Detection of Seedborne Plant Viruses Using Serologically Specific Electron Microscopy

R. H. Brlansky and K. S. Derrick

Plant pathologist and associate professor, respectively. Department of Plant Pathology, Louisiana State University, Baton Rouge, LA 70803. Present address of senior author: Department of Plant Pathology, Montana State University, Bozeman, MT 59717.

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ABSTRACT

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Serologically specific electron microscopy (SSEM) was used to detect and identify virus particles in crude extracts of seeds infected with tobacco ringspot virus (TRSV), barley stripe mosaic virus (BSMV), lettuce mosaic virus (LMV), and soybean mosaic virus (SMV). The seeds were halves and one half was assayed for virus using SSEM. The remaining seed halves were combined with healthy seed material in mixtures of 1:10, 1:100, 1:1,000, and 1:10,000 (w/w) and also were assayed using SSEM. Virus particles were detected in half seeds in assays using specific antiserum. Particles of TRSV, BSMV, and SMV were detected in one part of infected seed extract per 1,000 parts of healthy seed extract; LMV was detected in one part of infected seed extract per 100 parts of healthy seed extract.

A number of plant viruses are known to be transmitted through the seed of the infected plants (1,5,11), and the total number of known seedborne plant viruses has increased over the years. Fulton (5) listed about 36 viruses transmitted in 63 species of plants. Bennett (1) and Shepherd (11) expanded the list to about 50 seedborne viruses. Phatak (10) lists 85 viruses that are seed transmitted in one or more hosts. Seed transmission may be the most important means for the carry-over of virus in a crop from one season to the next and as a primary source of virus inoculum for vectors (11). Seed transmission has caused serious economic problems with certain crops where efficient vectors further spread the virus (14). Detection of seedborne plant viruses is often a difficult problem. Methods now used to detect seed infected by lettuce mosaic virus (LMV) include screening large numbers (to 30,000) of seedlings from given seed lots, assaying with local lesion indicator plants such as *Chenopodium quinoa* L. (8), conventional serology, and separation of infected seeds that are abnormally light in weight in airstreams (13). We have given preliminary reports on the use of serologically specific electron microscopy (SSEM) for the assay of viruses and mycoplasmas (4) and for the detection of seedborne plant viruses (2). Additional findings on the use of SSEM for the detection and identification of seedborne plant viruses are given in this report.

MATERIALS AND METHODS

Tobacco ringspot virus (TRSV) infected and healthy, virus-free 'Dare' soybean seed was provided by N. L. Horn, Louisiana State University. Healthy seed was obtained from virus-free plants grown in greenhouse facilities.The plants were tested for TRSV using SSEM and Ouchterlony gel immunodiffusion. 'Vantage' barley seed infected with barley stripe mosaic virus (BSMV) and virus-free seed were obtained from T. W. Carroll, Montana State University. Virus-free seed was from greenhouse-grown healthy plants tested serologically for BSMV using sodium dodecyl sulfate (SDS) disk gel diffusion. Virus-free lettuce seed, LMV-infected seed, and LMV antiserum were supplied by T. A. Zitter, University of Florida, Belle Glade. Virus-free lettuce seed was obtained from and tested by Ferry-Morse Research and certified from 30,000 seedlings tested to have 0% infection. Soybean mosaic virus (SMV) infected seed was obtained from J. P. Ross, North Carolina State University. Antiserum to TRSV NC-39 isolate was provided by G. V. Gooding, North Carolina State University. Antiserum to BSMV was obtained from R. J. Shepherd, University of California, Davis, and antiserum to SMV (ATCC PV A.S. 45) was obtained from the American Type Culture Collection.

