

The State and Infectivity of Tobacco Mosaic Virus in Flue-Cured Tobacco Tissue

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ABSTRACT

Tobacco mosaic virus (TMV) from flue-cured tobacco leaf tissue was 7-19% as infectious as TMV from fresh tissue. The ultraviolet absorption spectra of preparations from fresh and flue-cured tissues were similar, and absorption at 265 nm was an accurate measure of the amount of TMV present. Although purified TMV from flue-cured or fresh tobacco reacted similarly in serological tests, the lowest concentration of TMV that exhibited birefringence was about 4 times higher for TMV from flue-cured tobacco than for TMV from fresh tissue. *Phytopathology* 61:1032-1033.

Additional key words: heat inactivation, serology.

Tobacco mosaic virus (TMV) is the most prevalent viral pathogen of flue-cured tobacco in North Carolina (3), and may contribute as much as 2.3% of the dry wt of such tobaccos (2). Yet the effect of the flue-curing process on the virus is unknown. In this process, temperature is gradually increased from ambient to about 77 C over approximately a 120-hr period. During the final drying period, temperature is held at 77 C for about 30 hr (1). The purpose of this study was to compare concentration, serological activity, infectivity, and birefringence of TMV from fresh and from flue-cured tobacco leaf tissue.

All tissue was from *Nicotiana tabacum* L. 'Hicks', and consisted of leaf lamina. Samples of flue-cured tobacco were from the 1968 and 1969 crops, and were flue-cured in the conventional manner (1); samples of fresh tissue were from infected plants grown in the greenhouse. The detached leaf test and *N. glutinosa* L. were used for infectivity studies. TMV was purified by means of polyethylene glycol (4) followed by two differential centrifugations. Concentration of purified TMV was estimated by ultraviolet absorption at 265 nm (6). Dry wt and biuret determinations indicated that ultraviolet absorption, as previously reported for fresh tissue by Takahashi (6), was an accurate measure of viral material isolated from flue-cured tobacco. Ultraviolet absorption spectra of preparations from fresh and from flue-cured tobacco were similar from

240-300 nm, with similar extinction coefficients at 265 nm. All experiments were repeated twice with similar results.

Since conformational changes in the TMV protein may have occurred during flue-curing, serological activity was determined. Virus preparations purified from flue-cured tobacco harvested both years were assayed with an antiserum prepared against virus purified from fresh tissue. Both the agar-gel double-diffusion technique and the micro-precipitin test indicated that viruses from fresh and from flue-cured tissue were serologically identical; similar titers against TMV antiserum were obtained in the micro-precipitin test. Tests were not conducted using an antiserum prepared against TMV from flue-cured tobacco; therefore, it cannot be concluded that flue-curing does not induce antigenic changes.

Virus infectivity was greatly decreased by flue-curing. Only 15-18% as much infective TMV was extracted from flue-cured tobacco as from a comparable amount of fresh tissue. This result could be due to differences in the quantity of TMV in the different samples of tobacco tissue, as they were not grown under identical conditions. However, a similar relationship was found with purified TMV from fresh or flue-cured tobacco. Purified TMV from flue-cured tobacco was only 7-19% as infective as a similar concentration of purified TMV from fresh tissue (Fig. 1).

The virus preparations from fresh and from flue-cured leaves also differed in birefringent properties. The lowest concentration at which streaming could be seen was 0.1 mg/ml for purified TMV from air-dried or fresh tissue and about 0.4 mg/ml for TMV purified

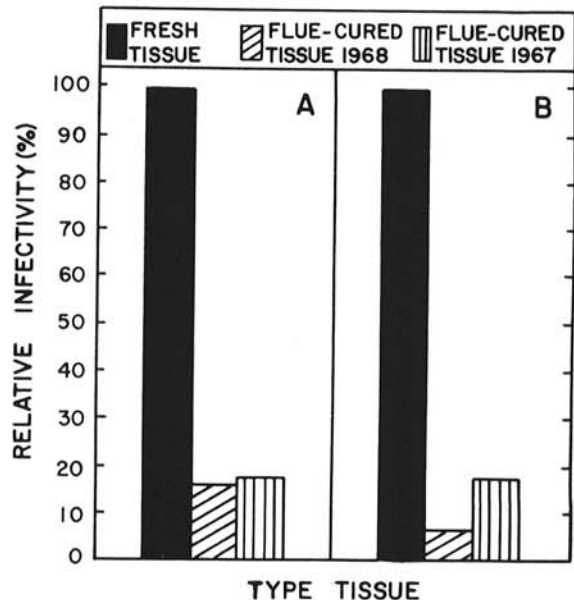


Fig. 1. Comparison of infectivity of tobacco mosaic virus (TMV) from fresh tissue and flue-cured tissue. A) Relative infectivity per g dry wt of fresh and flue-cured tissue from the 1968 and 1969 crops. B) Relative infectivity per mg of purified TMV from fresh and flue-cured tissue from the 1968 and 1969 crops.

from 1968 and 1969 samples of flue-cured tissues. This indicates some loss in linear shape of the TMV particle, perhaps by breakage into shorter pieces or by partial transformation of the particles into a sphere (5).

Based on our results, the flue-curing process as currently practiced does not completely inactivate TMV. Consequently, the use of flue-cured tobacco in tobacco products constitutes a source of infectious TMV for the initiation of new infections (3). Our results are not directly comparable to those of Thornberry et al. (7); they found that exposure of TMV-infected tobacco tissue to 80 C for 30 hr reduced infectivity about 95%. The reduction in amount of infectious virus as a result of flue-curing apparently resulted from a heating effect directly on the virus, and was not due to formation of an inhibitor of infection during flue-curing, because the reduction in infectivity of purified virus from flue-cured tissue was about the same as that for virus assayed directly. Desiccation can also be eliminated as a cause of changes in state and infectivity upon flue-curing. TMV purified from fresh tissue dried over P_2O_5 in a desiccator was similar to TMV purified from moist fresh tissue in terms of serological activity and birefringence. Infectivity of purified TMV from dried fresh tissue was reduced to about 70% of that from fresh

tissue, whereas infectivity of extracts made directly from dried fresh tissue was equal to or greater than that obtained with comparable amounts of fresh tissue.

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