Detection of Wheat Spindle Streak Mosaic Virus by Serologically Specific Electron Microscopy

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ABSTRACT

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Wheat spindle streak mosaic virus was readily detected by serologically specific electron microscopy (SSEM) in wheat plants suspected to be infected with this virus. About 1,000 times more particles were observed in crude sap from plants grown in the field and in a growth chamber by SSEM than by conventional transmission electron microscopy. Particles were 16 nm in diameter and ranged from 600 to 3,800 nm long, with a modal length of 1,775 nm. SSEM provides a routine rapid assay for detecting WSSMV-infected plants.

Wheat spindle streak mosaic (WSSM) was first observed in winter wheat in southern Ontario, Canada, in 1960 (10) and has since been reported in the United States (2,7,12,16,18) and other countries (1,12). The disease is caused by a soilborne virus transmitted by the plasmodiophoromycetous fungus *Polymyxa graminis* Led. Wheat spindle streak mosaic virus (WSSMV) particles are long flexuous rods that can be found in leaf ultrathin sections and in leaf-dip preparations only with difficulty (2,13,18). Attempts to purify infectious virus had been unsuccessful (6,13) until Usugi and Saito (15) reported purification of WSSMV using several cycles of differential centrifugation followed by cesium chloride density-gradient centrifugation.

At present, diagnosis of WSSM is based primarily on foliar symptoms and the presence of pinwheel inclusion bodies in leaf bundle sheath cells. Because virus concentration appears to be low in infected tissue and preparation of leaf ultrathin sections is time consuming, a sensitive and specific assay for rapid detection of virus particles in infected tissue was sought. Serologically specific electron microscopy (SSEM) has been reported to be even more sensitive than enzyme-linked immunosorbent assay or solid-phase radioimmunosorbent assay for detecting purified blueberry shoestring virus (4). Because of this and in order to conserve antiserum, SSEM was employed to detect WSSMV in field- and growth chamber-grown wheat.

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MATERIALS AND METHODS

Growth chamber studies. *Triticum aestivum* 'Ionia' seed was planted in wooden flats containing soil collected from fields in which WSSMV-infected plants were identified. Uninfected control plants were grown in sterilized greenhouse soil. Flats were placed in a greenhouse maintained at 20 ± 3 C and 3 days after planting, they were transferred to an outdoor cold frame for 60 days (17). After 2 mo, seedlings were transferred to a growth chamber at 10 C with 10,000 lux of light for 10 hr/day (11) and observed for symptom development.

Field studies. WSSMV-infected Ionia wheat plants at the four- to five-leaf stage were collected from fields in Allegan County, MI, in May 1982. Plants were transplanted into 20-cm-diameter plastic pots containing greenhouse soil and were placed in a growth chamber at 10 C, where they were maintained until heading.

SSEM. WSSMV antiserum with a titer of 1/320 in complement fixation was obtained from T. Usugi and Y. Saito (15). The Derrick technique (3) as modified by Milne and Luisoni (8) was used to coat grids with antiserum. Carbon-coated Parlodion-filmed 300-mesh grids were floated on 30-μl drops of a 1:500 dilution of antiserum in 0.06 M Na_2HPO_4-NaH_2PO_4 buffer at pH 7.0 (SSEM buffer). Grids were incubated at 37 C for 3 hr, then rinsed twice for 10 min in SSEM buffer. Virus from infected wheat roots, crowns, and leaves was obtained by first cutting 0.5–1.0 g of tissue into small pieces and grinding it in a mortar and pestle with liquid nitrogen. Three to 5 ml of SSEM buffer was added to the ground tissue to obtain an aqueous suspension, resulting in a final dilution of about 1 g tissue in 5 ml SSEM buffer. For controls, root, crown, and leaf tissues from uninfected plants were ground and prepared as before. Coated and rinsed grids were briefly drained and then floated on 30-μl drops of virus suspension. To achieve maximum trapping of virus particles, grids were incubated for a minimum of 7 hr (usually overnight for convenience) at 4 C, then drained, and negatively stained with 2% ammonium molybdate (AM). To enhance visualization of virus particles, most grids were coated again (decorated) with antiserum (8). For decoration, grids incubated overnight were drained and then floated on 30-μl drops of a 1:500 dilution of antiserum for 1–3 hr at 4 C. Grids were drained and negatively stained with AM.

