

ECOLOGY, EPIDEMIOLOGY, AND MOLECULAR BIOLOGY
OF FUNGUS VECTORED VIRUSES IN KANSAS

by

TIMMY LEE KENDALL

B.S., Purdue University, 1978

A THESIS

submitted in partial fulfillment of the
requirements for the degree

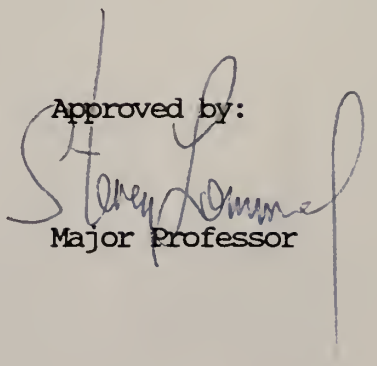
MASTER OF SCIENCE

Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1988

Approved by:


Major Professor

FUNGUS-VECTORED VIRUSES OF WHEAT IN KANSAS

T.L. Kendall and S.A. Lommel

Department of Plant Pathology, Throckmorton Hall
Kansas State University, Manhattan, KS 66506

SUMMARY

Since the beginning of intensive agriculture in the Great Plains of the United States, crops have been chronically infected with a range of soilborne viruses. Wheat, the dominant crop within the region is particularly susceptible to viruses that are naturally fungus transmitted. Wheat soilborne mosaic virus (WSBMV) is the most widespread and damaging of these viruses. Within the last 15 years, sources of single gene resistance have been identified and deployed for the effective control of WSBMV. However, the rapid spread of other soil-borne viruses and the danger involved in relying on a single resistance gene for disease control necessitates continued research on the etiology, epidemiology, vector relationships, and molecular biology of the disease causing agents. An example of these problems is the identification of a mixed infection of two wheat infecting, Polymyxa graminus transmitted viruses, WSBMV and wheat spindle streak mosaic virus (WSSMV). Together, these viruses break down the resistance to WSBMV resulting in a severe disease in the wheat cultivars once thought to be resistant. For these reasons there has been renewed interest in characterization of the viruses. WSBMV, the type member of the Furovirus group has been partially characterized at the molecular level. An interesting deletion mutagenesis phenomenon associated with the smaller RNA-II component may play a role in the epidemiology and transmission of WSBMV in the field. Additional viral structure can be surmised from the complete sequence of beet necrotic yellow vein virus, another member of the

Furovirus group. Further analysis of WSBMV is continuing, particularly in attempting to locate viral genes involved in biological properties such as systemic movement, host range, symptomatology, and the ability to be fungus transmitted. This research is being accomplished by pseudorecombination studies involving WSBMV and a newly isolated WSBMV-like virus that has an expanded host range and is readily mechanically transmissible. WSSMV is proving to be difficult to physically characterize because of the difficulty in purification and the presumably long length of the virion. The continued molecular characterization of these important viruses is prudent in light of recent advancements and concepts in virus disease control involving transformation of the host with specific viral genes to achieve virus disease control.

INTRODUCTION

Triticum aestivum (spring and winter wheat) has remained the dominant cereal grain crop in Kansas since the early twentieth century when wheat first exceeded corn in acreage harvested. Both soft spring and winter wheats were originally planted by settlers in Kansas in the early 1800s. However, it quickly became apparent that winter wheat was better suited to the harsh Kansas climate; therefore, spring wheat production was essentially eliminated by the late nineteenth century. Even early winter wheats were not hardy enough for prevailing weather conditions; consequently, the lack of winter hardiness in better soft winter wheats being used eventually led to widespread plantings of "Turkey" type wheats. Turkey, a hard winter wheat brought to Kansas

by the Mennonite settlers, originated in the Crimea area of Russia and readily adapted to the variable Kansas climate. This progressive shift from spring wheat to the widespread and exclusive use of winter wheat over large acreages generated the ideal conditions for introduction and establishment of fungus-vectored soil-borne wheat viruses in the state.

Wheat soilborne mosaic virus (WSBMV) and wheat spindle streak mosaic virus (WSSMV) currently cause serious diseases of winter wheat in several central and northeastern states. WSSMV has caused substantial losses of winter wheat in the eastern soft wheat region, particularly the states surrounding the Great Lakes. It was first reported in southern Ontario, Canada, and described by Slykhuis (1970) and Slykhuis & Polak (1971). Since then the disease has been reported in Michigan (Wiese, Saari, Clayton & Ellingboe, 1970), Indiana (Jackson et al., 1975), Kentucky (Williams, Pirone, Slykhuis & Tutt, 1975), Maryland, and New York in the United States, and southern France (Slykhuis, 1976) and India (Ahlawat, Majumdar & Chenulu, 1976). More recently, WSSMV has been reported in Nebraska (Brakke, Langenberg & Samson, 1982) and Kansas (Lommel & Willis, 1984; Lommel, Willis & Kendall, 1986). Wheat spindle streak mosaic (WSSM) caused by WSSMV was first observed in winter wheat in southern Ontario, Canada, in 1960 and has been a persistent disease problem in the Great Lakes Region due to the generally cooler temperatures. WSSMV has recently been reported as far south as Kentucky and as far west as Kansas and Nebraska, and it is distressing that the virus is moving to the south

and west. In Kansas, WSSM has so far been found only in counties in the southeastern and southcentral portion of the state.

