# Deterioration of Several Rod-Shaped Wheat Viruses Following Antibody Decoration

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## ABSTRACT

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Soilborne wheat mosaic, barley stripe mosaic, and wheat spindle streak mosaic viruses disintegrated to varying degrees after decoration with homologous antibodies on electron microscope specimen grids. Wheat streak mosaic virus was stable to decoration. Disintegration of wheat spindle streak mosaic virus in antisera to barley yellow mosaic virus from West Germany and Japan, affirmed the close relationship of these two viruses. Goat anti-rabbit immunoglobulin G labeled with colloidal gold positively identified otherwise unrecognized virus-antibody complexes. Instability of virus in antisera can be used as a measure of relatedness in the wheat yellow mosaic virus group and possibly in other groups.

Leaf-dip serology has become a fast, efficient, and popular procedure for virus identification since first reported by Ball and Brakke (1). Many uses, improvements, and amplifications have been published (3,5,7–10,11). Antibody attachment (decoration) to rod-shaped viruses in crude leaf-dip preparations and in purified or partially purified preparations is clearly visualized by electron microscopy as is similar decoration of partially purified icosahedral viruses. Decoration is more difficult to observe with unpurified icosahedral viruses, particularly when the virus concentration in crude sap is low, as with luteoviruses. Lin (9) has overcome poor visibility of specific rabbit immunoglobulin G (IgG) adsorption in leaf dip preparations by accentuating specific primary rabbit IgG attachment to virus particles by secondary staining with goat anti-rabbit IgG labeled with colloidal gold. This paper reports observations that some otherwise intact (stable) particles of wheat viruses disintegrate after antibody decoration.

## MATERIALS AND METHODS

**Viruses.** Soilborne wheat mosaic virus (SBWMV) was maintained by inoculation of wheat (*Triticum aestivum* L. 'Scout 66') via *Polymyxa graminis* Ledingham; and barley stripe mosaic virus (BSMV, type strain [ATCC PV 43]) and wheat streak mosaic virus (WSMV) were maintained by mechanical inoculation to wheat cultivar Centurk. Wheat spindle streak mosaic virus (WSSMV) was obtained from infected wheat (cultivar Vona) from a commercial field.

**Antisera.** Antisera to SBWMV, BSMV, and WSMV were from this laboratory; antiserum to WSSMV was received from K. Z. Haufler, Michigan State University, East Lansing; antisera to the NM and M strains of barley yellow mosaic virus (BaYMV) were received from W. Huth, Institut fur Viruskrankheiten der Pflanzen, Braunschweig, West Germany; and antiserum to BaYMV-J was received from T. Usugi, National Agriculture Research Center, Yatabe, Tsukuba, Japan. Antisera to WSSMV and BaYMV were absorbed with sap expressed from healthy wheat leaves. All antisera were used at dilutions of 1:200 in 0.05 M phosphate-buffered saline (30 mM $Na_2HPO_4$, 20 mM $NaH_2PO_4$, 147 mM NaCl, pH 7.2) containing 0.1% sodium azide. Electron microscope grids were prepared for immunologically specific electron microscopy (ISEM) as described by Derrick (3).

**Leaf-dips and decoration, ISEM and decoration, and colloidal gold enhancement.** Leaf-dips were prepared by crushing a 2- to 3-mm piece of wheat leaf in a drop of distilled water on Parafilm with a wooden dowel. Parlodion- or Formvar-covered electron microscope grids, with or without a coating of specific IgG (as specified for ISEM), were floated on drops of leaf extract for 1- to 30-min intervals, rinsed in phosphate-buffered saline, and the adhering virions were negatively stained either directly or after decoration by floating the grids on diluted antisera and washing them with phosphate-buffered saline. Potassium phosphotungstate (PTA, pH 7.0) and uranyl acetate (UAc, pH 3.5) stains were used. Colloidal gold enhancement of IgG decoration was as described by Lin (9).