For grids not pretreated with antiserum, uninfected and infected tissue was ground in a mortar and pestle with liquid nitrogen as described, then SSEM buffer was added to the ground material. Drops of the extracts were placed on carbon-coated Parlodion-filmed grids for 1 min. Grids were then drained and negatively stained with AM.

All grids were examined at ×20,000 in a Philips 201 transmission electron microscope (TEM) operated at 60 kV. An estimate of numbers of virus particles present was made by counting particles visible on 20 random 300-mesh grid squares on each of six grids in duplicate from six different extracts of each tissue assayed.

Tissue fixation. Segments of leaves bearing symptoms were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.0, rinsed in 0.1 M PB, and postfixed in 2% OsO_4 in 0.1 M PB. Fixed tissues were dehydrated in a graded ethanol series, transferred to acetone, and embedded in Spurr's resin (14). Ultrathin sections were positively stained with 5% uranyl acetate in ethanol followed by 0.4% lead citrate in water. Grids were examined with the Philips 201 TEM.

RESULTS

Growth chamber studies. Symptoms appeared on cold-frame-treated wheat 4–6 wk after transfer to the growth chamber at 10 C. Symptoms consisted of a diffuse chlorotic mottle, with a few chlorotic streaks on the foliage. In general, symptoms on plants grown in the growth chamber were less distinct and characteristic of WSSM than those found on infected wheat grown in the field. In addition, growth chamber-grown infected plants were slower to tiller and elongate and remained immature longer than field-grown plants. When leaves were

removed from plants for assay, a piece of leaf tissue (1–2 cm) was left attached to the culm. Until the plants approached maturity, new leaf tissue bearing symptoms similar to those on younger leaves was produced from intercalary meristems, causing the cut leaves to elongate to nearly their original length. New leaf tissue from intercalary meristems and growth of tillers provided a continuing source of virus-infected tissue throughout the year.

Ultrathin sections revealed characteristic pinwheel inclusion bodies in bundle sheath cells of infected leaves. Virions were not found in these cells, however.

Extracts of roots, crowns, and leaves of growth chamber-grown plants were examined for virus by SSEM. Virus was found in extracts of leaves but not in extracts from crowns and roots or in extracts from uninfected plants.

Sample preparations were examined on grids pretreated with WSSMV antiserum and decorated, grids pretreated with antiserum without decoration, and untreated grids. Only one virus particle was seen on all untreated grids. On serum-coated grids with and without decoration, however, an average of 12 virus particles per 300-mesh grid square was observed. Hence, use of SSEM resulted in more than a 1,000-fold increase in the number of particles detected. Because we found that decorated virus particles were seen more easily and could therefore be detected more rapidly, all subsequent virus preparations for SSEM were decorated.

Measurements were made of both decorated and undecorated particles. Lengths of particles from both treatments ranged from 600 to 3,300 nm, with a modal length of 1,800 nm (Fig. 1). Undecorated particles had an average diameter of 16 nm (Fig. 2A), whereas decorated particles were slightly larger (Fig. 2B).

Field studies. Field-grown wheat showed distinct and characteristic symptoms that continued to develop as the plants matured. Until the plants began to head, new basal leaf tissue replacing the apical portion removed for assay was produced, providing a source of virus for several months.

Pinwheel inclusion bodies (but not virions) were found in bundle sheath cells in ultrathin sections of infected leaves, as was reported for growth chamber-grown wheat.

Extracts of roots, crowns, and leaves were examined for presence of virus by SSEM. Three particles each were found in SSEM preparations from roots and crowns, resulting in an average of less than one particle per 300-mesh grid square. Many virus particles were detected in leaf tissue when SSEM was used, whereas particles were not detected in any tissue preparations on grids not treated with antiserum. An average of 13 virus particles per grid square was observed on pretreated grids of leaf tissue extracts. Based on the Student's *t* test, there was no significant difference at the 0.01 level of confidence (*unpublished*) between the number of virus particles on SSEM grids from cold-frame-treated plants and field plants. Particles were not detected in tissues from uninfected plants. Decorated particle lengths ranged from 600 to 3,800 nm, with a modal length of 1,750 nm (Fig. 3). The average diameter of undecorated particles was 16 nm.