Unlike WSSMV, WSBMV has probably been established in the Great Plains since the introduction of winter wheat varieties. The first reported epiphytotic of wheat soilborne mosaic (WSBM) caused by WSBMV was reported in Kansas in 1952 (Fellows, Sill & King, 1953) along the Missouri River and in southeast Kansas. It is now found almost as far west as the Kansas-Colorado border but is most prevalent and destructive in the southeast and southcentral areas of the state. WSBM was also reported in Oklahoma in 1952 (Wadsworth & Young, 1953).

Lommel and Willis (1984) found that large acreages of WSBM-resistant wheats in south central Kansas had developed disease symptoms that initially resembled WSBM. Yellowing turning to bronze, mosaics, stunting, and reduced tillering were observed in discrete areas within fields generally corresponding to lower terrain. Given the history of WSBMV in the area, the initial assumption was that the symptomatic wheat was infected with WSBMV. Closer inspection of the symptoms revealed spindle-shaped streaks and a bronzing of the tissue, which are not generally observed with WSBMV infections. Electron microscopic examination revealed WSBMV particles and other long, flexuous rods. Examination of thin sections revealed pinwheel and other amorphous inclusion bodies. From these observations, and with Western blot analysis (Lommel, Willis & Kendall, 1986), it was determined that the plants were infected simultaneously with WSSMV and WSBMV.

In the summer of 1986, Kendall and Lommel characterized a previously unreported virus found in sorghum in Reno County, Kansas. The disease situation was noticed due to the striking symptomatology of diseased plants. Most prominent was the occurrence of elongated and elliptical green islands and yellow mosaic of diseased tissues. The virus was first thought to be a warm temperature isolate of WSBMV, but, after further characterization, it was concluded that the virus should be considered a new member of the proposed Furovirus group.

HISTORICAL BACKGROUND

Wheat soilborne mosaic virus (WSBMV) was first observed in Illinois by McKinney in 1919. The virus persists in soil (Koehler, Bever & Bonnett, 1952; McKinney, 1923_a; McKinney, Eckerson & Webb, 1923) and is vectored specifically by the soil-inhabiting fungus Polymyxa graminis Led. (Brakke, Estes & Schuster, 1965; Brakke & Estes, 1967; Rao & Brakke, 1969). McKinney (1923_a) described two forms of the disease in Illinois, the rosetting and the leaf mottling forms, which are caused by the green and yellow strains of the virus, respectively. The green strain is not found in Kansas, but the yellow strain is endemic in Kansas and causes significant losses (Fellows, Sill & King, 1953). The yellow strain of WSBMV is also found in Florida (Kucharek & Walker, 1974), Illinois (Koehler, Bever & Bonnett, 1952), Nebraska (Brakke, 1971), Oklahoma (Wadsworth & Young, 1953), and Virginia (Roane, Starling & McKinney, 1954). The first WSBMV epiphytotic reported in Kansas was in 1952 (Fellows, Sill & King, 1953). By 1969, WSBMV had spread thoroughly from eastern to southcentral Kansas.

The first report of wheat spindle streak mosaic virus (WSSMV) was the mosaic disease of winter wheat described by Slykhuis (1961; 1970) in southern Ontario, Canada. Mosaic symptoms observed by Slykhuis had noticeable differences to symptoms attributed to mosaic disease of wheat caused by soil-borne viruses reported elsewhere (McKinney, 1923b; McKinney, 1931; Ikata & Kawai, 1937; Wada & Fukano, 1937; Sill, 1958) and, in particular, were distinct from symptoms associated with infection by WSBMV. Symptoms induced by the mosaic disease found in Ontario included chlorotic and necrotic dashes and prominent spindle-shaped streaks. The virus did not cause infection in WSBMV-susceptible rye or barley (Slykhuis, 1961) and symptom expression was suppressed at temperatures greater than 15°C, the optimum temperature at which symptoms of WSBMV infection are evident. Leaf dip preparations of diseased plants revealed long, flexuous particles completely unlike the short, stiff rods found in plants infected with WSBMV. Wheat spindle streak mosaic (WSSM) was confirmed to be a distinct soil-borne fungus-transmitted disease of wheat (Slykhuis, 1961; Slykhuis, 1964).

Slykhuis reported much variation in wheat spindle streak mosaic (WSSM) symptom expression, disease development, and severity from different locations and within the same location in different years (Slykhuis, 1960; Slykhuis, 1961; Slykhuis, 1964). The disease was found in most fields in 1961 where wheat had been grown the previous year, but was essentially absent from fields not previously planted to wheat.

