A New Disease of Maize and Wheat in the High Plains

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ABSTRACT

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Dent corn, sweet corn, and blue corn showing severe viruslike symptoms were found in Texas, Kansas, Colorado, Idaho, Nebraska, and Utah in 1993 and 1994. The disease can be devastating to susceptible genotypes, and was also found in wheat. Pathogen nucleoproteins from the infected tissue were concentrated by ultracentrifugation and analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis revealing the coat protein of wheat streak mosaic virus (WSMV) and a unique, approximately 32-kDa protein. Extraction of symptomatic tissue and purification by density gradient centrifugation yielded a product with a nearly pure 32-kDa protein but no defined viruslike structure. Neither electron microscopy of leaf dip preparations nor density gradient purification trials consistently revealed particles other than WSMV. An antiserum has been prepared to the 32-kDa antigen. The pathogen, which we have termed the high plains virus, is transmitted by eriophyid mites.

Maize plants with severe virus symptoms were received in mid-June 1993 for diagnosis. Serological analysis and mechanical inoculation identified only wheat streak mosaic virus (WSMV). The tests were negative for maize dwarf mosaic virus (MDMV), sugarcane mosaic virus (SCMV), johnsongrass mosaic virus (JgMV), sorghum mosaic virus (SrMV), maize stripe virus (MStpV), an unnamed tenui-like virus from oats, and maize chlorotic mottle virus (MCMV). WSMV was found in most of the samples, but symptom expression was too severe for WSMV alone; therefore, a second pathogen was suspected. Field observers noted that the disease was often worse on the side of the corn field near wheat, but by the time the disease was noted in corn the wheat was mature.

In September 1993, wheat samples with severe virus symptoms and necrosis were received from the same areas of Texas and Idaho that had diseased maize. In the late fall of 1993 and spring of 1994, affected wheat appeared in several additional areas of Kansas and Colorado.

In the spring of 1994, several new locations reported maize affected by this new disease. WSMV is common throughout this area but symptom severity suggested a second pathogen. We analyzed tissues to identify the second pathogen, which we have called the high plains virus (HPV), and determine its significance. Since the vector was initially unknown, most experiments were done with naturally infected maize and wheat sent by cooperators.

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MATERIALS AND METHODS

Viral nucleoproteins were isolated or concentrated by a small-scale purification procedure and the proteins were denatured in sodium dodecyl sulfate (SDS) and analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) (12). Two grams of infected tissue or appropriate control tissue was homogenized in 15 ml of 0.1 M (NH_4) citrate buffer, pH 6.5, containing 0.1% β-mercaptoethanol. The juice was expressed through Miracloth (Chicopee Mills, Millton, NJ) and centrifuged for 20 min at 10,000 rpm in an SS 34 rotor (Sorvall, Newtown, CT). Triton X-100 (1.5 ml of 20%) was added to the supernatant and it was centrifuged in a Ti50.2 rotor (Beckman Instruments, Fullerton, CA) for 2 h at 36,000 rpm through a 5-ml pad of 20% sucrose. The pellet was suspended in 300 µl of 0.05 M $NaPO_4$ buffer, pH 7.0. Aliquots were diluted into dissociation buffer containing 0.06 M Tris, 0.004 M HCl, 2% (wt/vol) SDS, 0.004% crystal violet, 10% (wt/vol) Ficoll, and 5 mM dithiothreitol, and heated for 3 min at 100°C. Occasionally, samples were subsequently alkylated by adding iodoacetamide to 15 mM and heating 5 min at 75°C.

The SDS-proteins were resolved by electrophoresis for 16 h at 80 V in 8 to 20% polyacrylamide gradient gels (11). Gels were silver stained by the method of Morrissey (14).

Nucleoproteins were further purified by the methods of Falk and Tsai (5). Centrifugation in linear 10 to 40% (vol/vol) sucrose density gradients for 3 h at 24,000 rpm in an SW27 rotor (Beckman Instruments) occasionally produced a dense zone containing WSMV at about 60% of the tube depth. In addition, a diffuse light-scattering zone occurring between 15 and 30% of the tube depth was collected, diluted, and then concentrated by centrifugation for 2 h at 75,000 gav (average gravitational force) in the Ti50 rotor (Beckman). Nucleoprotein samples from this zone were analyzed for proteins by PAGE or extracted by the methods of Falk and Tsai (5) to recover nucleic acids. The nucleoprotein was injected into a rabbit to produce antiserum by a series of three injections of 1 mg each. Serum was harvested 3 months after the initial injection. Enzyme-linked immunosorbent assay (ELISA) diagnostic procedures and Western blots (immunoblots) employed this antiserum.

