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INFLUENCE OF WASHING TIME ON INFECTION BY TOBACCO MOSAIC VIRUS

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Mechanical inoculation with a buffered virus inoculum from plant leaf tissues requires a washing of the leaves after inoculation to remove extraneous substances that might cause injury to the leaves, if allowed to remain on the plant. The influence on infection of this washing of inoculated leaves was investigated to obtain information that might be utilized to standardize further the virus assay procedure and to explain more fully the process of infection in the host tissues. These studies were aided by a contract between the Office of Naval Research, Department of Navy, and University of Illinois, NR 131-193.

Materials and Methods

The virus used throughout the investigations was a preparation of tobacco mosaic virus, *Marmor tabaci* Holmes, obtained from the expressed juice of diseased leaves of Turkish tobacco, *Nicotiana tabacum* L. The virus was partially purified by differential sedimentation in a model L. Spinco ultracentrifuge.

The assay tissues were those of the primary leaves of Scotia beans, *Phaseolus vulgaris* L. The plants were grown in sterilized soil in sterilized pots in a greenhouse, and the primary leaves were inoculated approximately 12 days after planting the seed.

The inoculum was a suspension of the virus in 0.1 molar phosphate buffer at pH 8.5 and contained 800-mesh aluminum oxide powder at a 7.0% concentration.

Mechanical inoculations consisted of applying the virus inoculum to the upper surface of the bean leaves by stroking the leaves with cheesecloth pads saturated with inoculum. After inoculation the leaves were washed by immersion in distilled water for stated periods of time.

Review of Literature

Yarwood (1951, 1952a) showed that the washing of bean leaves after mechanical inoculation with phosphate salts in the inoculum reduced the amount of infection. When injured leaves were inoculated by immersing them in aqueous virus inoculum, Yarwood (1952b) found that leaves injured by rubbing with a dry brush developed more lesions than leaves similarly rubbed with a wet brush. Dale (1953) reported that when injured bean leaves were inoculated by immersion in aqueous virus inoculum the amount of infection decreased as the immersion period increased. He also found that the amount of infection with the immersion method decreased with the duration of the aging of injured leaves in air prior to inoculation.

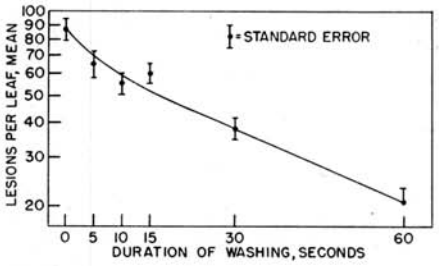

FIG. 1A.

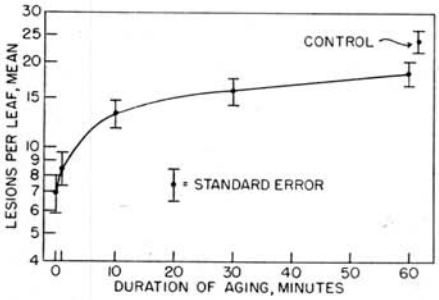

FIG. 1B.

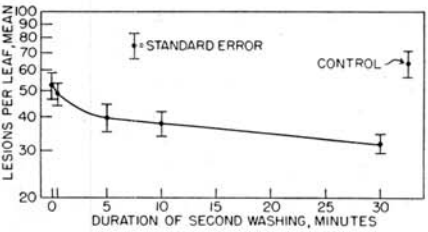

FIG. 1C.

FIG. 1.—The effect on amount of infection of washing inoculated bean leaves, when mechanically inoculated leaves were washed in distilled water: *A*. For varied periods of time immediately after inoculation (data at zero time of washing were obtained from inoculated plants that received a momentary dip in distilled water); *B*. For standard one-minute period of washing after standard ten-second preliminary washing and varied period of exposure to air; *C*. For varied periods of secondary washing after standard preliminary treatment consisting of 10-second wash and 60-minute period of aging in air.