Procedure for serologically specific electron microscopy. The procedure used was that of Derrick and Brlansky (4). Copper, 200-mesh, 74-μm, handle electron microscopy grids were used throughout this work. Grids were prepared by dipping them in a 1% solution of polybutene in xylene, air dried, and then coated with a film of Parlodion using a 0.5% solution dissolved in amyl acetate. The grids were then carbon-coated in a vacuum evaporator. The grids were floated on drops of the appropriate antiserum diluted 1:5,000 with Tris buffer (0.05 M, pH 7.2) for 30 min at room temperature. Unabsorbed serum proteins were removed by floating the grids on drops of Tris buffer. Experimental grids were immediately floated on crude seed extracts. Control grids were floated on either normal rabbit serum or on an antiserum to a virus serologically unrelated to the virus being tested. Fifty seeds of each type were tested for virus content. The suspected virus-infected seeds were halved and one-half of each seed was assayed for virus. The naturally dried seed halves were powdered in a mortar and pestle and then diluted with Tris-NaCl buffer (0.05 M, pH 7.2, 0.15 M NaCl). Soybean and barley seed were diluted 1:10 (w/v), but lettuce seed were diluted 1:1,000 (w/v) to give enough material to test. Antiserum coated grids then were floated on drops of the seed extracts for 30 min. Following the reaction the grids were washed to remove cellular debris by floating three times on drops of extraction buffer containing 0.4 M sucrose and floating twice in distilled water. Virus particles were stained by floating on a solution of 5% uranyl acetate in 50% ethanol for 3 min. The grids were then blotted and allowed to air dry.

The remaining seed halves from seed with virus were mixed in with healthy seed material to determine the ratios of infected: healthy seed at which SSEM could detect virus. For each virus tested, infected seed halves from the 50 seeds tested were ground to a powder and 100 mg of the infected seed material was mixed with 900 mg of healthy seed material. This 1:10 mixture was thoroughly homogenized, and then serial mixtures were made with healthy seed material in the same manner to obtain ratios of 1:100, 1:1,000, and 1:10,000. Infected seed extract/healthy seed extract samples from each of these mixtures were then diluted 1:10 (w/v) with extraction buffer and assayed. All assays were performed at room

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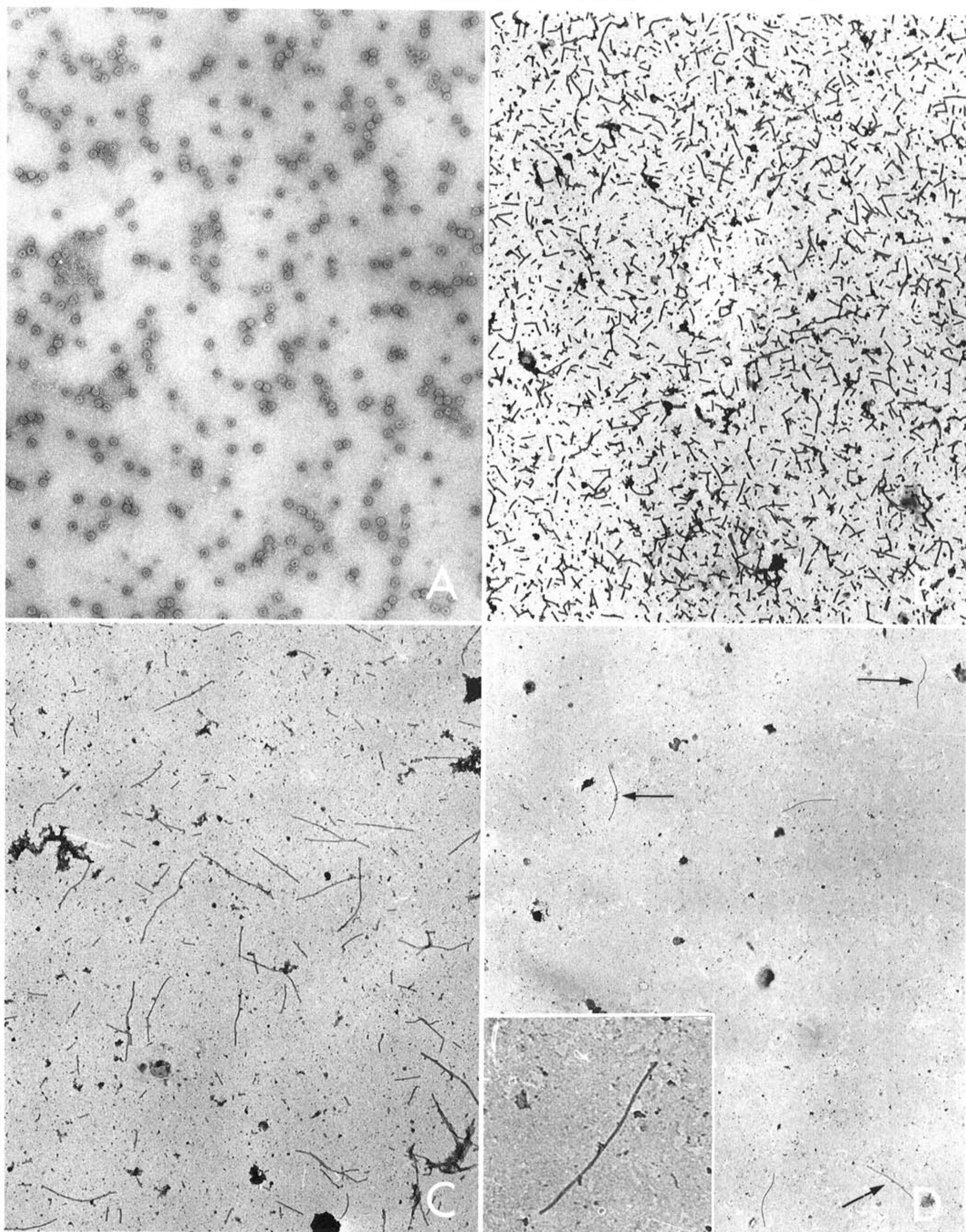

