Rapid Identification of Maize Stripe Virus

O. E. Bradfute and J. H. Tsai

Department of Plant Pathology, Ohio State University, Ohio Agricultural Research and Development Center, Wooster 44691; and University of Florida, Fort Lauderdale Research and Education Center, Fort Lauderdale 33314, respectively.

Salaries and research support provided by State and Federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. Journal article 38-87. Florida Agricultural Experiment Station Journal Series R00079.

Research supported in part by the Inter-American Institute for Cooperation on Agriculture, by the Cargill Foundation of Brazil, and by Pioneer Hi-Bred International, Inc.

We gratefully acknowledge photomicroscopy by R. Scott Poethig and technical assistance by Ms. G. Echenique.

Accepted for publication 25 January 1990 (submitted for electronic processing).

ABSTRACT

Bradfute, O. E., and Tsai, J. H. 1990. Rapid identification of maize stripe virus. Phytopathology. 80:715-719.

Maize stripe virus (MStpV) was identified in maize leaves by either diagnostic symptoms or needle-shaped crystals. The symptoms consisted of chlorotic patterns of overlapping circles with distinct margins when magnified 3–10×. The crystals were found in abundance by phase-contrast light microscopy in sap from symptomatic leaf areas. The crystals were similar to crystals of MStpV noncapsid protein in appearance and in differential solubility and reacted with antiserum to MStpV noncapsid protein in immunofluorescence microscopy. Diagnostic symptoms and the crystals could be found readily in naturally and experimentally MStpV-infected maize plants with a wide range of gross symptom types and throughout disease development, but not in maize infected by other maize viruses, or mycoplasmas, or in maize with sorghum downy mildew. The crystals were found in MStpV-infected sorghum, itchgrass, and two species of annual teosinte. By the presence of diagnostic symptoms and the crystals in leaf samples, the distribution of MStpV or a similar virus in maize was confirmed in Botswana, Mauritius, Nigeria, Peru, and Venezuela, where MStpV has been reported previously, and extended to Argentina, Brazil, and Puerto Rico. Similar needle-shaped crystals were found in maize leaves infected with rice stripe virus, confirming the similarity of this virus to MStpV and suggesting that direct observation of needle-shaped crystals may also indicate the presence of other viruses in the rice stripe virus group.

Additional keywords: rice stripe virus group, tenuiviruses.

Maize stripe disease occurs in many lowland, humid, tropical, and subtropical regions of the world (9). It is a major component of a severe maize (*Zea mays* L.) disease complex in southern Florida (5). Several symptoms of maize stripe disease are similar to those caused by other maize pathogens (11,14). Some attempts to find a pathogen have failed (14,24), and reports on the nature of the pathogen, maize stripe virus (MStpV), have been in conflict (13,19).

The disease was first described from East Africa by Storey (22) who recognized two symptom types: broad and narrow stripes in maize leaves. Kulkarni associated these symptoms with different diseases, reserving the name maize stripe for the disease with broad stripes (19). The pathogens of both diseases are transmitted by the corn delphacid, *Peregrinus maidis* Ashmead.

Maize mosaic virus, described by Herold in electron micrographs of maize leaves with a striping disease from Venezuela, is also transmitted by *P. maidis* (15). When Tsai (24) discovered a maize striping disease in Florida with a *P. maidis*-transmitted pathogen, electron microscopic examination of diseased leaves failed to reveal the abundance of rhabdovirus particles that are easily found in maize/mosaic-diseased leaves from Florida (4) and in maize leaves with similar striping symptoms from Texas and Hawaii (2,3). Instead, novel 3-nm filaments and needle-shaped crystals were found (O. E. Bradfute, *unpublished*) that are presumed to correspond, respectively, to the nucleoprotein filaments and noncapsid protein subsequently associated with MStpV (13). Similar filaments and noncapsid proteins as well as other common properties characterize a rice stripe virus group of viruses (23) or "tenuiviruses," as they are so named (12). This group includes rice stripe virus (17,18), rice hoja blanca virus (8,20), rice grassy stunt virus (16), Echinochloa hoja blanca virus (8), winter wheat mosaic virus (23), and European wheat striate virus (23), as well as maize stripe virus (11).