Allington and Laird (1954) reported that the amount of infection by tobacco mosaic virus on *Nicotiana glutinosa* was greatly reduced when wounded tissues were either soaked in water or exposed to air prior to inoculation by immersing wounded leaves in virus inoculum. Kalmus and Kassanis (1945) stated that leaf tissues of *N. glutinosa*, injured by rubbing with Celite powder, were resistant to infection by tobacco mosaic virus sprayed on the leaves three hours after the injury.

RESULTS

The influence of washing on the amount of infection resulting from mechanical inoculations was determined under three conditions of washing. The influence of washing of the leaves immediately after inoculation with virus was determined by inoculating the leaves in the previously described manner and then immediately immersing them in water for varied periods of time. The results, Figure 1A, show that the amount of infection decreased with the duration of the washing period.

The influence of a standard one-minute washing in water was determined after inoculated leaves were subjected to an initial ten-second washing followed by varied periods of exposure to air, aging. The results of this test, Figure 1B, when compared with the results in Figure 1A, indicate that the reduction in the amount of infection from the second washing after periods of aging was less than the reduction from washing immediately following inoculation. In addition the results in Figure 1B indicate that a

period of aging prior to washing influenced the amount of infection. This increased with the duration of the aging.

The influence of a secondary washing in water for various periods, after a standard initial 10-second washing in water and a standard 60-minute aging period in air, was determined. The results, Figure 1C, reveal that the amount of infection decreased with the duration of the second washing.

Discussion

Graphic analysis of the experimental data shows some relationships between the number of infections obtained and the duration of the washing or aging treatment of the inoculated leaves. When the data were plotted coordinately, with the number of lesions as ordinates on a logarithmic scale and time as abscissae on an arithmetic scale (Fig. 1A, B, C), the graphs indicate two regions of rate of change in the amount of infection with the duration of the treatments. In the first region the rate of change in the amount of infection is not constant, but in the second region, the rate is constant.

The results obtained fail to indicate specific mechanisms concerned in the influence of washing and aging on infection. However, the graphs do suggest that a physical phenomenon such as diffusion is involved. A mechanism involving growth processes of the host is unlikely, since the response occurs so rapidly. In the case of immediate washing after inoculation, water in bulk may disturb the mechanism of cellular recovery such as the formation of a surface film or membrane over the area of protoplasm injured by the inoculation. The aging of injured cells prior to a one-minute washing apparently allows any repair or healing process to progress to the extent that a recovered membrane is more resistant to the action of water in the washing period. This is supported by the fact that resistance to the effect of washing increases with the length of the aging periods. This concept of resistance is further supported by comparing the rate of change in the amount of infection shown in Figures 1A and 1C. When Kalmus and Kassanis (1945) applied virus subsequent to both injury and aging of leaves, the repair or healing became so fixed that the cells in *N. glutinosa* were resistant to infection after three hours.

The results obtained when Scotia beans were used as the assay plant suggest that definitely timed periods of washing of leaves following inoculation could further refine the local lesion method as presently used in measuring the relative infectivity of virus solutions. According to the curve in Figure 1A, maximal infection would be attained with a minimal period of washing immediately after mechanical inoculation. However, reproducible results might be more easily achieved if the virus assay were carried out with a washing period the length of which is shown within a region of the curve which follows a straight line relationship between the amount of infection and the duration of washing. This would tend to minimize the unavoidable variations encountered in the mechanics of washing.

SUMMARY

The washing of bean leaves inoculated with tobacco mosaic virus had a varied influence on the amount of infection. When washed immediately after mechanical inoculation, the amount of infection decreased with the duration of the washing period. When inoculated leaves were given an immediate ten-second washing, a varied period of exposure to air, and then a standard one-minute washing, the amount of infection increased with the duration of the aging period. When inoculated leaves were given an immediate 10-second washing and a subsequent, standard, 60-minute period of aging in air, the amount of infection decreased with the duration of the second washing. The data suggest that the initial cellular changes influencing infection are physical in nature.

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