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Identification of barley yellow mosaic virus by immuno-electron microscopy in barley but not in Polymyxa graminis or Lagena radicicola*

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Barley yellow mosaic virus (BaYMV) is a rod-shaped long flexuous virus infecting barley (*Hordeum vulgaris* L.) (Inouye and Saito, 1975) its only reported host. The virus is closely related to wheat yellow mosaic virus (WYMV) in Japan and wheat spindle streak mosaic virus (WSSMV) in North America (Usugi and Saito, 1976, 1979). A matter of concern is that BaYMV is spreading in Western Europe (Huth et al., 1984). Two strains have been recognized in West Germany, BaYMV-NM prevailing in winter, and BaYMV-M later in spring. In May and June Only BaYMV-M was present. BaYMV-M is mechanically transmissible (Huth et al., 1984), BaYMV-NM is not. We report here the occurrence of BaYMV-M in barley in the Netherlands.

Virus diseased barley plants (*Hordeum vulgare* L. cv. Igri) were carefully dug up from areas showing a yellow mosaic in a field on a southeast facing slope near Wittem in the Geul valley of Limburg in early March, 1984. Soil was carefully rinsed from roots of plants showing clear mosaic symptoms. *Polymyxa graminis* life cycle stages were found in root cortex cells by light microscopy. The same roots also contained thalli of *Lagena radicicola* Vanterpool and Led. Roots containing cystosori, plasmodia and zoosporangia, as well as leaf tissue of varying age were vacuum infiltrated with 0.1 M KPO_4-citrate buffer, pH 7.2, chilled and fixed as described (Langenberg, 1979). No osmic acid postfixation was used. Osmic acid has been shown to adversely affect antigenic reactivity (Roth et al., 1984). In the absence of OsO_4 postfixation all membranes in ultrathin sections are electron translucent. Tissues were dehydrated with a graded methanol series and embedded in Lowicryl HM20 or K_4M plastic (Lin and Langenberg, 1983).

Antiserum to BaYMV-NM and M strains was received from W. Huth, Braunschweig (West Germany), anti-BaYMV-J from T. Usugi, Tsukuba City (Japan) and anti-

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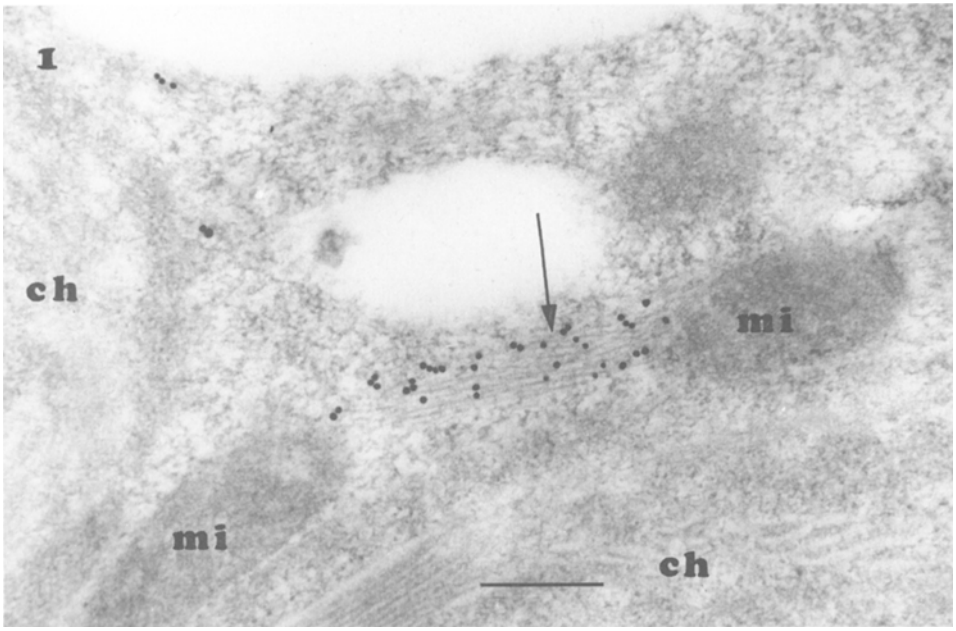

Fig. 1. Virus inclusion of BaYMV (arrow) stained positively with antiserum to BaYMV-M. Other antisera to BaYMV strains failed to stain virus. Membranes do not show because tissue was not post-fixed with OsO_4. Ch = chloroplast, Mi = mitochondrium. Bar represents 300 nm.

WSSMV from K.Z. Haufler, East Lansing, MI (USA). All antisera were absorbed (1 ml) with a healthy barley or wheat (WSSMV) preparation, (1 g tissue in 1 ml 0.1M phosphate buffer) and clarified by low speed.

Silver to gold sections of leaf and root tissue containing *P. graminis* and *L. radicicola* were stained according to Lin and Langenberg (1983) with 1 : 200 diluted antiserum followed by goat anti-rabbit IgG conjugated to colloidal gold. Sections were constrasted with uranyl acetate and lead citrate and viewed in a Zeiss EM10A.