Falk and Tsai have used antibodies to the 16,300 MW, noncapsid protein in enzyme-linked immunosorbent assays for MStpV-infected plants (9). We report here diagnostic symptoms for MStpV, a rapid assay for MStpV-infected plants by direct observation of noncapsid protein crystals in expressed leaf sap, the use of this rapid assay to confirm and possibly extend the known distribution of MStpV or a similar virus in maize, and the possibility this simple assay may also indicate the presence of other viruses of the rice stripe virus group.

MATERIALS AND METHODS

Sources of diseased plants. MStpV-infected plants included naturally infected maize found in field surveys in southern Florida (5), maize experimentally infected in Florida (5,24), or maize experimentally infected in Ohio with a Florida isolate of MStpV (13) supplied by L. R. Nault (Ohio State University, OARDC, Wooster). Other maize samples suspected of MStpV infection were from Argentina, collected by C. Martinez (Instituto Nacional de Technologia Agropecuaria, Pergamino), Alicia DeBiasi (Instituto Nacional de Technologia Agropecuaria, Buenos Aires), and O. E. Bradfute; Botswana, supplied by P. Jones (Rothamsted Experimental Station, Harpenden, Herts., England); Brazil, collected by G. Viegus (Cargill Foundation, Sao Paulo), D. Rodrigres (Cargill Agricola S. A., Sao Paulo), J. L. Dodd (Cargill Inc., Aurora, IL), and O. E. Bradfute; Mauritius, supplied by L. J. C. Autrey, (Sugar Industry Research Institute, Reduit); Nigeria, supplied by R. C. Muo (National Cereals Research Institute, Moor Plantation, Ibadan); Peru, supplied by J. Castillo Loayza, (Universidad Nacional Agraria, La Molina, Lima) and L. R. Nault; Puerto Rico, supplied by A. Sotomayor-Rios (USDA, ARS, Isabela) and W. R. Findley (USDA, ARS, Wooster, OH); and Venezuela, supplied by R. J. Lastra, (Instituto Venezolano de Investigaciones Cientifica, Caracas). Rice stripe virus-infected maize plants from Japan were supplied by S. Yamashita (University of Tokyo).

Control tests included maize leaves experimentally infected with a variety of other viruses and mycoplasmas. Samples infected with maize streak virus were supplied by V. D. Damsteegt (USDA, ARS, Frederick, MD); with maize chlorotic mottle virus by D. T. Gordon (Ohio State University, OARDC, Wooster); with maize chlorotic dwarf virus by J. K. Knoke (USDA, ARS, Wooster, OH); with maize white line mosaic virus and maize dwarf mosaic virus (strains A and B) by R. Louie (USDA, ARS, Wooster, OH); with maize rayado fino virus, corn stunt spiroplasma (*Spiroplasma kunkelii* (Whitcomb et al)[25]), and maize bushy stunt mycoplasmalike organism by L. R. Nault; and with maize mosaic virus, corn stunt spiroplasma, and maize bushy stunt mycoplasmalike organism by J. H. Tsai. Maize samples naturally infected with *Peronosclerospora sorghi* (Weston & Uppal) C. G. Shaw (causal agent of sorghum downy mildew) were supplied by R. W. Toler (Texas A & M University, College Station).

Symptoms. Leaf symptoms were examined with a 3×, 7×, or 10× hand lens and recorded with transmitted illumination on Ektachrome film in a 35-mm Nikon camera with a Micro-NIKKOR lens.

Assays. Leaf samples were cooled, but not frozen, and mailed in sealed, insulated containers when possible and maintained at 4 C until assayed. Symptomatic samples, 0.2–0.5 cm^2, excluding green tissue and the midrib or primary vein, were selected from fully expanded, more mature regions of the leaf laminae. A 0.2–0.3 μl droplet of sap was expressed directly from a leaf sample onto a microscope slide with small needle-nosed pliers. A cover glass was then applied with sufficient pressure to spread the droplet, wetting the cover glass, and reducing the thickness of liquid sap. The wet area under the cover glass, about 10 mm across, was examined with a Carl Zeiss light microscope equipped with high-dry, Zernike phase-contrast optics. Alternatively, a few samples were examined with a Carl Zeiss light microscope equipped with an oil immersion, 100× objective lens with iris diaphragm and an oil immersion dark-field condenser. Observations of samples from three plants were made in each test unless stated otherwise.