DISCUSSION

At present, diagnosis of WSSM cannot be made easily and results obtained from leaf-dip or tissue preparations may be inconclusive. The disease can be confused with soilborne wheat mosaic because of similarities in symptomatology and mode of transmission and with wheat streak mosaic because of pinwheel inclusions and thin flexuous virions in infected tissues. We have found that SSEM with decoration is a sensitive and specific technique for rapid detection of WSSMV in infected tissues. In preparations using grids pretreated with antiserum, about 1,000 times more particles were detected in infected wheat than in preparations on grids that were not pretreated. SSEM has been reported to be as much as 1,000 times more sensitive than conventional

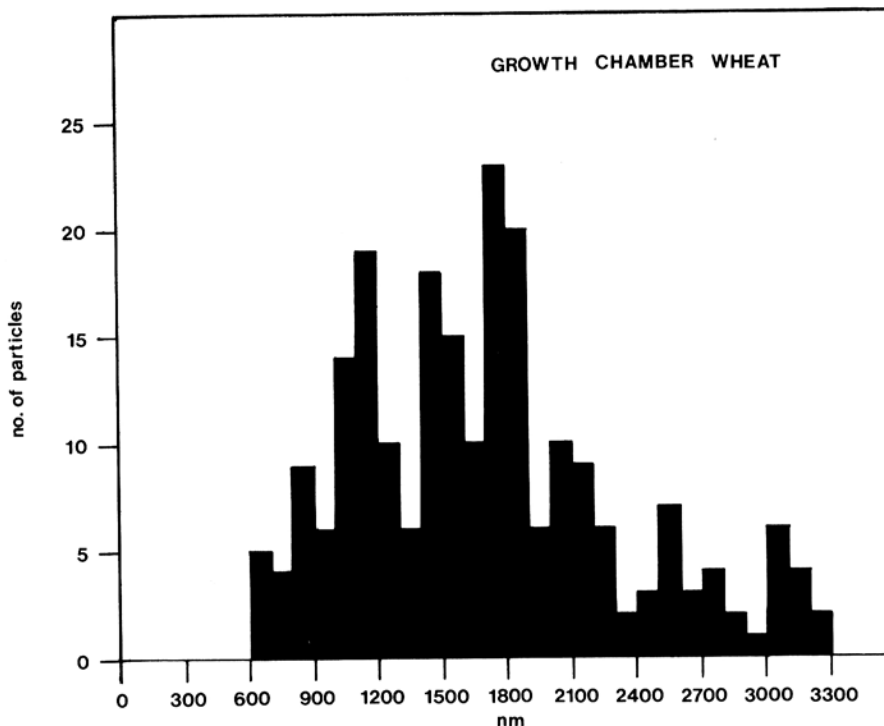

Fig. 1. Distribution of lengths of 225 WSSMV particles found in wheat leaf tissue from cold-frame-treated plants grown in a growth chamber at 10 C. Modal particle length = 1,800 nm.

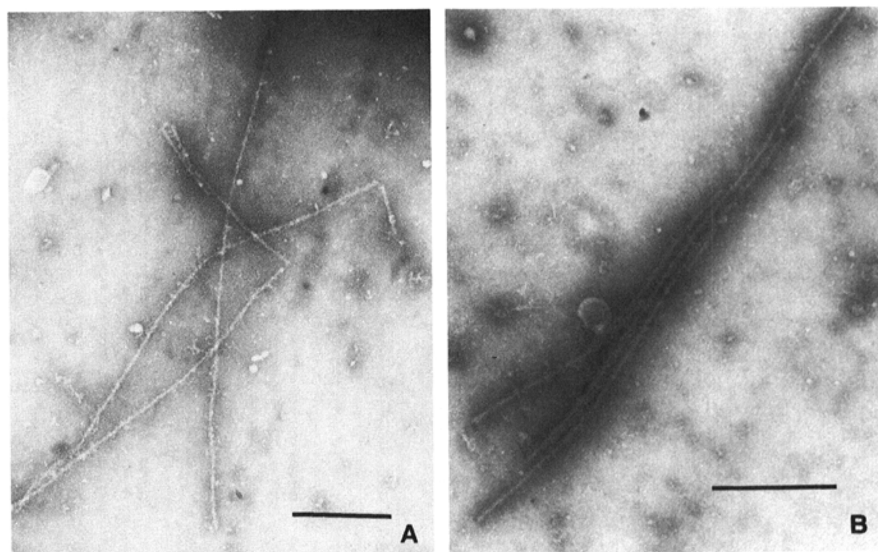

Fig. 2. Wheat spindle streak mosaic virus (WSSMV) particles from leaves of wheat plants grown in a growth chamber at 10 C following cold-frame treatment: **(A)** Particles not coated with antiserum (undecorated) (×37,500). (B) Particles decorated with antiserum (×48,260). Scale bars = 500 nm.