EPIDEMIOLOGY

WSBMV

WSBMV is composed of short, stiff, rod-shaped particles of two lengths, 280 nm and 90-140 nm (Brakke, Estes & Schuster, 1965; Gumpf, 1971; Tsuchizaki, Hibino & Saito, 1973; Brakke, 1977), and is vectored through soil by the plasmodiophoraceous fungus Polymyxa graminis Led. (Brakke, Estes & Schuster, 1965; Brakke & Estes, 1967; Rao & Brakke, 1969). Polymyxa graminis is an obligate pathogen of wheat roots, and the virus is carried internally in the zoospores and resting spores of the fungus (Rao & Brakke, 1969).

The natural host range of Polymyxa graminis includes not only the grasses in which WSBMV infects, but also several species of Bromus as well as corn and sorghum (W. G. Langenberg, unpublished). Several genera of the Gramineae and Chenopodium species comprise the host range of WSBMV (McKinney, 1930; Paulsen, 1970; Tsuchizaki, Hibino, & Saito, 1973), wheat being the crop of economic importance.

Chenopodium species are artificial hosts for the virus, as these plants are not susceptible to Polymyxa and hence the natural infection process. Mechanical transmission of WSBMV has been documented (Tsuchizaki, Hibino & Saito, 1975; Shirako & Brakke, 1984_a and _b) using leaf extracts, purified virions, and isolated RNA, which explains the inclusion of Chenopodium in the host range of WSBMV. Symptoms of WSBM infection include mosaics, reduced tillering and plant height, and reduced yield and kernel weight. Significant reductions in yield have been recorded almost every year in eastern

and southcentral Kansas. When the virus and fungus relationship has become established in the soil at a given location, the soil at that location will remain infective indefinitely, even in the absence of a suitable host crop. W. G. Willis (unpublished) has observed WSBM infection in wheat from a field not planted to wheat in the previous 15 years.

Polymyxa infection occurs in the fall and symptom development and expression occur in early spring as virus-infected plants resume growth after winter dormancy. It has been determined experimentally that 15-17°C is the optimum temperature range for disease development (Brakke & Rao, 1967), and this reflects the temperature profile for maximum infection of virus and fungus and the subsequent symptom expression in the field. The length of the cool period in spring and the amount of moisture in a given year are the two critical factors profoundly affecting severity of WSBM for that year. Viral infection is generally associated with areas of the field having lower terrain and areas where ground water is directed, as wet soil is necessary for the spread of the viruliferous Polymyxa zoospores. Infected plants display variable degrees of recovery from infection depending on temperatures in early spring. As temperatures increase, symptoms disappear and if the temperature increase is early during the period of time plants break winter dormancy, little effect on plant development and eventual yield will occur.

At present, the disease is controlled by the use of resistant varieties and by planting late in the fall in order to decrease the

infection period before winter dormancy. Resistance to WSBMV may operate on several levels. WSBM resistance incorporated into some varieties is controlled by a single dominant gene (Miyake, 1938; Modawi, Heyne, Brunetta & Willis, 1982; Merkle & Smith, 1983). Originally, it was thought that the resistance conferred by this gene was to the virus. However, based on disease patterns observed in Nebraska (Brakke, Langenberg & Samson, 1982) and Kansas (Lommel & Willis, 1984; Lommel, Willis & Kendall, 1986) and the fact that WSBMV mechanically inoculated to leaves of the resistant varieties (Larsen, Brakke & Langenberg, 1985) can result in infection, it is evident that resistance is not to the virus but to the fungus vector. Although the mechanism of field resistance to WSBM is unclear, it undoubtedly involves a root-vector interaction. This is because WSBMV infection may cause a hypersensitive reaction that results in reduced secondary root growth in resistant varieties (Larsen, Brakke & Langenberg, 1985). The results could indicate that the fungal vector is unable to colonize the roots of resistant plants or that the virus is not able to move from the vector to the colonized roots. In addition, Larsen, Brakke & Langenberg, (1985) speculated that resistance may be to the wild-type WSBMV and not to observed deletion mutants, or that the Polymyxa zoospores can only transmit the wild-type virus and are unable to transmit the deletion mutant forms.

Regardless of the mechanism of resistance, basing breeding programs on the use of the single dominant resistance gene is inadequate to control the disease as field resistance to WSBMV breaks

down in the presence of WSSMV (Brakke, Langenberg & Samson, 1982; Lommel & Willis, 1984; Lommel, Willis & Kendall, 1986). Lommel & Willis (1984) reported extensive infection of WSBMV resistant cultivars carrying the single dominant resistance gene to WSBM by a combination of WSBMV and WSSMV. The synergistic relationship of WSSMV and WSBMV and the subsequent interaction with Polymyxa results in a loss of resistance to WSBM in those cultivars previously considered WSBMV resistant.