Double-antibody sandwich enzyme-linked immunosorbent assays (ELISA) as previously described (9) were used in comparative tests.

Crystal identification. For the following tests a small droplet of sap from a symptomatic region of a leaf lamina was expressed directly onto a microscope slide as before, spread with the edge of a cover glass, and allowed to dry at room temperature. The presence of crystals in the dried sap was confirmed by phase-contrast light microscopy as before.

Crystal solubility was partially characterized by introducing 1-μl droplets of different solutions in separate tests to the edge of a cover glass placed on dried sap and monitoring the crystals with phase-contrast light microscopy as the solution spread between the slide and cover glass.

Indirect immunofluorescence microscopy was used to test the crystals for a serological relationship to MStpV noncapsid protein. Primary antibody in the form of rabbit antiserum to MStpV noncapsid protein (supplied by R. E. Gingery, USDA, ARS, Wooster, OH) was serially diluted in phosphate-citrate buffer, pH 5.5, and a 1-μl droplet applied to the dried sap in separate tests. The buffer was prepared by mixing 0.1 M Na_2HPO_4 and 0.05 M citric acid. After incubation in a moist chamber at room temperature for 2 hr, a 1-μl droplet of a secondary antibody, fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Miles-Yeda, Ltd., Miles Laboratories, Inc.) diluted 1:32 in phosphate-citrate buffer, pH 5.5, was added. A cover glass was applied and the slide was examined with a Carl Zeiss light microscope equipped with an incident-light fluorescence system (mercury HBO 100 W/2 light source, DF 485/22 exciter filter, DR 500 LP dichromatic beam splitter, and No. 50 barrier filter) and transmitted-light, phase-contrast optics. With this microscope a specimen field could be viewed in phase contrast or fluorescence alternately in rapid succession, or in combined phase contrast and fluorescence, permitting the association of fluorescence with specific microscopic structures. Experimental controls consisted of incubation of dried sap in separate tests with buffer alone, with primary antibody solution alone, with secondary antibody solution alone, incubation of the primary and secondary antibody solutions together without the dried sap, and incubation of dried sap from healthy control maize incubated sequentially with primary and secondary antibody solutions.

RESULTS

Symptoms. In experimentally infected maize plants, initial maize stripe symptoms were circular to oval chlorotic spots, 0.5 mm in diameter to 1 × 2 mm, respectively, with the longer dimension parallel to the veins (Fig. 1). Viewed with transmitted light, the spots appeared translucent, usually light green or light yellow green in color; white or yellow was less common. The margins of the spots were smooth and distinct, even when viewed at a magnification of 3–10×. The spots were scattered or aligned in rows parallel to the veins, and usually between, but occasionally on, secondary veins. As the disease progressed (Figs. 1 and 2), the spots became more numerous and appeared superposed, giving an outline of overlapping circles with distinct edges similar to the outline of cumulus clouds against an otherwise clear sky. Chlorotic patterns appeared in parallel streaks (irregular edges), stripes (smooth edges), or bands that varied in width (some exceeding half the width of the lamina), or a leaf was totally chlorotic (see previous reports [11,14]). Some of these symptoms were similar to the gross leaf symptoms resulting from infections of maize rayado fino virus, maize mosaic virus, corn stunt spiroplasma, *Peronosclerospora* spp. (agents of downy mildews), and possibly other pathogens (1,4,5,21). However, when viewed at 3–10×, the chlorotic areas in the form of overlapping circles with smooth, distinct margins (best seen in Fig. 2) were unique in comparison to symptoms of other maize virus, mycoplasma, or downy mildew diseases (specifically diseases caused by maize mosaic virus, maize rayado fino virus, maize streak virus, maize dwarf mosaic virus [strains A & B], maize chlorotic dwarf virus, maize chlorotic mottle virus, maize white line mosaic virus, corn stunt spiroplasma, maize bushy stunt mycoplasmalike organism, or *P. sorghi*). With few exceptions, these distinctive symptoms were found in naturally infected maize plants.