## RESULTS

All viruses, before they were decorated, were stable to both negative stains. All viruses were also stable to decoration in heterologous antisera and either of the negative stains, except for WSSMV which was unstable in BaYMV antisera. The results of reacting the homologous and heterologous antisera with the wheat-infecting viruses is summarized in Table 1.

**Soilborne wheat mosaic virus.** The virus was stable in ISEM. The virus particles disintegrated after decoration with homologous antiserum both in standard leaf-dips and after ISEM. Both PTA and UAc gave the same result. Some particles seemed to survive decoration (Fig. 1A). It was not possible to determine the percentage of virus particles disrupted by decoration because of the different numbers of stable virions seen from one leaf-dip to another. However, the numbers of particles from the same leaf-dip droplet were clearly less with decoration than without. Immunogold staining of morphologically indistinct clumps was interpreted as staining of the disintegrated missing virions (Fig. 1B).

**Barley stripe mosaic virus.** This virus was reported to partly disintegrate in leaf-dips followed by decoration (1). Immunogold and PTA staining revealed more extensive degradation than reported earlier because it was then possible to detect capsid protein from completely disintegrated virus. The scattered gold particles in Fig. 1C are more numerous than found in control grids and indicate presence of capsid protein. This capsid protein is thought to arise almost entirely from disintegration of virions because no "free antigen," or nonsedimentable antigen, could be

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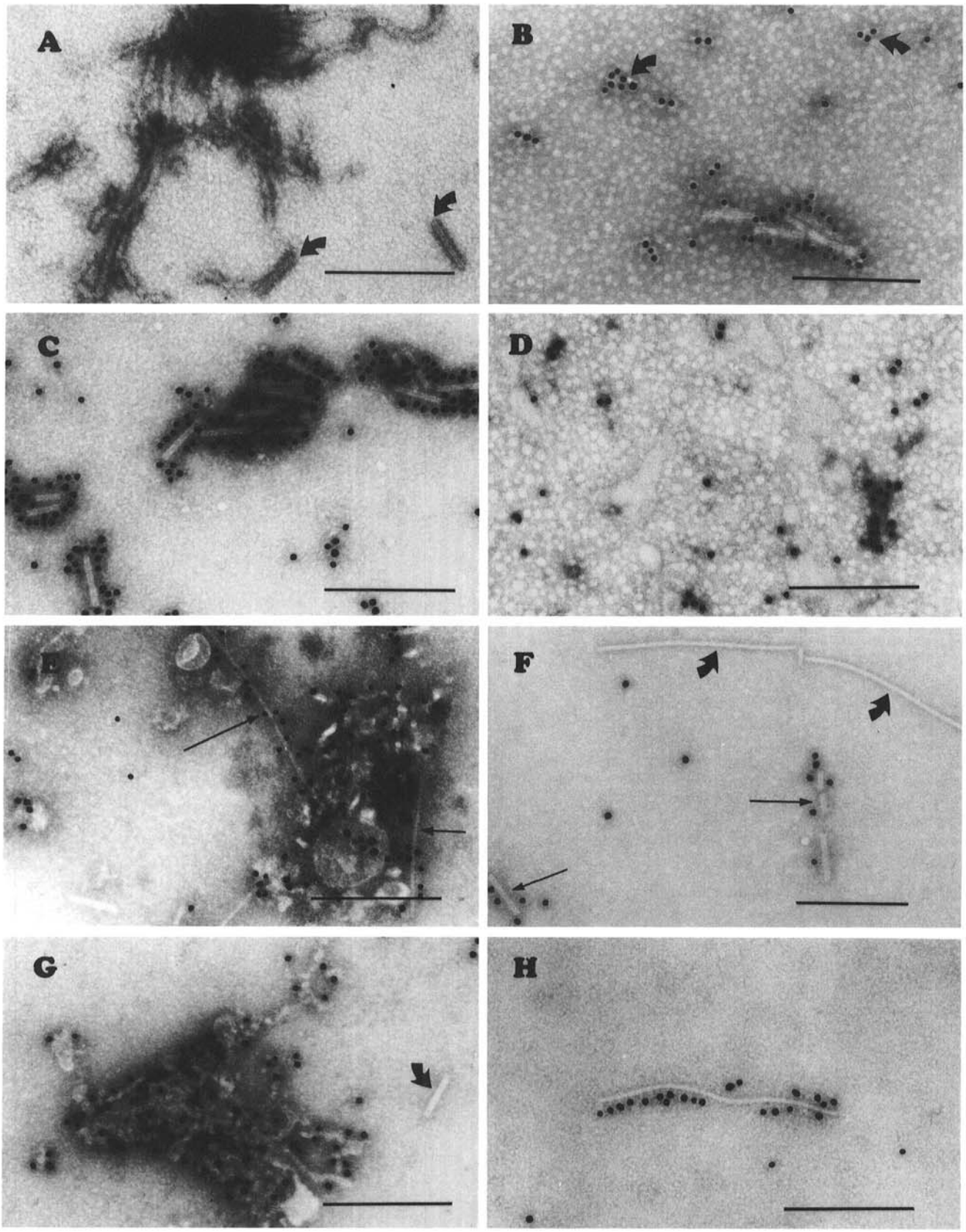


