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The occurrence of two strains of barley yellow mosaic virus in England

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Barley yellow mosaic virus (BaYMV), first reported in the United Kingdom in 1980 (Hill and Evans, 1980), has now spread and become established throughout England and Wales (S.A. Hill, personal communication). In the field BaYMV infection is confined to winter cropped barley, however, spring cultivars are also susceptible (Plumb et al., 1986). The virus is soil-borne and is almost certainly transmitted by a fungus belonging to the Plasmodiophoromycetes, *Polymyxa graminis* (Toyama and Kusaba, 1970).

Huth et al., (1984) first described the occurrence of a mixture of two strains of BaYMV in field infected barley in the Federal Republic of Germany. The two strains were reported to differ in serology, buoyant density and mechanical transmissibility but not particle morphology or symptomatology. Whilst strain BaYMV-M was mechanically transmissible to barley, the other, BaYMV-NM was not. The two strains are serologically distinct but, BaYMV-NM is serologically related to a Japanese isolate of BaYMV and another German strain (BaYMV-S_0) (Ehlers and Paul, 1986) which is mechanically transmissible with difficulty and is not serologically related to BaYMV-M. In our studies on English isolates of BaYMV six isolates were investigated. Five field isolates of BaYMV, all in the same cultivar of barley cv. Maris Otter were collected during the spring of 1985. The isolates originated from sites in Oxfordshire (BaYMV-I_1, and BaYMV-W_1), Gloucestershire (BaYMV-Fa_1), Berkshire (BaYMV-F_1) and Essex (BaYMV-C_1). The sixth isolate, from a site in Bedfordshire, (BaYMV-S_1) was maintained by the inoculation of barley seedlings with a viruliferous culture of *P. graminis*, isolated from virus infected plants (Adams et al., 1986).

Antisera against the two German strains of BaYMV (M and M + NM, supplied by W. Huth) were cross adsorbed with an acetone powder of healthy barley leaves prepared as described previously (Tucker and Fairbrothers, 1970). Gammaglobulin (IgG) fractions from the antisera were precipitated with ammonium sulphate and resuspended at a concentration of 1 mg ml^{-1}, prior to conjugation with alkaline phosphatase for use in enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977). ELISA tests were performed in duplicate or triplicate with diluted extracts (1 : 10) of healthy 'Maris Otter' and BaYMV infected leaves. The mean absorbance at 405 nm plus three times the standard deviation ($\mu + 3\sigma$) obtained with a healthy barley plant extract was used to calculate an upper threshold value to distinguish positive results.

All six isolates were tested for mechanical transmissibility. Inoculations were performed using sap extracted from infected plants buffered with 0.01 M sodium phosphate (pH 7.1) containing 1% (w/v) sodium sulphite. 'Maris Otter' barley plants were inoculated with each isolate at the three leaf stage (Friedt, 1983) and maintained for a

minimum of nine weeks at 15 °C with a 12 h photoperiod at 16 000 lux.

All isolates with the exception of BaYMV-W_1 were mechanically transmissible with varying degrees of difficulty and produced characteristic symptoms of infection (Inouye and Saito, 1975). BaYMV-I_1 was more difficult to transmit reproducibly and those plants that did become infected were designated as being infected with the mechanically transmissable component of this isolate, BaYMV-I_m.

Plants infected with the BaYMV-Fa_1, F_1, C_1 or S_1 isolates gave positive results in ELISA tests using IgG raised against BaYMV-M while extracts from plants infected with BaYMV-I_1 or W_1 did not. In separate ELISA tests extracts from plants infected with BaYMV-I_m consistently gave negative results with IgG raised against BaYMV-M while extracts from plants infected with BaYMV-S_1 gave positive results under the same conditions. In ELISA tests using mixed IgG raised against BaYMV-M + NM, extracts of BaYMV-S_1 infected plants gave positive results while extracts from plants infected with BaYMV-I_m gave low positive values.

Clarification of a serological relationship between the BaYMV-S_1 and I_m isolates and the German BaYMV-M and NM virus strains, respectively, was achieved following immunogold labelling of virus particles. Formvar covered grids with or without a precoating of virus-specific IgG raised against BaYMV-M + NM were used in either immunosorbent electron microscopy (ISEM) (Roberts and Harrison, 1979) or leaf dip preparations. Both treatments were followed by decoration with IgG and protein-A gold (PAG) labelling. Leaf tissue was extracted, (lg to 2 ml) in 0.1 M potassium phosphate buffer (pH 7) with a small amount of 600 mesh carborundum using a mortar and pestle. Extracted virus particles were decorated for 15 min at 37 °C with IgG diluted 1 : 50 in phosphate buffered saline (0.1 M potassium phosphate, 0.15 M sodium chloride, pH 7.4) containing 0.05% (v/v) Tween 20 (PBS-Tween). IgG decorated particles were labelled using a 1 : 20 dilution of PAG (Bioclin Biological Services Ltd., U.K.) diluted in PBS-Tween and incubated at room temperature for 30 min. Thereafter grids were stained with 2% potassium phosphotungstate and examined in the electron microscope. Between each step of the protocol grids were washed with PBS-Tween and dried with filter paper. For each grid all particles found on a minimum number of four grid squares were counted.