Crystals. Needle-shaped crystals were observed by phase-contrast microscopy in sap expressed from the chlorotic regions of MStpV-infected leaves. The crystals were usually abundant and readily found by scanning the droplet of sap spread between the slide and coverslip with a 16× phase-contrast objective lens and 12.5× eyepiece. The detailed morphology of many crystals was obvious in a single microscope field of view produced with a 40× phase-contrast objective lens and 12.5× eyepiece (Figs. 3 and 4). They varied in length and width, with the thinner crystals appearing curved (Fig. 4) as a consequence of positioning or flexing in the flow of sap between the microscope slide and cover glass. Our experiments were not adequate to determine any consistent association between crystal length or flexibility and host plant species, conditions of sample storage, or possibly virus type or strain. Occasionally, samples from young leaves with high water content required examination of many fields before a few crystals were found. However, crystals were readily found in these same leaves after a few hours of storage, slight desiccation, or examination of more mature symptomatic leaf areas. Crystals were not found in green areas of MStpV-infected leaves or in leaves of healthy control maize plants. The presence or absence of crystals was consistent in replicate samples of plants, except they were not always found in MStpV-infected plants if sap was expressed from the midrib or primary vein or from symptomatic regions intermixed with green tissue.

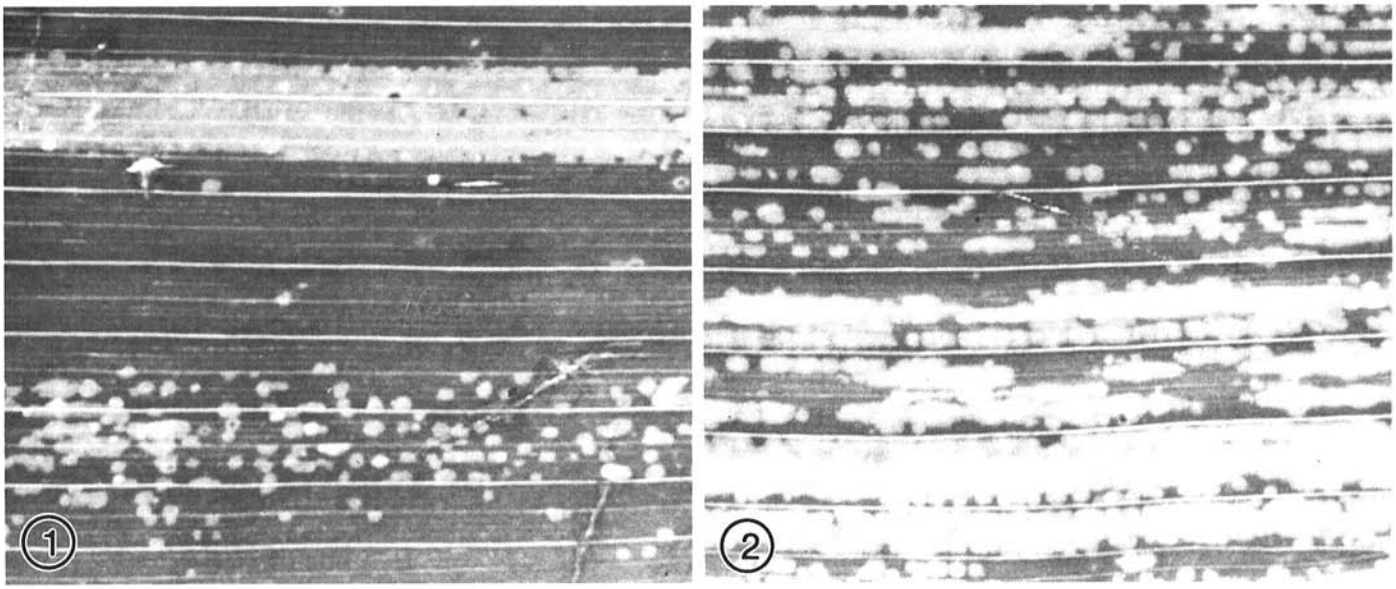
Needle-shaped crystals were also readily observed in oil immersion, dark-field, light microscopy. This provided a single test for both spiroplasma (7) and crystals in leaf samples where advanced symptoms of corn stunt spiroplasma were not always distinguishable from advanced symptoms for MStpV.

