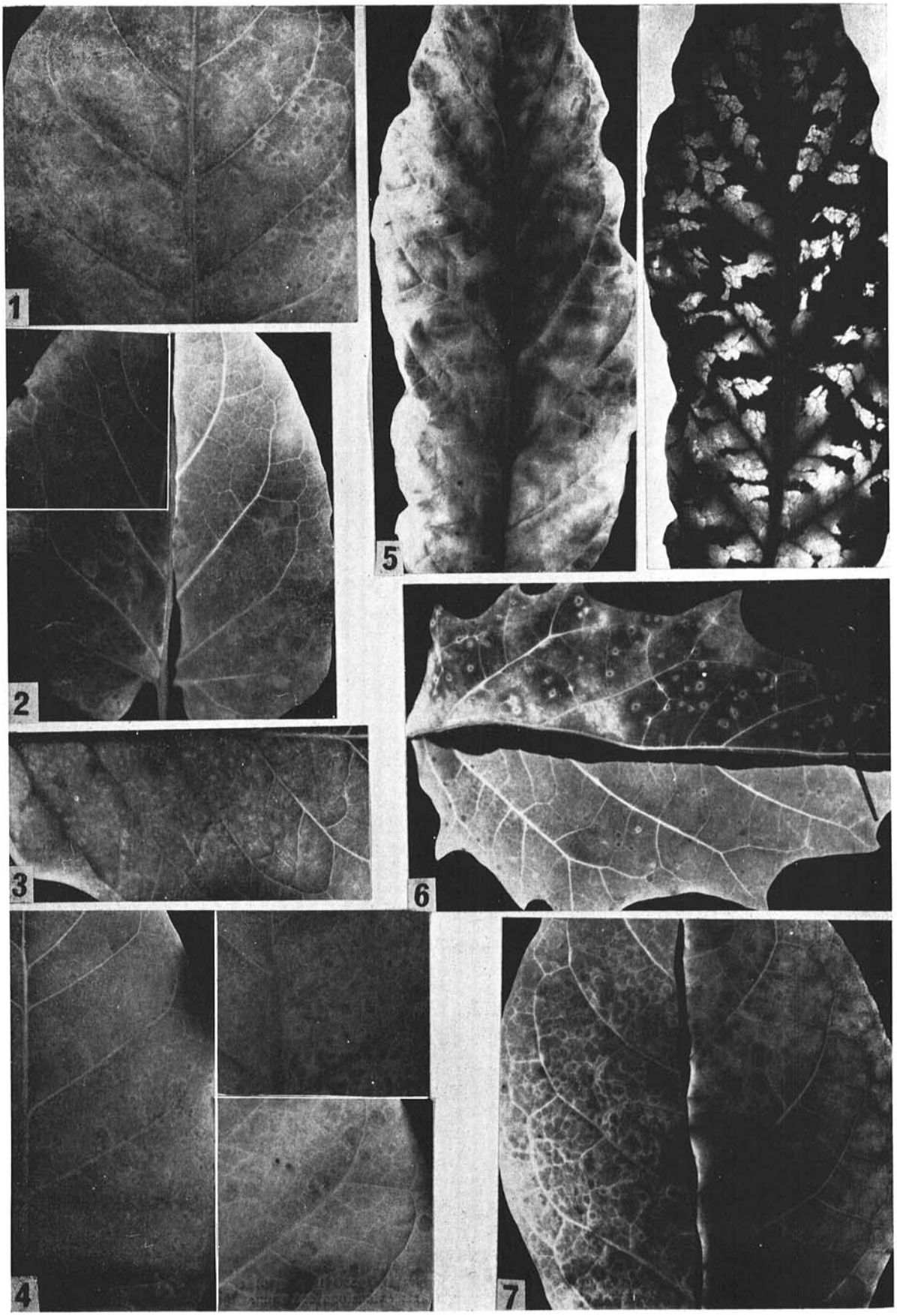
Fig. 1-7. **1)** Radioautogram of *Nicotiana tabacum* 'Bright Yellow' leaves infected with ordinary strain of tobacco mosaic virus (TMV-O) for 2 days and exposed to $^{14}CO_2$ for 30 min. Left half: inoculated with 5 $\mu g/ml$; right half: 50 $\mu g/ml$. **2)** Radioautogram of *N. glutinosa* leaves infected with TMV-O for 2 days and exposed to $^{14}CO_2$ for 30 min (right half) and chased for 23 hr (lower left half). Upper left half: 3 days after inoculation. **3)** Radioautogram of systemically infected leaves of *N. tabacum* 'Bright Yellow' leaves inoculated with TMV-O and exposed to $^{14}CO_2$ for 30 min. Only the lower leaves were inoculated with TMV-O. **4)** Pattern of development of radioactive lesions on *N. tabacum* 'Bright Yellow' leaves inoculated with TMV-O and exposed to $^{14}CO_2$. Left half: 2 days after inoculation; upper right half: 3 days after inoculation; lower right half: 5 days after inoculation. **5)** Radioautogram (left) and photograph taken by transmitted light of *N. tabacum* 'Bright Yellow' leaves showing typical mosaic symptoms. **6)** Radioautogram of *Datura stramonium* leaves infected with TMV-O for 2 days and exposed to $^{14}CO_2$ for 30 min. Lower half: chased for 23 hr. **7)** Radioautogram of *N. tabacum* 'Bright Yellow' leaves infected with TMV-O for 3 days and exposed to $^{14}CO_2$ for 30 min. Right half: chased for 23 hr.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with TMV-O for 2 days were exposed in $^{14}CO_2$. Figure 1 indicates that radioactive lesions appeared on the inoculated leaves which showed no visible symptoms, but did not on the leaves rubbed with distilled water and Carborundum without virus. The number of lesions increased as the concentration of virus inoculum increased (Fig. 8). Firstly, small compact lesions appeared 1 day after inoculation, then each lesion enlarged and showed the presence of a ring 2 days after inoculation. This ring consisted of a periphery having high radioactivity and a center that showed low radioactivity. These lesions enlarged with the infection stages and, about 5 days after inoculation, became diffused and the peripheral radioactivity disappeared (Fig. 4). The diam of the ring showing high radioactivity increased linearly until 4 days after inoculation, and reached a maximum about 5 days after inoculation. The diam of the center showing low radioactivity also increased until 5 days after inoculation. These lesions developed at the rate of about 1 mm/day on the average. Enlargement of the lesion area followed a typical sigmoid curve approximately coinciding with the curve plotting total lesion areas against time (Fig. 9).