WSSMV

Natural transmission of WSSMV, a long, flexuous, filamentous particle frequently exceeding 2000 nm in length, is the same as that of WSBMV, namely through the fungal vector Polymyxa graminis (Barr and Slykhuis, 1969; Barr and Slykhuis, 1976; Slykhuis & Barr, 1978; Nolt, Romaine, Smith & Cole, 1981). Olpidium brassicae (Wor.) Dang., Rhizophydium graminis Led., Lagenia radiculicola Vanterpool and Led., and several Pythium species were also found on roots of diseased wheat plants and were therefore investigated as being possible vectors of WSSMV. However, Polymyxa was the only fungus found in constant association with WSSM infection. Slykhuis & Polak (1971) were able to get limited infection through mechanical inoculation of plants with infected tissue extracts and no infection with purified virus. Usugi & Saito (1979) reported moderate success with inoculations using purified virus. These conflicting results may be due to strain differences in the virus. The virus has a very limited host range with common winter and spring wheats (Triticum aestivum) and durum

wheat (Triticum turgidum) the only known hosts (Slykhuis, 1970; Slykhuis, 1976). WSSMV, like WSBMV, is persistent in soil in conjunction with its fungal vector, even in the absence of a suitable host plant. The level of WSSMV inoculum in soil increases dramatically in those fields in which wheat has been continuously planted (Slykhuis, 1970).

Symptoms of WSSM are short, chlorotic, and necrotic dashes and spindle-shaped streaks, reduced tillering and plant height, and reduced yields in years when temperature favors disease development. The virus induces a variety of inclusion bodies within infected tissue, most notably distinctive cylindrical pinwheel inclusions. Temperature is the major determinant in disease development and symptom expression (Slykhuis, 1970; Slykhuis, 1974; Slykhuis, 1975a & b). WSSMV infection, like that of WSBMV, occurs after planting and emergence in the fall, and symptoms are expressed when plants resume growth in the spring. However, temperature requirements for infection of WSSMV are markedly different from WSBMV, with temperatures of 10°C or less necessary for symptom expression. Symptoms are not expressed at temperatures higher than 15°C (Slykhuis, 1970; Slykhuis, 1974; Slykhuis, 1975a and b), and plants in which disease symptoms were prominent when the temperature was approximately 10°C appeared to recover as the temperature increased during the growing season, although infected plants still produced fewer heads and subsequently a lower grain yield. When temperatures remained cool throughout May and June, mosaic and even necrotic symptoms remained

evident through heading. Interestingly, the optimum disease development temperature of 10°C is deleterious to the fungal vector which requires 15–22°C for maximum development (Slykhuis & Barr, 1978). The optimum temperature of 15°C for disease transmission appears to be a compromise between the temperatures required for disease development and vector development, and therefore it is not surprising that movement of the virus and introduction of WSSM into new locations is slow and probably occurs within a narrow temperature range.

The WSBMV-WSSMV Complex

In the presence of WSSMV, field resistance to WSBMV breaks down (Brakke, Langenberg & Samson, 1982; Lommel and Willis, 1984), resulting in a new disease in hard red winter wheat (Lommel, Willis & Kendall, 1986). Before the movement of WSSMV into Kansas, the WSBM-Polymyxa interaction was relatively stable, with control centering on the use of resistant varieties. However, the situation has dramatically changed with movement and establishment of WSSMV across the state. Lommel and Willis (1984) reported that extensive acreages of WSBM-resistant wheats in southcentral Kansas developing disease symptoms normally associated with WSBMV infection. Severe mosaic, yellowing, bronzing, stunting, and reduced tillering were observed in areas within fields corresponding to lower terrain and waterways. Infected tissues exhibited not only symptoms typical of WSBM but also spindle-shaped streaks not associated with symptoms induced by WSBMV. Electron microscopic examination revealed not only the long and short,

stiff rods typical of WSBMV, but also long, flexuous particles (Fig. 1). Examination of thin sections revealed distinctive cytoplasmic inclusions including cylindrical, pinwheel inclusions. From these observations, and with confirmation from Western blot analysis, it was determined that these plants were infected with both WSBMV and WSSMV (Lommel, Willis & Kendall, 1986), and that the two viruses had interacted synergistically to result in the new disease complex observed in the previously WSBMV-resistant cultivars.