Solubility tests showed that needle-shaped crystals dissolved in distilled water, sap from healthy maize leaves, and phosphate-

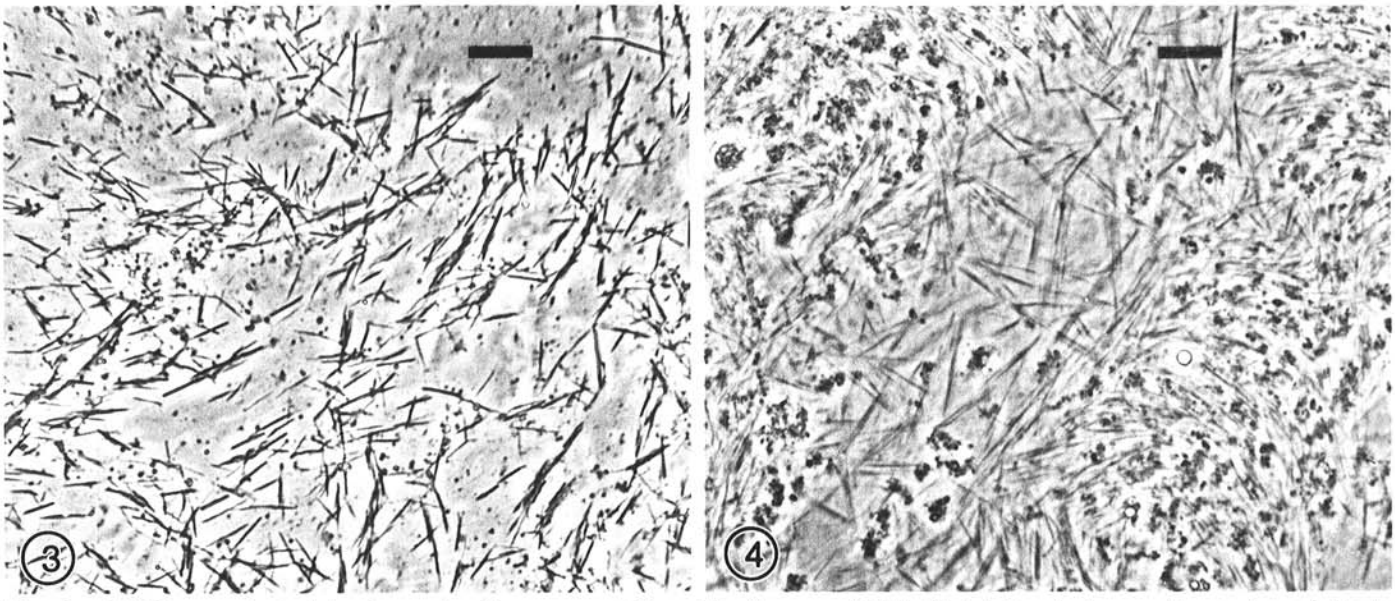
citrate buffer pH 6.5, but not in phosphate-citrate buffer pH 5.5. Fluorescent, needle-shaped crystals were found by indirect immunofluorescence tests of sap from symptomatic maize leaves that included rabbit antiserum to MStpV noncapsid protein (diluted 1:100 or 1:500) and the fluorescent labeled secondary antibody to rabbit IgG, but not in any of the control tests.

Assays. Needle-shaped crystals were found in experimentally infected maize in all stages of maize stripe symptom development: initial symptoms (8/8), well-developed symptoms (6/6), senescent leaves with advanced symptoms (9/9), but not in healthy maize plants (0/6). (Test results are expressed as [number of plants with crystals/number plants tested].) In a double blind test, needle-shaped crystals were found in all of 10 experimentally infected maize plants and in none of 10 healthy control maize plants. In a comparison of assay methods, all of 20 experimentally infected and none of 20 healthy control maize plants were found to contain needle-shaped crystals and to be positive for MStpV noncapsid protein as determined by ELISA.