Inoculated leaves of Bright Yellow tobacco and *Datura* infected with TEV produced lesions more compact and more concentrated than those caused by TMV. Bright Yellow leaves inoculated with PVX produced lesions 3-4 days after inoculation. On inoculated Samsun leaves, CMV-Y lesions developed much faster than those caused by other viruses. *Phaseolus* leaves infected with TMV-B produced lesions on young secondary leaves, but not on the primary leaves.

Radioautography of noninoculated but systemically infected leaves whose lower leaves were inoculated.—Lower leaves of Bright Yellow tobacco inoculated with TMV-O and the upper leaves were detached at intervals after inoculation, then were exposed in $^{14}CO_2$. Radioautograms of the symptomless upper leaves revealed that, although radioactive lesions appeared 4-5 days after inoculation of lower leaves, radioactivity in the lesion center did not disappear as it did on the inoculated leaves (Fig. 3). Leaves showing a typical mosaic symptom produced a high rate of radioactivity localized in the yellow-green areas rather than in the dark-green areas (Fig. 5).

Radioautography of locally infected leaves.—*Nicotiana glutinosa* and *Datura* leaves were inoculated with TMV-O. Necrotic local lesions appeared on leaves of both hosts about 2 days after inoculation. Before lesion appearance, 24 and 36 hr after inoculation, the leaves were exposed to $^{14}CO_2$. The same pattern of radioactive lesions like that on the inoculated leaves of systemically infected hosts was observed on locally infected leaves (Fig. 2, right half). When the leaves having visible local lesions were exposed to $^{14}CO_2$ 3 days after inoculation, however, no radioactive lesions appeared on the leaves (Fig. 2, upper left).

Effect of chasing on the pattern of radioactive lesions.—Since radioactive lesions might be caused by the translocation of fixed $^{14}CO_2$, we investigated whether or not $^{14}CO_2$ fixed in the leaves translocates during virus infection. Leaves of Bright Yellow tobacco were inoculated with TMV-O and, 2 days later, the half-leaves were exposed to $^{14}CO_2$, then chased for