The endemic nature of WSBMV and WSSMV in Kansas, and the fact that WSBMV and WSSMV are vectored by the same persistent fungus, indicate that the potential for development of the new disease situation has existed for some time and will continue in successive wheat crops. The reason the disease had not been observed prior to 1984 is based on many factors, not least among them the highly variable Kansas climate. WSSMV was identified in four counties in 1984 and 43 counties in 1985. Undoubtedly, this dramatic increase in the number of counties reporting WSSMV infection is a reflection of more intensive surveying for the virus and not movement of the virus into new areas. However, the wide planting of presumed WSBM-resistant wheats, coupled with the conducive spring weather conditions, certainly contributed to the sudden development of the disease. The new situation has generated considerable concern since WSBM-resistant cultivars planted in Kansas, particularly the widely used wheats such as "Newton", are fully susceptible to WSBMV in the presence of WSSMV. The use of the single dominant gene in breeding for resistance to

WSBMV and the subsequent, almost exclusive planting of these cultivars has resulted in limited genotypic variation in Kansas wheat, a situation conducive to the development of WSBMV-WSSMV synergism.

It is interesting to speculate why this disease situation is relatively widespread but has previously escaped notice. Antiserum to WSSMV produced in Michigan and used in this laboratory in Western blot studies (Lommel, Willis & Kendall, 1986) reacted at low levels to WSBMV, suggesting that there might be similar antigenic determinants between WSBMV and WSSMV, but more likely that the cultures of WSSMV used to generate antiserum also contained low levels of WSBMV. WSBM-like symptoms have periodically been reported on WSBMV-resistant cultivars (W. G. Willis, unpublished), particularly "Newton", in Kansas in the past, indicating that the synergistic reaction probably occurred prior to published reports. WSBMV-resistant wheat varieties from Kansas breeders have often given ambiguous results when grown in other states, again indicating that the WSBMV-WSSMV synergism may be more common than currently reported. Given the years of historical accounts, and the development of resistance to WSBMV about 20 years ago, it is apparent that, at least in Kansas, WSSMV moved in and became established within the last 15 years. These observations illustrate the rate of movement at which fungal transmitted viruses can enter a new location. Once again, it is important to note that once fields are infested with the virus and fungus they remain so indefinitely.

VIRUS PROPERTIES

WSBMV

Wheat soil-borne mosaic virus is the type member of the proposed Furovirus group (fungus-borne rod-shaped virus) (Shirako & Brakke, 1984a). Other possible members include: beet necrotic yellow vein virus (BNYVV) (Tamada, 1975; Richards et al., 1985, and Ziegler et al., 1985), potato mop-top virus (Roberts & Harrison, 1979), and peanut clump virus (Thouvenel, Dollet & Fauquet, 1976). The group, and specifically WSBMV, are typified by rod-shaped virions and a bipartite genome. The genome is separately encapsidated by a single capsid protein of 19.7 kDa, generating virions 20 nm in diameter. Component I virions are always observed to be the same length, 281 nm (Brakke, 1977; Tsuchizaki, Hibino & Saito, 1973; Hsu & Brakke, 1985a). However, component II virions have at least three discrete lengths 138 nm, 110 nm, and 92 nm (Gumpf, 1971; Brakke, Estes & Schuster, 1965; Tsuchizaki, Hibino & Saito, 1973; Brakke, 1977). Shirako & Brakke (1984a and b) and Shirako & Ehara (1986) proposed a deletion mutagenesis phenomenon in which portions of the genome are sequentially deleted from near the 3' terminus of RNA-2, resulting in component II virions of reduced size. The RNA from the component I virion is termed the 1.0L RNA by Shirako & Brakke (1981, 1984a and b) and is approximately 2.28×10^6 Da or 6700 bases. The wild type component II virion RNA is approximately half the size of the 1.0L RNA and, consequently is termed the 0.5L RNA and is 1.23×10^6 Da or 3300 bases. The two deletion mutants of the wild-type component II RNA are the 0.4L (2700 bases) and 0.35L (2300 bases) RNAs.

Apparently there is a temporal progression of the size of the component II virions under field conditions (Hsu & Brakke, 1985a). In the early spring WSBMV-infected wheat plants are always infected with the wild-type virus, that is the 1.0L and 0.5L virions. After several months plants can be observed to be infected with the 1.0L virions and a combination of the 0.5L with the 0.4L or 0.35L, or the 0.4L and 0.35L virions alone (Shirako & Brakke, 1984a and b). These observations were consistent when plants were either mechanically inoculated with the wild-type virions originally or infected via Polymyxa graminis in the field. Shirako and Brakke (1984b) concluded that a minimum of two deletion events occurred consecutively and spontaneously. Both component II deletion mutants were stable and maintainable; however, infections involving the 0.35L RNA-II component exhibited more severe symptoms than those of the wild-type virus (Shirako & Brakke, 1984a and b; Shirako & Ehara, 1986). Shirako & Brakke speculated that the reason the 0.35L form of component II did not predominate in nature was that it could not be transmitted by its natural vector. If this is true, then the region of the genome controlling fungal transmission is within the region of RNA-II that is deleted.

Gumpf (1971) & Tsuchizaki, Hibino & Saito (1975) reported that the component II particle were not infectious while the component I particles were infectious (Gumpf, 1971). However, more recent reports (Tsuchizaki, Hibino & Saito, 1975; Shirako & Brakke, 1984a and b) state that neither the 1.0L particle nor 1.0L RNA are infectious. In

planta, both RNAs are required for infectivity. The conflicting results reflect component I preparations in the earlier investigations were contaminated with component II particles. It is currently assumed that both the 1.0L RNA and either the 0.5L, 0.4L or 0.35L RNAs are required for infection.