The crystals were found in naturally and experimentally infected maize leaves with all types of maize stripe symptoms: scattered or aligned spots, streaks, stripes, bands, and totally chlorotic leaves. They were not found in maize plants experimentally infected with maize mosaic virus, maize rayado fino virus, maize streak virus, maize dwarf mosaic virus (strains A and B), maize chlorotic dwarf virus, maize chlorotic mottle virus, maize white line mosaic virus, corn stunt spiroplasma, maize bushy stunt mycoplasmalike organism, or in field-collected maize plants with sorghum downy mildew. However, needle-shaped crystals were found in field-collected maize plants coinfected with MStpV and spiroplasma (two plants) or maize rayado fino virus (one plant). Needle-shaped crystals were found in all field-collected maize plants with the distinctive symptoms of MStpV (66/66), but also in six field-collected maize plants without distinctive symptoms of MStpV.



Figs. 1 and 2. Close-up photographs of maize leaves with distinctive symptoms of MStpV. **1,** Scattered chlorotic spots and chlorotic bands typical of leaf areas with initial and advanced symptom development, respectively. **2,** Chlorotic patterns typical of leaf areas with intermediate symptom development. Chlorotic areas with an outline of overlapping circles and with margins that remain distinct when magnified were unique in comparison to symptoms of other maize virus, mycoplasma, and downy mildew diseases (magnification approximately 3×).



Figs. 3 and 4. Phase-contrast photomicrographs of sap expressed from chlorotic areas of MStpV-infected maize leaves with symptoms similar to those shown in Figures 1 and 2. **3,** Masses of high-contrast, needle-shaped crystals typically observed. **4,** Cellular debris and needle-shaped crystals showing variation of crystal length, width, and curvature (scale marker represents 10 μm).

Needle-shaped crystals were found in MStpV-symptomatic maize leaves tested directly from the field or greenhouse, or in leaves stored at room temperature for several days, at 4 C for several weeks, or at 37 C for 1 hr. They also were found in wilted maize leaves, providing the cells regained turgor after rehydration. They were not found after leaves were permanently wilted, were stored at 60 C for 1.5 hr, were frozen and thawed, or had become necrotic or rotten during transport or storage.

Needle-shaped crystals were also found in MStpV-experimentally infected plants other than maize: sorghum (*Sorghum bicolor* (L.) Moench.), itchgrass (*Rottboellia exaltata* L. fil.), and two species of annual teosinte (*Zea mays* subsp. *mexicana* (Schrader) Iltis and Z. luxurians (Durieu & Asch.) Bird).

Needle-shaped crystals were detected in maize leaves from Argentina (2/2), Botswana (1/1), Brazil (2/2), Mauritius (4/4), Nigeria (3/3), Peru (5/5), Puerto Rico (2/2), and Venezuela (2/2). (Test results are expressed as [number of plants with crystals/number of plants with unique MStpV symptoms tested].)

Similarly appearing crystals were found in field-collected maize leaves from Japan naturally infected with rice stripe virus (2/2) and in maize leaves from Mauritius experimentally infected with maize chlorotic stripe virus (3/3). (Test results are expressed as [number of plants with crystals/number of plants tested].) The size and condition of the leaf samples received were insufficient to determine if the maize plants infected with rice stripe virus or maize chlorotic stripe virus had exhibited symptoms similar to those we describe as diagnostic for MStpV.

DISCUSSION

We find the unique symptoms of maize stripe virus described in this report are diagnostic for MStpV infections of maize. Although a few natural infections escape detection by symptomatology alone, these unique symptoms are useful in conducting field surveys and selecting samples to be tested for MStpV. Additional research would be required to establish if these symptoms are useful in diagnosing MStpV in plant species other than maize or in diagnosing any other viruses of the rice stripe virus group. In preliminary examinations of magnified symptoms of virus-infected leaves, we found subtle differences more difficult to distinguish on Gramineae with leaves smaller than those of maize.