Transmission of the pathogen to maize seedlings by insects collected in diseased maize fields was attempted. Insects were not identified to species but plant-hoppers, leafhoppers, aphids, and beetles were represented in the field collections.

Electron microscope visualization was attempted with leaf-dip and semipurified preparations, and fixation, embedding, and thin-sectioning of infected maize and wheat tissues. For transmission electron microscopy, leaf tissue of barley infected only with HPV was fixed in 0.5% PIPES [piperazine-*N*-*N*′-bis(2-ethanesulfonic acid)] pH 8.0 buffer made to 5% with glutaraldehyde and post fixed in 2% osmium tetroxide in 0.18 M PIPES, pH 6.8 buffer (17). Thin sections were stained with 5% uranyl acetate in 50% ethanol followed by Reynolds lead citrate, and then viewed with a Philips EM 201 electron microscope at 60 and 80 kV.

RESULTS

Minipurification of the diseased tissue extracts followed by PAGE always showed a unique protein of 32 kDa (Fig. 1). Other virus coat proteins were also observed in many of the samples. WSMV coat protein was seen in most field samples of maize and wheat. WSMV coat protein breaks down in older infected tissue (4), and we occasionally found the smaller, degraded, WSMV coat protein components. A few samples of both crops contained no detectable WSMV bands and may represent single infections by HPV.

Viral coat proteins in the 35 to 39 kDa size range were found in several samples and were probably from strains of the potyviruses maize dwarf mosaic virus and/or sugarcane mosaic virus strain MD-B, which are common in the area. Mechanical transmission of some samples to sorghum supported this supposition. One sample had two coat proteins in addition to the unique 32-kDa protein and probably had a triple infection.

Younger diseased plants generally contained substantial amounts of the 32-kDa protein. However, as the plants matured the amount of 32-kDa protein declined. At maturity it was barely detectable.

Density gradient centrifugation with techniques developed for tenuiviruses (5) successfully purified a nucleoprotein carrying the 32-kDa protein associated with the disease. The nucleoprotein sedimenting in a diffuse band would be consistent with a small or highly asymmetric (filamentous) particle.

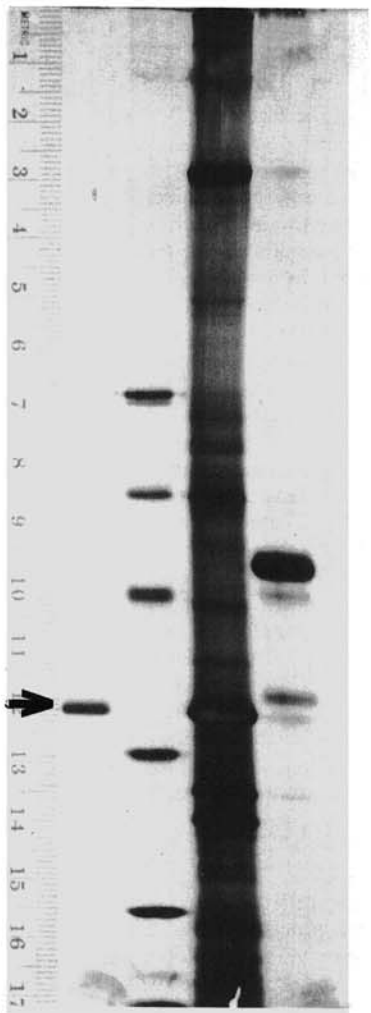

Fig. 1. Silver stained proteins in a 5 to 12% polyacrylamide gradient gel. Lane 1: 32-kDa protein (arrow) purified through density gradient centrifugation from high plains virus (HPV)–infected maize. Lane 2: Protein standards. Lane 3: Minipurifed proteins from HPV-infected maize, with a large band at 32 kDa. Lane 4: Purified wheat streak mosaic virus, with capsid breakdown products.