It is possible that one RNA component encodes a functional replicase and can replicate independent of the other component but is restricted to the initially inoculated cells. This situation exists with several other multicomponent and bipartite genomed viruses such as cowpea mosaic virus (Goldbach, Rezelman & van Kammen, 1980), tobacco rattle virus (Lister & Bracker, 1969) red clover necrotic mosaic virus (Paje-Manalo & Lommel, in press; Osman & Buck, 1987), and nepoviruses (Robinson, Barker, Harrison & Mayo, 1980). The inoculation of the separated WSBMV RNAs or virions to plants would not address this possibility. The separated RNAs need to be inoculated to protoplasts and assayed for the replication of the input RNA. Consequently, it is not clear which WSBMV RNA component encodes either the replicase or cell-to-cell movement genes.

Classical pseudorecombination experiments between different strains of WSBMV indicate that the RNA-II encodes capsid and inclusion body proteins and RNA-I controls virus concentration and infectivity on tobacco (Tsuchizaki, Hibino & Saito, 1975). The 19.7 kDa capsid protein is synthesized in vitro from the 0.5L RNA as well as from the 0.4L and 0.35L mutants (Hsu & Brakke, 1985_b; Shirako & Ehara, 1986), indicating that the deletions do not occur within the capsid protein

gene. A 90 kDa polypeptide observed from the in vitro translation of 0.5L RNA-II is presumably the viral inclusion body protein observed in infected tissue (Hsu & Brakke, 1985b; Hibino, Tsuchizaki & Saito, 1974). The 90 kDa polypeptide is not synthesized from either the 0.4 or 0.35L deletion mutants (Hsu & Brakke, 1985b). The 0.4L RNA directs the synthesis of a 66 kDa polypeptide and the 0.35L RNA directs the synthesis of a 55 kDa polypeptide (Fig. 2). Given this data, the deletions must occur within the 90 kDa inclusion body protein gene. The 90 kDa polypeptide is immunoprecipitated by capsid protein antiserum, and peptide mapping experiments indicate that the two proteins are at least partially homologous. In addition to these polypeptides, all forms of RNA-II direct the synthesis of a 28 kDa polypeptide which is also immunoprecipitated by capsid protein antiserum. Given this data, Hsu & Brakke (1985b) proposed a map of the genome organization and a model for the deletion mutagenesis of WSBMV RNA-II (Fig. 2). For the 0.5L wild-type RNA the observed 19.7, 28 and 90 kDa polypeptides are all homologous and 5' linear. The 28 kDa polypeptide and the 90 kDa polypeptide are synthesized by translation through one and two amber stop codons respectively. Consequently, the two discrete deletion events occur near the 3' terminus resulting in the stepwise reduction of the 90 kDa open reading frame leaving the 19.7 and 28 kDa domains of the open reading frame intact (Fig. 2). Assuming that this map is correct, the proteins encoded by the 0.5L RNA-II (3.3 kb) would require approximately 2.46 kb of coding capacity leaving an additional 1.14 kb of non-coding RNA. The excess sequence

seems a bit extravagant when compared to the relative high utility of other plant viruses.

RNA-I encodes polypeptides of 180 kDa, 152 kDa, 135 kDa, 80 kDa, and 45 kDa as determined by in vitro translation analysis (Hsu & Brakke, 1985_b; Shirako & Ehara, 1986). The maximum coding capacity for RNA-I, assuming 6.7 kb, are a polypeptide or polypeptides totalling 246 kDa. Obviously, most of the observed polypeptides are homologous and arise through a readthrough mechanism.

In summary, the biological information and genome organization of WSBMV is fragmentary. RNA-I encodes several large polypeptides whose expression strategy are uncharacterized. No biological properties associated with RNA-I have yet been firmly established. Although one may speculate that the replicase function is encoded by RNA-I. To establish this concept, the replication of purified RNA-I would need to be established in wheat protoplasts. If RNA-I can replicate in protoplasts, but is not detected in planta (Tsuchizaki, Hibino & Saito, 1975; Shirako & Brakke, 1984_a and _b), a cell-to-cell movement function could be tentatively assigned to RNA-II. There is more biological and organizational information regarding RNA-II, partly as a result of analysis of the curious spontaneous deletion phenomenon. The wild-type form, 0.5L RNA-II encodes a 90 kDa polypeptide which is presumed to be the inclusion body protein. All forms of RNA-II encode the capsid protein. Also, by comparing the various deletion forms, the sequence near the 3' end may control the ability to be Polymyxa transmitted. Many of the questions alluded to can be answered by

sequence analysis of the WSBMV genome as well as protoplast infection studies and further recombination studies with various strains to address the genetic control of fungus transmission.