Fig. 1-(A to D). Four seedborne viruses detected with serologically specific electron microscopy from infected seeds. **A)** Tobacco ringspot virus from infected soybean seed (×46,840). **B)** Barley stripe mosaic virus from infected barley seed (×9,000). **C)** Soybean mosaic virus from infected soybean seed (×13,500). **D)** Lettuce mosaic virus from infected lettuce seed (×9,000). Insert ×21,000.

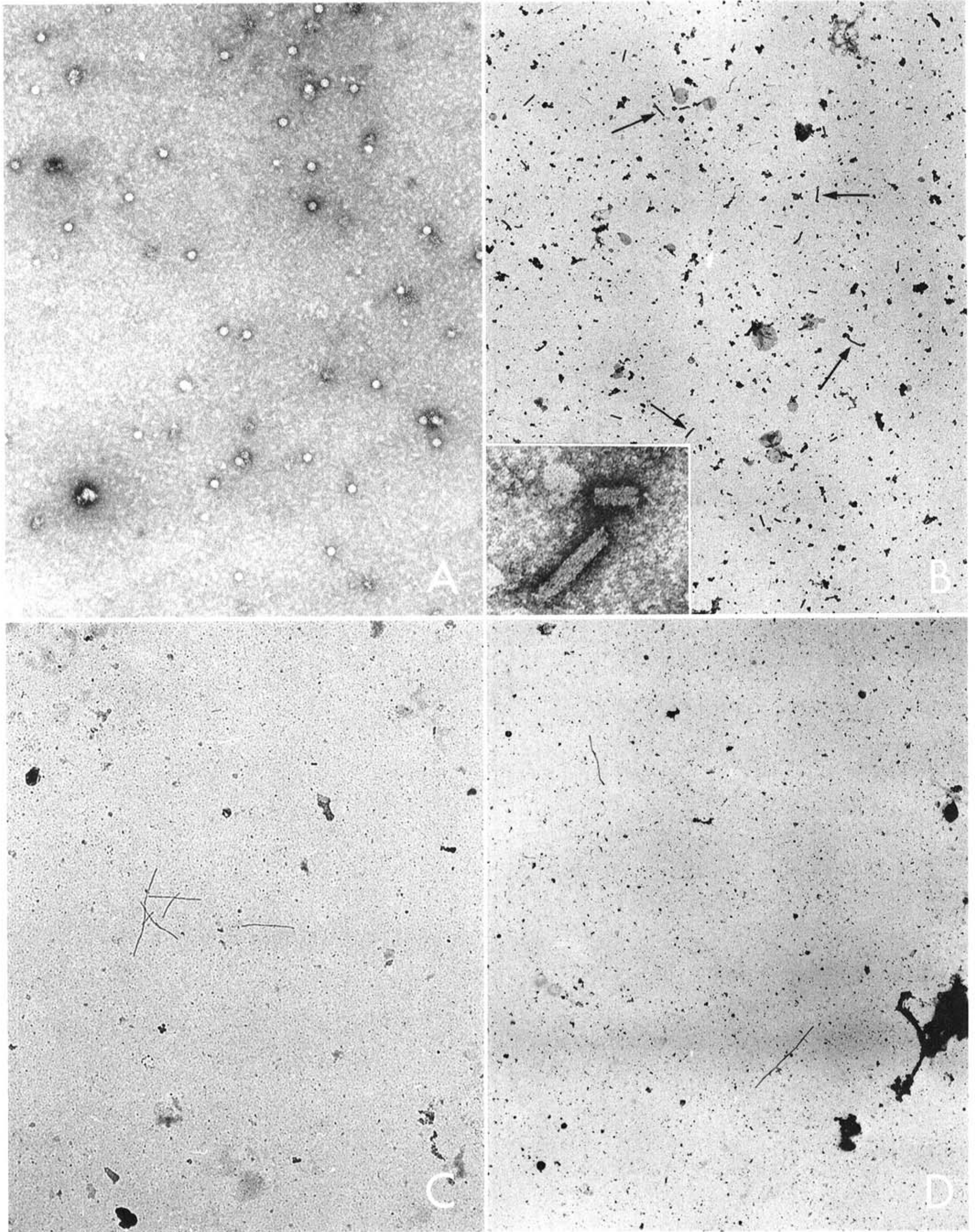

Fig. 2-(A to D). Detection limits of four seedborne viruses using serologically specific electron microscopy. **A)** Tobacco ringspot virus detected in a mixture of one part infected seed to 1,000 parts of healthy seed (×46,840). **B)** Barley stripe mosaic virus detected in a mixture of one part infected seed to 1,000 parts of healthy seed (×9,000). Insert ×117,600, **C)** Soybean mosaic virus detected in a mixture of one part infected seed to 1,000 parts of healthy seed (×9,000). **D)** Lettuce mosaic virus detected in a mixture of one part infected to 100 parts of healthy seed (×13,500).