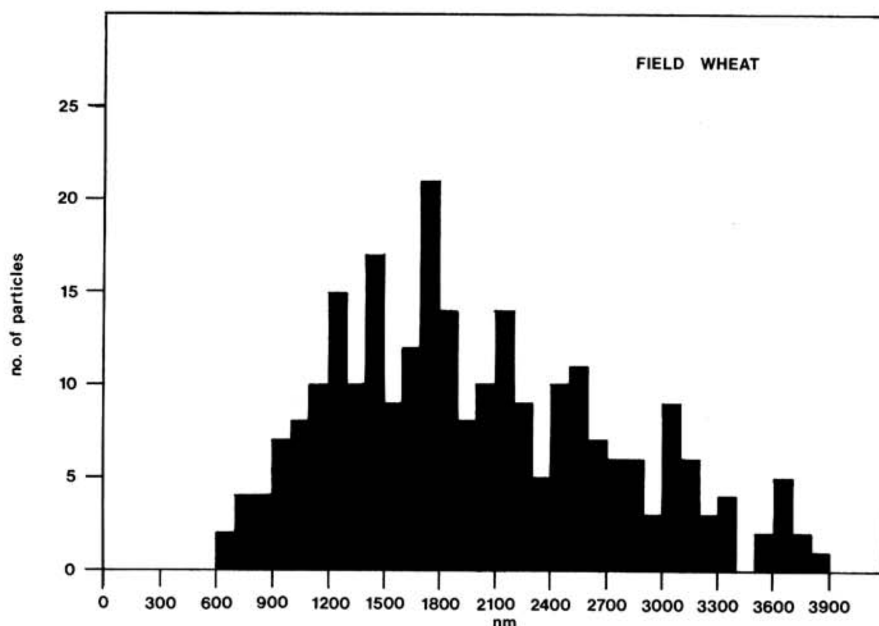

Fig. 3. Distribution of lengths of 225 WSSMV particles found in wheat leaf tissue from plants transferred from the field to a growth chamber at 10 C. Modal particle length = 1,750 nm.

methods of electron microscopy for detecting certain other plant viruses (5). Addition of decoration to the procedure provided enhanced visualization of virus particles and a high degree of specificity.

It was interesting to note the differences between growth chamber and field wheat in relation to WSSM. Growth chamber wheat plants were smaller and had narrower leaves than plants brought in from the field, characteristics that facilitated easier tissue grinding and virus extraction. Symptoms on growth chamber wheat, however, were less distinct than those on field wheat, which may indicate that growth chamber conditions were less than optimal for virus replication.

Age of host tissue may have had an effect on virus location within the plant. A few virus particles were found in extracts from roots and crown of young field-grown plants. Attempts to isolate particles from the same tissues at a later date were unsuccessful, whereas particles could readily be isolated from leaf tissue, suggesting movement of virus from roots up to the terminal leaves. Particles were not found in roots and crowns from growth chamber-grown plants, perhaps because when tissues from these plants were assayed for virus, the plants had been growing in 10 C 2–3 mo longer than field wheat had been growing so the disease may already have been in the latter stages of its cycle.

Several ranges in length and diameter measurements of WSSMV particles have been reported. Reports on diameter measurements range from 12 to 20 nm (2,6,9); our report of 16 nm falls within this range. Variations in length measurements are greater, however, with reported measurements ranging from 100 to 3,200 nm (2,6,9,13,15,18). We have reported a range of 600–3,800 nm, with a modal length of 1,775 nm, for particles extracted from infected leaves by grinding in liquid nitrogen. Because WSSMV particles are long and thin, they are fragile and subject to breakage during preparation of leaf dips and extraction from tissue. Although some short particles were obtained in our tissue extracts, we found that grinding in liquid nitrogen was a less tedious and more satisfactory procedure than grinding in buffer for quick release of relatively intact virus particles from infected tissues.

Results of this investigation provide evidence favoring SSEM as a rapid and sensitive technique for detecting WSSMV in plants showing suspicious symptoms.

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