The characterization of another member of the Furovirus group, namely beet necrotic yellow vein virus (BNYVV), allows for speculation about the organization of the WSBMV genome and in particular RNA-II. In vitro translation (Ziegler et al., 1985) and sequence analysis (Bouzoubaa et al., 1986) have clearly shown that the 21 kDa capsid protein is the 5' terminal open reading frame on BNYVV RNA-II, and the 85 kDa polypeptide is produced by a partial readthrough of an amber termination codon at the end of the capsid protein open reading frame (Fig. 2). This is more than likely the same mechanism employed for the synthesis of the capsid protein and 90 kDa protein from WSBMV RNA-II. BNYVV RNA-II possess several other open reading frames. A 42 kDa open reading frame was identified that partially overlaps with the 3' end of the 85 kDa open reading frame. There is also evidence for a protein of this size in infected plants. Near the 3' end there are three additional open reading frames which encompass approximately 1.15 kb (Fig. 2). No polypeptides have been identified that could account for these open reading frames. The location of these open reading frames coincides with the location of the deletions of WSBMV (Fig. 2). As mentioned earlier, there is approximately 1.14 kb of unutilized sequence available at the 3' end of the WSBMV 0.5L RNA-II. Again, this observation coincides with the sequence data for the RNA-II of BNYVV. There may be an uncharacterized association between the

observed WSBMV RNA-II deletions and three possible small open reading frames, assuming they are present. Once again, these speculations can be clarified by sequencing clones of the 0.5L, 0.4L and 0.35L RNAs.

A single large open reading frame of 237 kDa was deduced from the RNA-1 sequence of BNYVV (Bouzoubaa et al., 1987). Proteins of approximately 200 kDa, 150 kDa, and 50 kDa were observed from in vitro translation studies (Ziegler et al., 1985). Bouzoubaa et al. (1987) proposed that the observed polypeptides had the same N terminus, with the shorter products arising by premature termination of translation. Once again, this strategy fits with the existing data for WSBMV RNA-I. Various domains of deduced amino acid from the 237 kDa open reading frame of BNYVV RNA-I are homologous to polypeptides implicated as replicase for tobacco mosaic virus as well as several other plant viruses. Again assuming an organizational relationship between BNYVV and WSBMV, the RNA-I of WSBMV may well be involved in replication. This association again highlights the interest in determining if WSBMV or any Furovirus RNA-I can replicate independent of RNA-II in an appropriate protoplast system.

Many associations and assumptions have been made herein on the similarities between WSBMV and BNYVV. It is prudent to reflect on the significant differences. First, BNYVV has two smaller RNA components associated with the virus (Bouzoubaa et al., 1985) which are not homologous to WSBMV RNAs-I or -II. The smaller RNAs appear to be unnecessary for infection in Chenopodium (Kuszala et al., 1986). Secondly, WSBMV is not readily mechanically transmissible whereas

BNYVV is. Finally, WSBMV does not have 3' terminal poly-A tracts whereas BNYVV does (Bouzoubba et al., 1986).

WSBMV-like Virus

In the summer of 1986, a single hybrid sorghum line from a breeder's plot in southcentral Kansas developed virus-like symptoms. Analysis of the plants indicated the presence of a virus with morphological properties similar to WSBMV. Western blot analysis using WSBMV mono- and polyclonal antisera indicated no serological affinity to WSBMV. However, as is the case of WSBMV with its homologous sera (Langenberg, 1986) the virions from sorghum dissociated in the presence of WSBMV antisera. This observation suggests a minor serological relationship between the two viruses, possibly within a conserved epitope associated with protein-protein or protein-RNA interactions. These structural domains would presumably be the more conserved domains of the capsid protein.

The sorghum-infecting virus was purified as described by Shirako & Brakke (1984a) and physicochemical properties determined. Electron microscopic examination revealed particles of approximately 260 nm and 140 nm, almost identical to the particles of WSBMV (Fig. 3). The virions are composed of a single capsid protein species of 20.5 kDa and two single-stranded RNAs of 2×10^6 Da (6400 bases) and 1.2×10^6 Da (3300 bases). In WSBMV-infected plants there are consistently about 20 component II particles for every one component I particle. This ratio is reversed for the sorghum virus, where there are approximately 10-15 component I particles for every component II

particle.

Preliminary in vitro translation studies indicate that the overall translational profiles are similar to, but distinct from the translational products from WSBMV. The relative concentration of the largest product and capsid protein are opposite from those observed for WSBMV. This could be due to the reverse in relative RNA ratios previously mentioned. WSBMV antisera did not immunoprecipitate any of the sorghum virus-translation products.