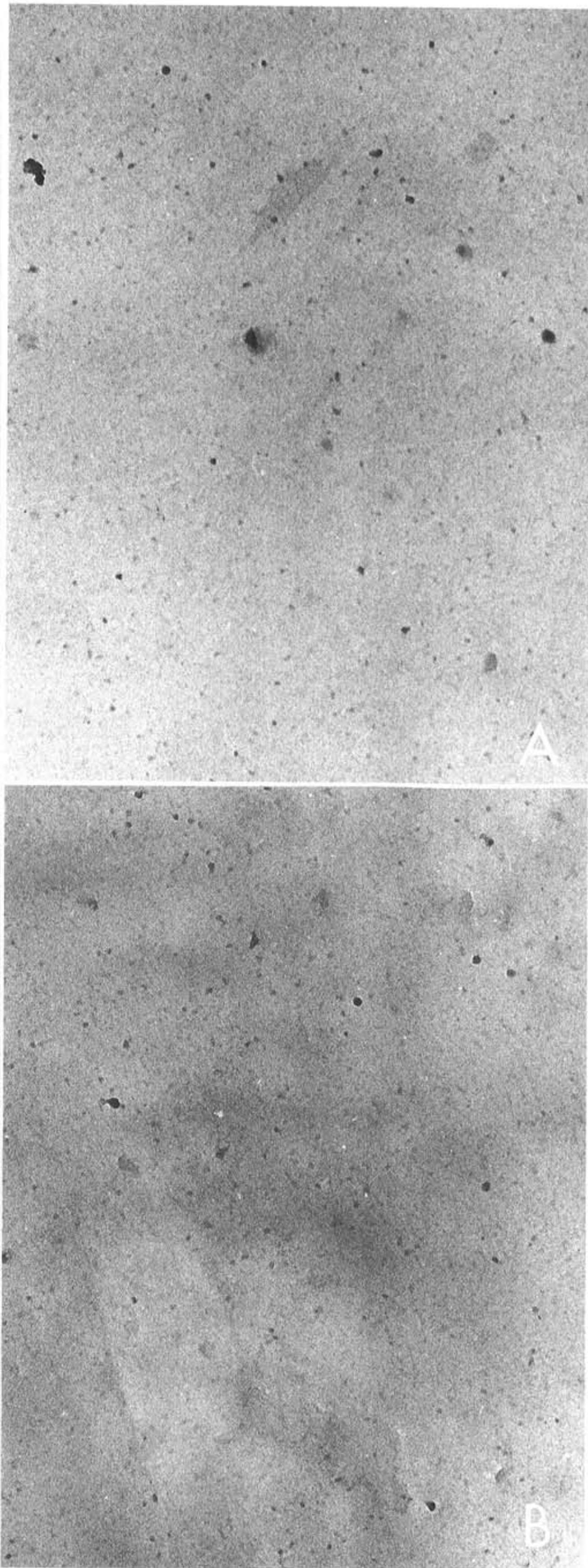

Fig. 3-(A,B). Control grids. **A)** Tobacco ringspot virus infected seed material reacted on a barley stripe mosaic virus antiserum coated grid (×46,840). **B)** Tobacco ringspot virus infected seed material reacted on a grid containing normal serum (×46,840).

temperature. Attached virions were viewed with an Hitachi HU 11-A electron microscope and photographs were taken. Low magnification photographs (×3,000–23,420) were made and particle counts of the resulting 7 × 8 cm micrographs were done to determine the number of virus particles attached at each dilution. High magnification photographs (×7,000–39,500) were made of selected particles for positive identification as virus particles.

RESULTS AND DISCUSSION

All four seedborne viruses were detected in half seeds by SSEM. Particle counts for 10 half-seed assays of TRSV ranged from 1,160 to 1,184 virions at a magnification of 23,420 (Fig. 1-A). Assays of 10 BSMV infected half seeds showed 1,928–1,960 virions attached at a magnification of 3,000 (Fig. 1-B). The number of SMV particles in 10 soybean seed halves ranged from 512 to 544 at ×3,000 and the number of LMV virions in five half-seed assays ranged from 26 to 30 at ×3,000 (Fig. 1-C,D). Tests of 50 seeds of each type showed that 75–80% of the soybean seeds from infected plants contained TRSV, 40–42% of the barley seeds were infected with BSMV, 10% of the lettuce seeds were infected with LMV, and 20% of the soybean seeds contained SMV. These percentages are consistent with known values for these viruses (3,7,9,12).

Virus particles were detected in TRSV-infected soybean seed halves, SMV-infected seed halves, and BSMV-infected seed halves in 1:10, 1:100, and 1:1,000 mixtures (Fig. 2-A,B,C). The 1:1,000 mixture represents one infected seed per 1,000 healthy seeds. At the 1:1,000 mixture, the range of the number of virions attached were: 56–139 for TRSV, 23–26 for BSMV, and 10–22 for SMV. Detection of LMV at 1:100 in mixtures tested (Fig. 2-D) represented one infected seed per 100 healthy seeds. The number of LMV particles per field was four to seven at ×3,000. No virions were attached to control grids (Fig. 3).