Of the three BaYMV antisera, no reaction (no stain) was obtained with anti-BaYMV-NM, BaYMV-J or WSSMV, but a moderate positive reaction with BaYMV-M (Fig. 1). This finding agrees with the reactions of the M. strain of BaYMV in West Germany (Huth et al., 1984). The BaYMV inclusion is thus positively identified as that of the M strain. A light gold deposit over pinwheels (Fig. 2) indicated entrapped virions or attached virion coat protein. Background stain was light. Virus induced coat of mail inclusions (Fig. 2) did not stain with anti-BaYMV coat protein. None of the cell's organelles stained above background.

Sections containing *P. graminis* plasmodia, zoosporangia and cystosori were stained with each of the three BaYMV antisera. None of the stages of *P. graminis* in the root stained above background.

None of the many *L. radicicola* thalli stained for BaYMV either. The thallus of *L. radicicicola* was long and thread-like and doubled back on itself from one to several times within the infected cell.

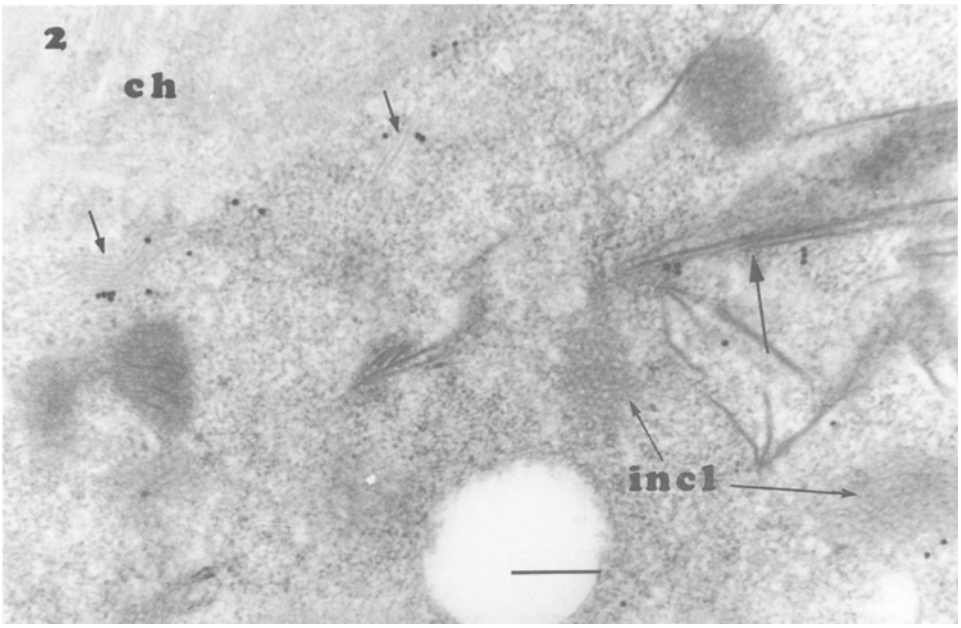

Fig. 2. Cytoplasm of Igri barley leaf cell containing small inclusions of BaYMV positively stained with antiserum to BaYMV-M (light arrows), and also some antigen association with the pinwheels (heavy arrow) but other virus-induced inclusions (incl.) did not stain. Ch-chloroplast. Bar represents 300 nm.

Virus infection of the barley field was readily recognized from the road. The field was located on a slope, where water could not collect, yet infected areas of approximately 3 m diameter were widespread throughout the field. The field was known to be sown to wheat cv. Okapi before the Igri barley crop but its cropping history was otherwise not known.

It is unclear at this time if the M strain arises by mutation from the NM strain and if it can be maintained naturally by soil transmission. In another *P. graminis* transmitted virus of cereals, soil-borne wheat mosaic virus (SBWMV), the wild type strain (SBWMV-WT) is found early in spring in the field as symptoms appear. Shirako and Brakke (1984) have shown that SBWMV-WT gives rise by deletion mutation to a mechanically transmissible SBWMV strain by sampling individual plants during the growing season.

L. radicicola thalli were also numerous in roots of BaYMV-infected barley. The thalli were sectioned and stained for BaYMV because it is still possible that *L. radicicola* serves as a vector of soil-borne viruses. We agree with Barr and Slykhuis (1969) that, unless proven otherwise, this obligate zoosporic fungal parasite of graminaceous plants should also be considered a possible vector especially since it is commonly encountered and also prefers cool moist conditions.

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Samenvatting

Identificatie van gerstegeelmozaïekvirus met immuno-elektronenmicroscopie in gerst maar niet in Polymyxa graminis of in Lagena radicicola

Gerstegeelmozaïekvirus (BaYMV) werd in een perceel wintergerst (cv. Igri) gevonden by Wittem in Limburg, Nederland. Het virus werd met immuno-elektronenmicroscopie geïdentificeerd in bladweefsel als BaYMV. In de wortels van geïnfecteerde planten werden alle vormen die van de schimmel-vector *(Polymyxa graminis* Led.) bekend zijn aangetroffen nl. cystosori, plasmodia en zoosporangia. Inwendig kon geen BaYMV worden aangetoond of waargenomen in *Polymyxa* of *Lagena* spp.

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