**Fig 1. A,** Soilborne wheat mosaic virus (SBWMV) virions decorated with homologous immunoglobulin G (IgG) after immunologically specific electron microscopy. Virions became flexuous and stretched before disintegrating; some (arrows) remained intact. Uranyl acetate (UAc) negative stain. **B,** As in **A,** except followed by gold-labeled IgG. Gold-labeled SBWMV aggregate and disrupted viral coat protein (arrows). Phosphotungstic acid (PTA) negative stain. **C,** Barley stripe mosaic virus (BSMV) decorated with homologous IgG followed by gold-labeled IgG enhancement. PTA negative stain. **D,** As in **C** except UAc was used as the negative stain. Almost all decorated BSMV aggregates disintegrated. Gold particles indicate BSMV capsid protein-IgG complexes. **E,** Wheat spindle streak mosaic virus (WSSMV) decorated with anti-WSSMV and gold-labeled IgG enhanced. Disruption of decorated virus is severe. Disrupted virus-IgG aggregates are only identifiable after gold enhancement. Some WSSMV particles were intact (small arrows). UAc negative stain. **F,** WSSMV (arrows) remained stable in anti-SBWMV and gold-labeled IgG enhancement of SBWMV-IgG showed little evidence of disrupted viral coat protein of WSSMV. Intact SBWMV (small arrows) are also shown. **G,** WSSMV in anti-barley yellow mosaic virus (BaYMV-NM strain) IgG and gold-labeled IgG enhanced to show that the aggregate consists of WSSMV-anti BaYMV-NM IgG. SBWMV (arrow) was unaffected. UAc negative stain. **H,** Wheat streak mosaic virus-homologous IgG complex enhanced with gold-labeled IgG showed that the virion-IgG complexes were stable. UAc negative stain. All bars represent 300 nm.

TABLE 1. Summary of reactions between homologous and heterologous antisera with several viruses on electron microscope grids after negative staining

| | Antiserum to: | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | BaYMV[b] | | | |
| Virus[a] | SBWMV | BSMV | WSSMV | NM | M | J | WSMV |
| SBWMV | D[c] | – | – | – | – | – | – |
| BSMV | – | D | – | – | – | – | – |
| WSSMV | – | – | D | D | D | D | – |
| WSMV | – | – | – | – | – | – | – |

[a] SBWMV = soilborne wheat mosaic virus, BSMV = barley stripe mosaic virus, WSSMV = wheat spindle streak mosaic virus, WSMV = wheat streak mosaic virus.

[b] Abbreviations: NM stands for the nonmechanically transmissible strain and M for the mechanically transmissible (both antisera from a West German source), and J indicates a Japanese source of anti-BaYMV.