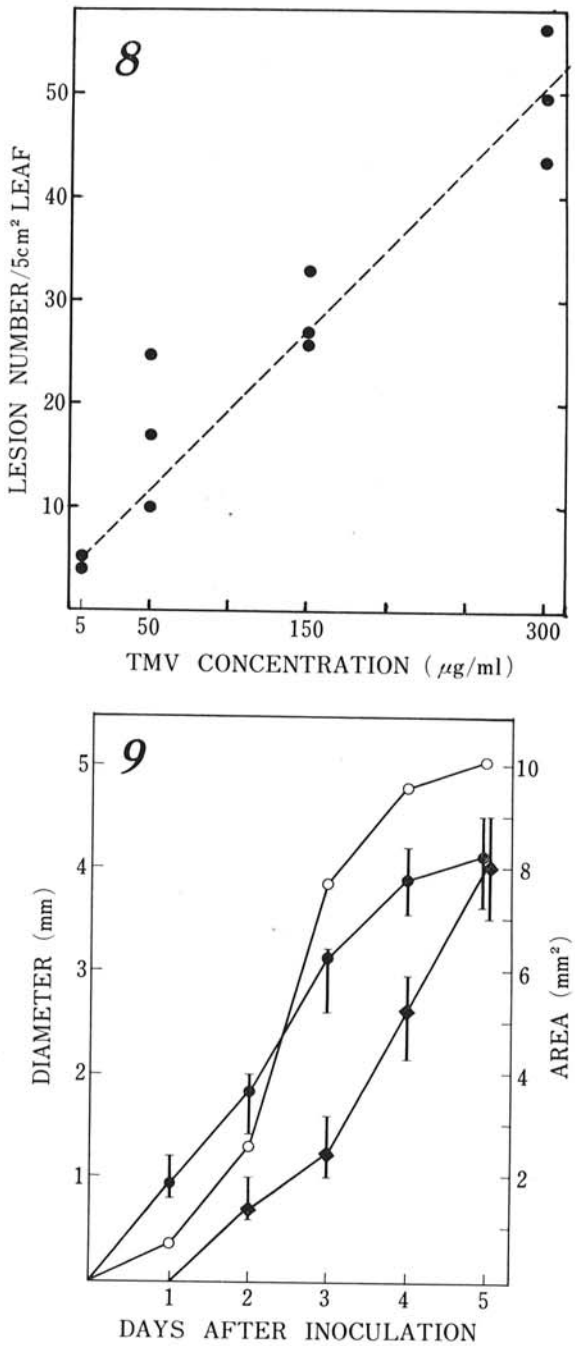

Fig. 8-9. 3) Relation between number of radioactive lesions in *Nicotiana tabacum* 'Bright Yellow' exposed to $^{14}CO_2$ for 30 min and concentration of tobacco mosaic virus, ordinary strain in inoculum. **9)** Development of radioactive lesions by ordinary strain of tobacco mosaic virus infection on *Nicotiana tabacum* 'Bright Yellow' leaves. Solid circles: diam of the periphery showing high radioactivity; solid squares: diam of the center showing low radioactivity; open circles: total lesion areas. The Y axis extensions are ranges.

23 hr in nonradioactive air. Other half-leaves were exposed to $^{14}CO_2$ and were not chased but were quickly sampled. Figure 7 shows that the rate of radioactivity in the lesion periphery was considerably diminished by chasing. In the local-lesion hosts, *N. glutinosa* and *Datura,* however, radioactive substances accumulated around the local lesions even after chasing (Fig. 2, 6). Leaves were exposed to $^{14}CO_2$ for 30 min before virus inoculation with TMV-O. Radioactive lesions appeared on the leaves of local-lesion hosts, *N. glutinosa* and *Datura,* and the radioactivity accumulated around the visible lesions. The systemically infected host, Bright Yellow tobacco, however, produced no radioactive lesions.

DISCUSSION.—Localized high radioactivity was found in inoculated leaves of systemically infected hosts that showed no visible symptoms at an early stage of virus infection. Owen (2, 3) stated that photosynthesis of tobacco leaves infected with TMV increased over the control 30 min after inoculation. However, Zaitlin & Hesketh (9) could not confirm this by estimating the over-all photosynthetic activity of inoculated leaves. We reported that the rates of photosynthesis of tobacco leaves inoculated with TMV and noninoculated leaves were almost identical before symptoms appeared (1). In the current report, however, localization of high radioactivity in the lesions and low radioactivity in the inter-lesion areas of the inoculated leaves was demonstrated by using the radioautographic technique. Thus, although the over-all rate of photosynthesis was similar between inoculated and noninoculated leaves, a given inoculated leaf had localized regions of high and low photosynthetic activity.

Leaves showing mosaic symptoms of TMV infection had a high radioactivity level which was localized in the yellow-green areas. This may indicate that yellow-green areas showed high photosynthetic activity per given unit area. We have recently confirmed that the yellow-green area produced higher radioactivity per unit of chlorophyll and higher virus titer than the dark-green area (Doke & Hirai, *unpublished data*). In virus-infected leaves, virus particles are unable to fix $^{14}CO_2$, except that $^{14}CO_2$ fixed by host leaves incorporates into the virus components (1, 7). Only a small per cent of total radioactivity fixed in the inoculated leaves when the leaves were exposed to $^{14}CO_2$ for 30 min was incorporated into TMV and the soluble antigen (Doke & Hirai, *unpublished data*). Therefore, this high level of radioactivity in the yellow-green areas or in the inoculated leaves was not attributable to the presence of viruses. It was reported that the inoculated leaves of systemically infected host showed a high respiratory rate at the early infection stage (5). Virus infection sites seem to cause a metabolic activation that is localized within the leaf in systemically infected hosts. We conclude that leaf areas of high radioactivity contain cells more actively producing virus, and fix more CO_2 than leaf areas of low radioactivity.

By chasing of the inoculated leaves of local-lesion hosts which had been exposed to $^{14}CO_2$, radioactive substances accumulated around the visible local lesions. This result is almost consistent with those previously reported (4, 6, 8). This phenomenon was not found in systemically infected leaves and seems to be caused by the translocation of the radioactive substances from other leaf areas to the periphery of local lesions.

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