Nucleic acids extracted from the density gradient fraction yielding the 32-kDa nucleoprotein were separated by electrophoresis on agarose gels. A consistent pattern of four bands was obtained from preparations from corn or wheat. The largest and smallest nucleic acids were thin bands while the two middle bands were broader and more diffuse and may represent doublets. Therefore, there may be four to six species of nucleic acids (8,9).

Rabbit immunization yielded an antibody that reacted strongly to the 32-kDa protein but only weakly with other proteins in heavily loaded Western blots. The antiserum has a titer end-point of about 1/50,000 in indirect ELISA. We currently use the antiserum to diagnose the pathogen in field samples.

Attempts to transmit the pathogen to maize seedlings mechanically or with field-collected insects failed. However, the wheat leaf curl mite (*Aceria tosichella*, Keifer) did transmit the pathogen to wheat, barley, and maize (D. Seifers and T. Harvey, *unpublished*).

Electron microscopy of leaf-dip or semipurified preparations was not definitive. Thin-sectioned wheat, barley, and maize tissue revealed, in addition to the filamentous virus particles and the pinwheel-shaped cytoplasmic inclusions characteristic of WSMV, many spherical or ovoid double-membrane-bound vesicles 100 to 200 nm in diameter (Fig. 2). Details of these observations have been presented by Ahn et al. (2). The results of the observations suggest that HPV represents a potentially new group of plant viruses.

DISCUSSION

By the end of 1995, HPV had been confirmed in samples of maize and wheat tissue received from nearly 100 counties in an area extending from the Texas panhandle to eastern Nebraska, to central South Dakota, to western Idaho and back through Colorado to eastern New Mexico and Texas.

Only three dent corn hybrids were found to be highly susceptible to the HPV, Golden Harvest 2544, Funks 4292, and ICI 8310. Several sweet corn hybrids, including How Sweet It Is, and one blue corn variety were severely affected. In some fields all plants were affected. Dent corn yields were reduced by 25 to 75%. Severely infected sweet corn was not marketable and was a total loss.

The first symptoms appeared when the plants were small, at the 30- to 45-cm stage. The incidence of symptomatic plants and symptom intensity increased for several weeks. For example, in one report, the incidence of symptomatic plants increased from 20 to 30% to 70 to 80% over 3 to 4 weeks. In another field, disease incidence increased from a trace to 5% up to 10 to 30%. The disease incidence then appeared to decline, but actually the smaller, more severely infected plants died while the healthy and less severely infected plants survived and the appearance of the field improved.

Symptom expression in the field varied with the time of infection. When susceptible field corn and sweet corn were infected early the first symptoms were stunting and pronounced chlorosis in the youngest leaves. The chlorosis was in the form of a general mosaic and flecking or streaking. There was no sharp delimitation between symptomatic and nonsymptomatic areas of the leaf. However, the symptoms were not as uniform as a typical MDMV reaction. As the plant developed, the new growth continued to be chlorotic while the older tissue reddened at the leaf margin beginning at the tip. The reddening progressed down the leaf followed by necrosis that began at the tip and moved down the leaf. In the most severe cases the plants died. In the most striking example we have directly observed, all plants in 12 rows of two hybrids of sweet corn, each row 400 m long, were killed before reaching 1 m in height. In the mildest cases some plants eventually recovered from the shock phase and grew to sexual maturity. In sweet corn this usually meant an unacceptable ear, while in field corn the ear matured but was often stunted or deformed. Ears were not sterile, but ear and kernel size were reduced. At maturity the infected dent corn plants were stunted, 2 m tall versus the normal 2.7 m tall, and displayed striking chlorosis with red stripes, sectors, and flecks. Mature plants approaching harvest had clearly defined red sectors and stripes on the leaves.

