Determination of diffusion coefficient of turnip yellow mosaic virus by the gel-precipitin technique

The determination of diffusion coefficients of viruses by optical methods may be difficult owing to the relatively large amounts of purified virus required. The gel-precipitin technique offers a partial solution to this problem by allowing accurate diffusion coefficients to be determined with relatively small amounts of virus.

The TYMV was prepared from infected Chinese cabbage plants by the method of MARKHAM AND SMITH[1]. The antiserum was obtained from 2 rabbits which both received three consecutive weekly intravenous injections of about 10 mg TYMV. The precipitin apparatus used was similar to the one described by POLSON[2]. Antiserum diluted 4-fold with saline was placed in the cups in the lowest section of the apparatus: 0.5 % agar was inserted in the cavities of the middle section and after this had set, a series of 2-fold dilutions of TYMV was placed in the top sections. Precipitin bands could be seen after 2 days and broadened thereafter, their position and widths being recorded daily. The apparatus was kept at room temperature because removal from an incubator for measurement involves temperature fluctuations which may cause

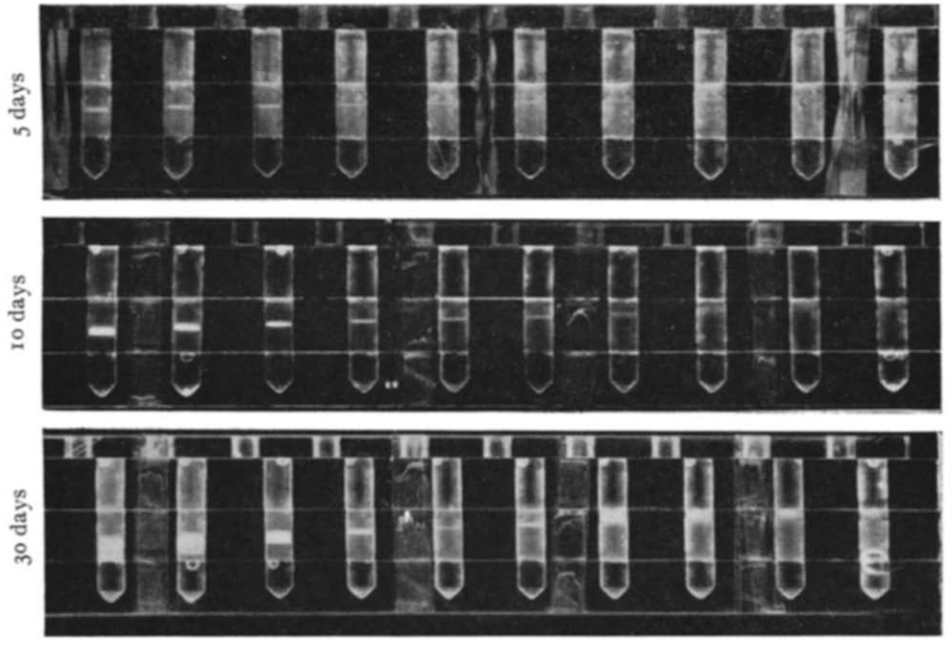

Fig. 1. Photograph of the precipitin bands formed by serial dilutions of TYMV against constant antiserum after 5, 10 and 30 days.

band splitting. Fig. 1 shows the apparatus with the positions of the bands after 5, 10 and 30 days respectively. In Fig. 2 the broad diagonal lines were obtained by plotting the distances of the middle of the precipitin bands from the antibody meniscus after 4 and 8 days against the dilution of virus and the 3 V-shaped curves were obtained by plotting the widths of the precipitin bands after 4, 6 and 8 days against the dilution of virus. Since the bands are stationary and narrowest when

Abbreviation: TYMV, turnip yellow mosaic virus.

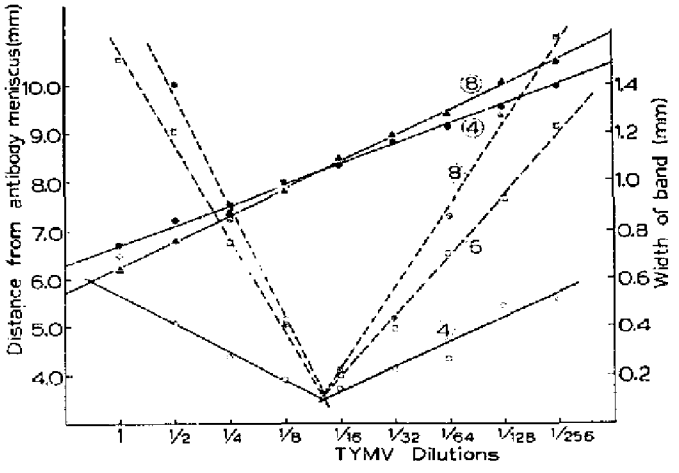

Fig. 2. Gel-precipitin test on TYMV. The diagonal curves show the positions of the precipitin bands after 4 and 8 days plotted against virus dilution. The V-shaped curves show the variation in band width after 4, 6 and 8 days, also plotted against virus dilution.

the concentration of antigen and antibody in the initial solution are optimal for precipitation[2], the points at which the families of curves intersect refer to this position. Interpolation reveals that the band formed by solutions initially at optimal proportions would be 8.25 mm from the antibody meniscus. It has been shown by POLSON[2] that under these circumstances:

$$\frac{x^2_g}{x^2_b} = \frac{D_g}{D_b}$$

where x_g and x_b are the distances of the bands from the antigen and antibody menisci respectively and D_g and D_b the diffusion constants of antigen and antibody. In this experiment, $x_g + x_b$ was 12,958 mm. If D_b is assumed to have the value found by LARGIER[3] for tetanus antitoxin, namely $4.81 \cdot 10^{-7}$ cm/sec at 20°, the diffusion constant of TYMV may be calculated to be 1.56 Fick Units in good agreement with MARKHAM's[4] value of 1.51–1.55 Fick Units. Insertion of the value 1.56 Fick Units in Stokes-Einstein equation:

$$r = \frac{RT}{N} \cdot \frac{1}{6\pi D\eta}$$

gives a value of 27.4 mμ for the diameter of the TYMV particle.

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