[c] Symbols: "D" stands for virion disintegration, and "–" stands for virion stability.

detected in extracts of BSMV-infected plants (*unpublished*). After UAc negative staining very few virus particles remained. The few remaining particles were only recognizable as BSMV after gold enhancement of virus-IgG complexes (Fig. 1D).

**Wheat spindle streak mosaic virus.** As with SBWMV and BSMV, WSSMV was stable in ISEM. Secondary decoration by homologous antiserum (Fig. 1E) or antiserum of related viruses disintegrated most virus particles. SBWMV remained intact in BaYMV antisera (Fig. 1G, arrow ), and WSSMV remained intact in anti-SBWMV-serum (Fig. 1F, heavy arrows). However, WSSMV was as unstable after decoration in anti-BaYMV antiserum as it was in WSSMV antiserum. All three sources of BaYMV antisera severely disrupted WSSMV in as little as 15 sec. Only gold enhancement of specific IgG attachment allowed detection of residues of WSSMV particles (Fig. 1G, WSSMV in anti-BaYMV-NM). PTA and UAc negative stains gave similar results. Disruption of WSSMV in all three anti-BaYMV antisera serves as additional evidence that WSSMV is closely related to BaYMV. Fixation of WSSMV leaf-dip preparations in 2% glutaraldehyde and 4% formaldehyde for 10 min followed by washing and neutralization in neutral ammonium phosphate did not prevent subsequent disruption by specific IgG in decoration attempts.

**Wheat streak mosaic virus.** This virus was stable to decoration in leaf-dips or after ISEM (Fig. 1H) in both negative stains, PTA or UAc.

## DISCUSSION

The instability to decoration of several wheat viruses may interfere with detection and with determination of relationships by ISEM or leaf-dips. Use of immunogold staining for reliable detection of unstable strains is advised. On the other hand, instability to decoration can be used as a measure of relatedness as shown in the break-up of WSSMV in anti-BaYMV antisera. Huth et al (6) have shown that two major types of BaYMV occur in West Germany: the NM strain is the wild type, early spring form, which gives way to the M strain which is obtained after mechanical inoculation. The NM strain is replaced by the M strain later in the growing season as the weather warms. Partial coating by M strain capsid protein of NM strain virions was shown by decoration. Huth et al (6) noted instability of BaYMV virions to decoration. However, the German BaYMV was not as unstable to decoration as the North American WSSMV to homologous or BaYMV antisera from West Germany and Japan. WSSMV and BaYMV should be considered host-specific strains of the wheat yellow mosaic virus (WYMV) isolate from Japan. Close relationships between WYMV, WSSMV, BaYMV, and rice necrotic mosaic virus have been demonstrated by Usugi and Saito (12,13). Moreover, some, but not all, West German strains of BaYMV reacted strongly with anti-WYMV from Japan (6). Host specificity has separated the strains in the WYMV group.

Differential stability of viruses to negative stain is well known (2,4). While pH often affects stability, the instability of the wheat viruses to decoration was not related to pH except for BSMV which was less stable in UAc than in neutral PTA.

The mechanism of disruption of viruses in homologous antiserum or antiserum to related strains is not known but may be due to antibodies reacting with cryptotopes (epitopes hidden in intact virions) once virions are destabilized by attachment of homologous IgG to epitopes and neotopes on the virion surface. Ionic and hydrophobic bonds between homologous IgG and virion subunits may neutralize or change the forces that hold the subunits together. Permanent separation of subunits by IgG to cryptotopes makes the destabilizing activity irreversible. Since WSMV was not affected by homologous IgG, WSMV either was not destabilized by homologous IgG, its cryptotopes were not reactive, or this WSMV antiserum had no IgG to cryptotopes. The hypothesis presented here only affects virions bound to a surface such as the supporting membrane on the electron microscope grid. The ionic and hydrophobic reactions of homologus IgG with the virion surface in solutions is inherently different. In solution, charges and stresses on surface areas can be neutralized by crosslinking of IgG to other virions and thus the virions are not destabilized.

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