Seedborne virus has been successfully detected using SSEM. The size of the seed being tested was, however, a limiting factor. Lettuce seeds were very difficult to assay because of their small size. The different starches and oils in the seeds presented some problems in grinding the extracts, but debris was removed easily from the grids with successive washings in extraction buffer containing 0.4 M sucrose followed by washing with distilled water.

Hamilton and Nichols (6) compared immunodiffusion in SDS-agar gels, enzyme-linked immunosorbent assay (ELISA), and SSEM for the detection of pea seedborne mosaic virus (PSMV) in both seeds and leaves. They concluded that both ELISA and SSEM have potential applications for detecting seedborne viruses in homogenates of seeds. Comparisons of the two techniques in detecting PSMV in homogenates of infected seed showed that SSEM could detect virus in seed lots having only 1–5% infected seed, whereas ELISA gave negative results with such seed lots.

The results of this investigation provide further evidence for use of SSEM as a sensitive technique for detection and identification of seedborne plant viruses. The success of SSEM in mixtures of one infected seed per 1,000 healthy seeds suggests its possible use, along with other serological techniques such as ELISA, in screening seed lots for virus. The detection limits with SSEM on small seed such as that of lettuce as well as with almost any seedborne virus of low titer might be enhanced by combining centrifugal and chemical extraction methods to concentrate the virus in the homogenate before assaying.

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