When BaYMV-M specific IgG was used for primary decoration, a second decoration step using BaYMV-M + NM specific IgG was included followed by PAG labelling prior to staining. Particles were labelled using a different sized PAG colloid following primary and secondary decoration. In this procedure, virus particles were trapped with or without IgG specific for BaYMV-M + NM and differentially labelled with 20 nm (PAG 20) and 5 nm (PAG 5) gold particles depending on the ability of isolates to cross react with BaYMV-M or M + NM specific IgG. When IgG specific to BaYMV-M + NM was used in primary decoration and PAG 20 labelled, no secondary decoration step was used.

Following either the production of leaf dip preparations or ISEM of BaYMV-S_1 and subsequent decoration with BaYMV-M followed by M + NM specific IgG, successful primary labelling with PAG 20 was achieved but on occasion PAG 5 particles were also found attached to heavily PAG 20 labelled BaYMV particles (Fig. 1A). Decoration of BaYMV-I_m with BaYMV-M + NM specific IgG also resulted in successful primary labelling of particles (not shown). However, decoration of BaYMV-I_m with IgG raised against BaYMV-M, Followed by PAG 20 labelling and then a second antibody decora-

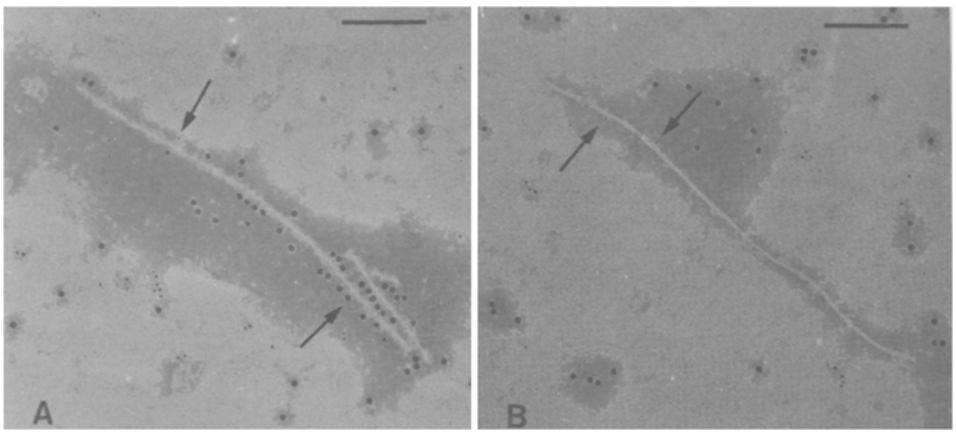

Fig. 1. Electron micrographs of BaYMV particles trapped by ISEM. Two differnt isolates BaYMV-S_I (panel A) and BaYMV-I_m (panel B) were examined. Particles immunogold labelled with 5 nm, and 20 nm gold colloids, are indicated following treatment as described in the text. The bar marker represents 200 nm.

tion with IgG raised against BaYMV-M+NM followed by PAG 5 labelling, resulted in PAG 5 labelled particles only (Fig. 1B). While ISEM increased the number of trapped particles on grids with both BaYMV-S_I and I_m, as compared to leaf dips on uncoated grids, increases in background labelling and the occurrence of unlabelled particles did result, particularly with BaYMV-I_m. Nevertheless, differences in the ratio of PAG 5 labelled to PAG 20 labelled particles for BaYMV-S_I and BaYMV-I_m were consistently found with both procedures and in good agreement. Problems of non-specific PAG labelling in the absence of viral specific IgG with BaYMV (Louro and Lesemann, 1984) were not encountered in our studies and no virus labelling was found to occur when pre-immune serum was used for decoration.

On the basis of the results presented here it would appear that in England, as in the Federal Republic of Germany (Huth et al., 1984; Ehlers and Paul, 1986) at least two strains of BaYMV isolate exist. These are serologically related respectively to the German M and NM virus strains. the former includes the BaYMV-S_I, Fa_I, and C_I isolates on the basis of their serology and relative ease of mechanical transmission. The latter group includes BaYMV-I_I and I_m by virtue of their differing serology and relative difficulty of mechanical transmission. However, the failure to transmit BaYMV-W_I and the absence of more complete serological data for this isolate suggests it requires further investigation. Presently, there is insufficient evidence to indicate any relaionship between BaYMV-W_I, and either BaYMV-I_I or I_m. Whilst it remains possible that low absorbance values found in ELISA tests with BaYMV-I_I, I_m and W_I are a reflection in part of virus concentration and lability, the results given by PAG labelling substantiate our findings. The results presented here do not preclude the occurrence of further BaYMV strains present either in mixture or as individual strains in England. It is also possible that the proportion of the two strains described alters during the growing season as described by Huth et al. (1984) for BaYMV-M and NM. Following submission of this manuscript Adams et al. (1987) have demonstrated the occurrence of two strains of BaYMV in England, similar to ones described in this report.

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Samenvatting

Het voorkomen van twee stammen van het gerstegeelmozaïekvirus in Engeland

Een aantal isolaten van het gerstegeelmozaïekvirus, afkomstig van verschillende plaatsen in Engeland, werden met elkaar vergeleken. Uit de resultaten, verkregen met ELISA, goudmerking en mechanische overdracht, kan worden geconcludeerd, dat tenminste twee stammen van het virus in Engeland voorkomen. Deze stammen zijn, wat betreft de wijze van overdracht en de serologische eigenschappen, gelijk aan die welke in de Duitse Bondsrepubliek zijn beschreven.

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