The needle-shaped crystals found by phase-contrast light microscopy in sap of symptomatic regions of maize leaves appear to provide a reliable assay for MStpV over a wide range of conditions, including experimental transmissions, field collections, and double infections. The crystals occurred in all symptomatic maize plants tested from the two MStpV cultures used in virus characterization and assay development studies (8–10,13,24). Although no direct, systematic comparisons with immunological, biochemical, or transmission assays of naturally infected plants are available, the test was equivalent to ELISA in identifying experimentally infected maize plants. Furthermore, the rapidity, simplicity, and ease of evaluation of this test should provide a useful complement to more elaborate methods of identification. The test is faster and requires less skill than electron microscopy of negatively stained leaf dip preparations for virus particles, light microscopy for virus inclusions in stained epidermal strips (6), or dark-field light microscopy for spiroplasmas (7). This test could be executed in minimally equipped laboratories and should be useful in disease surveys and in monitoring breeding programs for resistance.

We conclude the needle-shaped crystals found in this work are composed of the 16,300 MW, virus-associated, noncapsid protein (10,13). The crystals reacted to antiserum to MStpV noncapsid protein, and both the crystals and the protein are found in relatively large amounts in plants infected with MStpV. The noncapsid protein has been estimated to compose up to about 10% (w/w) of infected maize leaf tissue (10). They are neither found nor reported in healthy maize plants. The two also are not found in maize plants infected with other viruses or mycoplasmas, with the exception of rice stripe virus (17,18) and possibly maize chlorotic stripe virus (if it is found to differ from MStpV). Moreover, the solubility of purified and crystallized noncapsid protein in phosphate-citrate buffer at pH 6.5, but not at pH 5.5, and its appearance in phase-contrast light microscopy are the same as the crystals we found by direct examination of plant sap.

The geographical distribution of MStpV suggested from our data is consistent with previous reports (11) for the occurrence of MStpV in Botswana, Mauritius, Nigeria, Peru, Venezuela, and southern Florida. Our data suggest an extension of the known distribution of MStpV or a similar virus to Argentina, Brazil, and Puerto Rico, where the virus could be economically significant, but previously not distinguished from the other viruses infecting maize. Although the addition of these new areas to the known distribution of MStpV would be consistent with the wide tropical and subtropical distribution of the MStpV vector (*P. maidis*), positive identification of MStpV in these new areas should await confirmation.

Our limited data from tests of plant species other than maize are consistent with the established host range of MStpV (11) and suggest that the presence of needle-shaped crystals may provide a useful assay for MStpV in a variety of plant species. However, the appearance of similar needle-shaped crystals in rice stripe virus-infected maize plants suggests that this test may not uniquely identify MStpV, but may instead identify viruses of the rice stripe virus group. If further direct microscopic examination of leaf sap establishes a consistent association of needle-shaped crystals with other viruses in this group, then consideration of host range and geographical distribution could be used with this rapid test to indicate the presence of an individual virus or a subgroup of the rice stripe virus group in a particular locality. For example, MStpV is the only virus of this group known to infect maize in the tropics and subtropics of the western hemisphere (8,11,20).