Several aspects of symptomatology distinguish this pathogen from other maize viruses found in this area. Symptoms of this disease are often most severe on lower leaves and develop more slowly on upper leaves. Other viruses are typically more noticeable on the upper leaves. MDMV, SCMV (also known as MDMV-B), JgMV, MCMV, WSMV, and maize chlorotic dwarf virus all produce some chlorosis and mosaic but do not significantly stunt under normal field conditions. Single infections by any of these viruses, which are found in the high plains or the central corn belt, will not produce the severe chlorosis seen with the HPV on susceptible genotypes. None of these viruses produce the discrete, small, white spots, in linear clusters or rows roughly following the veins, that are seen with HPV. None of these viruses alone produce the pronounced red striping or sectoring seen in the mid- to late stages of this disease. Mixed infections with some of these viruses can produce some or all of the above symptoms, especially the stunting. Reddening in a uniform or mosaic pattern is common with severe corn lethal necrosis, which is a double infection by MCMV and SCMV-MDMV-B (16). The spiroplasma-caused disease corn stunt will cause reddening of the leaves and stunting

that resembles HPV. However, corn stunt also causes proliferation of ears, which is not characteristic of HPV. Corn stunt is commonly found in the southern U.S. and Mexico.

Only one of several maize plants transplanted from the field to a growth chamber at 22°C survived. Tissues were eventually free of symptoms but still contained the 32-kDa protein. Several attempts to transplant affected winter wheat from the field to the greenhouse were unsuccessful and the wheat died quickly. This was probably due to transplanting stress but the root system also seemed stunted and damaged. In the field both maize and wheat suffered some mortality and stand reduction but larger plants did not die so suddenly. Three weeks from first symptoms to complete collapse and death would be the typical rate of disease onset in susceptible sweet corn 0.5 m in height. In growth chamber inoculations of HPV by mites into susceptible seedlings in the third-leaf stage, small chlorotic spots appeared in 4 days and the plants were dead in 10 days at 27°C.

Symptoms of HPV on wheat are not as easily distinguished from other virus symptoms as they are on maize. In field-infected wheat the first symptoms are small chlorotic spots. These rapidly expand into a mosaic and then into a general yellowing of the plant. In our experience field infections very often are mixed infections of WSMV and HPV.

This new pathogen found in maize and wheat fields of the high plains has caused concern because it had not been recognized in this area before. In some fields it was devastating. Several aspects of the molecular characterization of the pathogen suggested similarity to a tenuivirus. A coat protein of 32 kDa is consistent with a tenuivirus. Tenuivirus-like filaments were occasionally seen in the electron microscopy of semipurified preparations. A broad light-scattering zone, similar to that expected for tenuivirus, was obtained with preparations from symptomatic corn. The isolation of 4 to 6 species of nucleic acids would also be indicative of the tenuiviruses that have 5 species of RNA (5). Also, like tenuiviruses, it could not be mechanically transmitted. However, the 32-kDa protein did not react in Western blots with antisera to maize stripe virus or to an unnamed tenuivirus from oats. Also, we did not find the large amounts of 16-kDa noncapsid proteins typically associated with the tenuivirus infections. Furthermore, the tenuiviruses are all transmitted by delphacid plant-hoppers, and although they all produce cellular inclusion bodies those structures do not resemble the spherical double-membrane-bound bodies associated with this disease (Fig. 2) (2).

In some field samples of corn and wheat we could detect only the 32-kDa protein and not WSMV protein. These samples reacted only with antiserum to the 32-kDa protein. Therefore, we believe the 32-kDa-associated pathogen may produce severe symptoms by itself. Unfortunately, only controlled infection from pure cultures can assure pure infections. Controlled infections of either wheat or maize with WSMV alone have never produced such severe symptoms. Transmission by mites of the 32-kDa pathogen in mixed infection with WSMV in a growth chamber at 27°C yields symptoms that were generally less severe than those of typical field samples. However, very susceptible genotypes were killed in only a few days even at 27°C. We speculate that, in general, higher field temperatures or the fluctuating day/night temperatures may exacerbate symptom expression.

Mite transmission and host range of this pathogen are reminiscent of the wheat spot mosaic pathogen described by Slykhuis (18,19). Electron microscopic observation of small, spherical, double-membrane vesicles 100 to 200 nm in diameter also fits the description of wheat spot chlorosis disease assumed to be similar to wheat spot mosaic virus (3,15). However, we have been unable to locate a verified culture of wheat spot mosaic virus or the wheat spot chlorosis pathogen to make comparisons.

It is possible to group the mite-transmitted pathogens with cytology similar to that of wheat spot mosaic (18,19): the mite transmitted diseases of wheat spot chlorosis (3,15), rose rosette (6,10), thistle mosaic (1), fig mosaic (3), and redbud yellow ringspot (2) form a natural biological group for which no etiological agent has yet been identified by Koch's postulates. Characterization of the HPV pathogen of maize and wheat should facilitate identification of pathogens of this group. The 32-kDa-protein–associated pathogen resembles tenuiviruses, but it also resembles tospoviruses, of which tomato spotted wilt (TSWV) is the type member. Indeed, tospoviruses share many properties with tenuiviruses (7) and both are plant infecting members of the Bunyaviridae (13). The 32-kDa-protein–associated pathogen more closely resembles TSWV in having a nucleocapsid that is difficult to see by electron microscopy and in producing intracellular vesicles, albeit larger ones than those of TSWV.

The widespread distribution of the disease suggests that it is not new but probably endemic. In wheat it may have easily been mistaken for WSMV, which is trans-

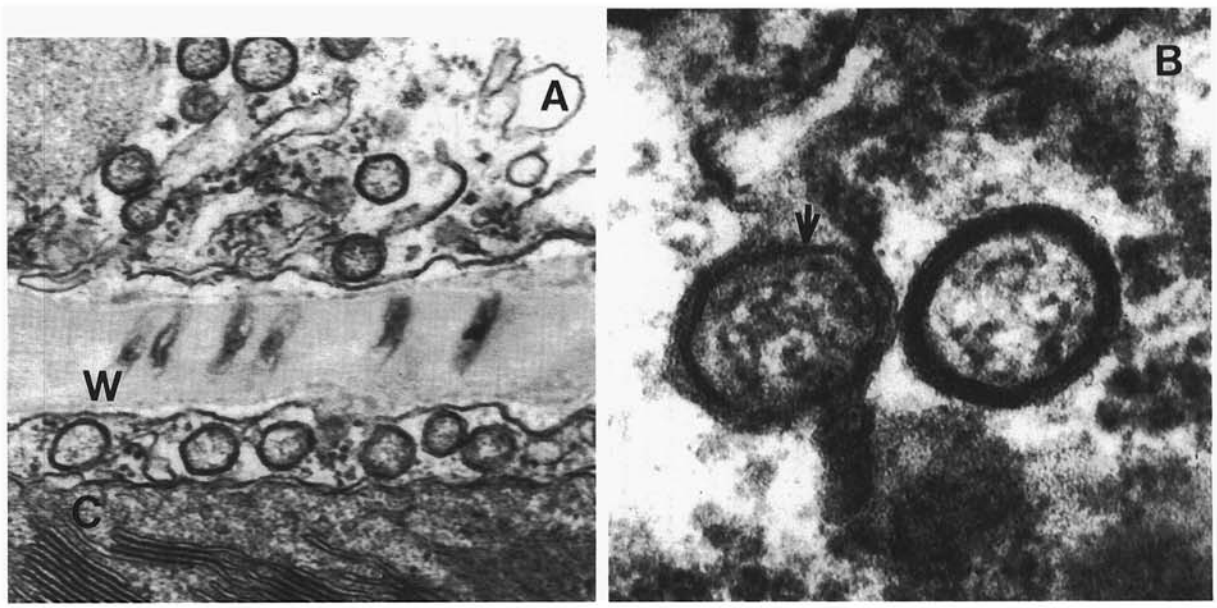

Fig. 2. Electron micrographs of high plains virus–infected barley leaves. **(A)** (×60,000) Numerous spherical bodies in the cytoplasm between the chloroplast (C) and the cell wall (W). **(B)** (×250,000) The spherical bodies have been enlarged and the double membrane is apparent. The particle with the arrow appears to be budding through the endoplasmic reticulum.

mitted by the same eriophyid mite. In maize the disease may have been mistaken for a severe strain of a common virus. The epidemiology probably depends strongly on the biology of the mite. The reappearance of the disease in 1993, 1994, and 1995 in some of the same areas confirms that the pathogen is established and endemic in those areas. Among susceptible genotypes this disease can have a severe economic impact on two major crops, maize and wheat; therefore, the disease will need to be controlled.

Work is continuing on vector relationships, cytological effects, host range, genotype interactions, and possible synergistic interaction with WSMV. We emphasize that Koch's postulates have not been fulfilled and that the disease may prove more complex than we have portrayed it.

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