LITERATURE CITED

1. Bradfute, O. E., Nault, L. R., Gordon, D. T., Robertson, D. C., Toler, R. W., and Boothroyd, C. W. 1980. Identification of maize rayado fino virus in the United States. Plant Dis. 64:50-53.
2. Bradfute, O. E., and Robertson, D. C. 1974. Rhabdovirus-like particles in stunted maize from Texas and Hawaii. Pages 266-267 in: Proc. Electron Microsc. Soc. Am., 32nd. St. Louis, MO. C. J. Arceneaux, ed.
3. Bradfute, O. E., and Robertson, D. C. 1974. Possible occurrence of maize mosaic virus in the continental United States. Proc. Am. Phytopathol. Soc. 1:36.
4. Bradfute, O. E., and Tsai, J. H. 1983. Identification of maize mosaic virus in Florida. Plant Dis. 67:1339-1342.
5. Bradfute, O. E., Tsai, J. H., and Gordon, D. T. 1981. Corn stunt spiroplasma and viruses associated with a maize disease epidemic in southern Florida. Plant Dis. 65:837-841.
6. Christie, R. G., and Edwardson, J. R. 1977. Light and electron microscopy of plant virus inclusions. Fla. Agric. Exp. Stn. Monogr. 9. 150 pp.
7. Davis, R. E., and Worley, J. F. 1973. Spiroplasma: Motile, helical microorganism associated with corn stunt disease. Phytopathology 63:403-408.
8. Falk, B. W., Morales, F. J., Tsai, J. H., and Niessen, A. I. 1987. Serological and biochemical properties of the capsid and major noncapsid proteins of maize stripe virus, rice hoja blanca, and Echinochloa hoja blanca virus. Phytopathology 77:196-201.
9. Falk, B. W., and Tsai, J. H. 1983. Assay for maize stripe virus-infected plants by using antiserum produced to a purified noncapsid protein. Phytopathology 73:1259-1262.
10. Falk, B. W., Tsai, J. H., and Lommel, S. A. 1987. Differences in levels of detection for the maize stripe virus capsid and major noncapsid proteins in plant and insect hosts. J. Gen. Virol. 68:1801-1811.
11. Gingery, R. E. 1985. Maize stripe virus. No. 300 in: Descriptions of Plant Viruses. Assoc. Appl. Biol., Natl. Veg. Res. Stn., Wellesbourne, Warwick, U.K. 4 pp.
12. Gingery, R. E. 1988. The rice stripe virus group. Pages 297-329 in: The Filamentous Plant Viruses. R. G. Milne, ed. Plenum Press, New York.
13. Gingery, R. E., Nault, L. R., and Bradfute, O. E. 1981. Maize stripe

virus: Characteristics of a member of a new virus class. Virology 112:99-108.
14. Greber, R. S. 1981. Maize stripe disease in Australia. Aust. J. Agric. Res. 32: 27-36.
15. Herold, F. 1972. Maize mosaic virus. No. 94 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
16. Hibino, H., Usugi, T., Omura, T., Tsuchizaki, T., Shohara, K., and Iwasaki, M. 1985. Rice grassy stunt virus: A planthopper-borne circular filament. Phytopathology 75:894-899.
17. Koganezawa, H. 1977. Purification and properties of rice stripe virus. Pages 151-154 in: Symposium on Virus Diseases of Tropical Crops. Tropical Agriculture Research Series No. 10, Tropical Agriculture Research Center, Tsukuba, Ibaragi, Japan.
18. Koganezawa, H., Doi, Y., and Yora, K. 1975. Purification of rice stripe virus. Ann. Phytopathol. Soc. Jpn. 41:148-154.
19. Kulkarni, H. Y. 1973. Comparison and characterization of maize stripe and maize line viruses. Ann. Appl. Biol. 75:205-216.
20. Morales, F. J., and Niessen, A. I. 1983. Association of spiral filamentous viruslike particles with rice hoja blanca. Phytopathology 73:971-974.
21. Shurtleff, M. C., ed. 1980. Downy mildews. Pages 28-37 in: Compendium of Corn Diseases. 2nd ed. American Phytopathological Society, St. Paul, MN. 105 pp.
22. Storey, H. H. 1936. Virus diseases of East African Plants. IV. A survey of the viruses attacking the Gramineae. East Afr. Agric. J. 1:333-337.
23. Toriyama, S. 1982. Characterization of rice stripe virus: a heavy component carrying infectivity. J. Gen. Virol. 61:187-195.
24. Tsai, J. H. 1975. Occurrence of a corn disease in Florida transmitted by *Peregrinus maidis*. Plant Dis. Rep. 59:830-833.
25. Whitcomb, R. F., Chen, T. A., Williamson, D. L., Liao, C., Tulley, J. G., Bove, J. M., Mouches, C., Rose, D. L., Coan, M. E., and Clark, T. B. 1986. *Spiroplasma kunkelii* sp. nova.: Characterization of the etiological agent of corn stunt disease. Int. J. Syst. Bacteriol. 36:170-178.