Northern blot hybridization studies under high stringency conditions were negative, indicating that if homology exists between the two viruses it was less than 90 percent. Physiochemically, the newly isolated virus from sorghum and WSBMV are very similar; however, they are serologically- and genome homology-specific and, therefore, should be considered distinctive members of the furovirus group. From a physical point of view this virus appears much more closely related to WSBMV than to BNYVV or any of the other proposed Furoviruses.

The sorghum virus possesses unique biological properties which make it valuable in studies of the genetic control of various viral phenotypes. Unlike WSBMV, the virus is readily mechanically transmissible to several monocotyledonous and dicotyledonous hosts. After initial isolation the virus was mechanically inoculated to corn (Zea mays) N28ht. A bright yellow mosaic and elongated ringspot symptoms appeared after several weeks (Fig. 4). Approximately 10 percent of the plants became infected. N28ht corn is used as a maintenance and purification host for this virus. Upon inoculation,

both Chenopodium quinoa and C. amaranticolor develop numerous yellow chlorotic rings after 4 days, with the centers turning into large, white necrotic lesions after approximately 6 days. The virus does not infect Chenopodium systemically. Interestingly, the virus could not be reinoculated mechanically to several different commercial hybrid sorghum varieties. The virus also could not infect WSBMV-resistant or -susceptible varieties of wheat, indicating that, at least for sorghum, another mode of transmission occurs, possibly by P. graminis.

Soil from the location of the original isolation was collected and under glasshouse conditions a variety of corn, wheat, and sorghum varieties were sown. Again, no symptoms appeared on any of the plants. The intent of this experiment was to see if a soil factor, presumably Polymyxa graminis would transmit the virus. The experiment did not eliminate the possibility that P. graminis is present and viruliferous in the soil. Currently, inoculation experiments are being conducted. As of yet, there is no conclusive evidence that this virus is or can be fungus transmitted even though it was isolated from sorghum and cannot be mechanically inoculated to sorghum.

The fact that this Furovirus is very similar to WSBMV and can be readily inoculated to corn and Chenopodium presents the opportunity to perform pseudorecombination experiments with WSBMV in order to identify which RNA component may control vector and mechanical transmission properties, host range, and symptoms. These experiments are currently in progress. Host range studies are currently being conducted in the field in which the virus was identified in 1986.

Surprisingly, the virus has been located in the past in both Nebraska and Kansas. Slide files from the extension groups at both the University of Nebraska and Kansas State University contain photographs of the distinctive ringspot symptoms this virus produces on corn and sorghum. The virus had been incorrectly identified as maize chlorotic mottle virus by both groups and therefore was not studied further.

WSSMV

WSSMV is a long, flexuous, rod-shaped particle 16 nm in diameter and approximately 2,000 nm in length with a capsid protein of molecular weight 36 kDa (Haufler & Fulbright, 1985; Kendall & Lommel, 1986). Buoyant density of the virion in cesium chloride has been reported as 1.281 g/cm³ (Usugi & Saito, 1979) compared to 1.294 g/cm³ for barley yellow mosaic virus (BaYMV) and 1.28 g/cm³ for wheat yellow mosaic virus (WYMV), (Usugi & Saito, 1976). The nucleic acid composition is unknown. When the virus is dissociated in RNA disruption buffer (1.0 M urea, 0.05 M 2-mercaptoethanol, 5 percent sucrose, and 1 percent SDS) for 10 minutes at 50°C and assayed electrophoretically, two RNA species of approximately 6500 and 4000 bases are seen. The size of the larger RNA is identical to that of WSBMV, while that of the smaller RNA is slightly larger than the RNA-2 of WSBMV. This pattern of RNAs is seen with phenol-extracted RNA preparations but is difficult to repeat between different purified viral preparations or even between different RNA extractions of the same viral preparations. As most of the viral cultures of WSSMV are generally contaminated to some degree with WSBMV, the RNAs visualized during gel electrophoresis

of dissociated virus may be those of WSBMV, although the larger size of RNA-2 is unexplained.

The exact length of the WSSMV particle has been disputed in the literature, although it is generally accepted to be close to 2,000 nm. Slykhuis & Polak (1971) reported particles in partially purified preparations to be about 13 nm wide with lengths less than 1,000 nm and having peaks of particle length distribution at 275-300 nm and 600 nm. However, in leaf dip preparations, particles varied from 190-1975 nm in length, indicating that fragmentation or aggregation may have occurred. These measurements correspond closely with measurements reported in Japan. Usugi & Saito (1976; 1979) reported two predominant particle lengths, 200-300 nm and 600 nm, in partially purified preparations of WYMV, BaYMV, and WSSMV. Haufler and Fulbright (1983a) report particle lengths of 600-3,800 nm with a modal length of 1,750 nm. Particles less than 1,000 nm in length were not infectious (Slykhuis & Polak, 1971), indicating that infectivity is associated with longer particles and is not retained during purification. Infectivity could be associated with extremely long particles, such as those of citrus tristeza (Bar-Joseph, Loebenstein & Cohen, 1970).

Most recent reports pertaining to WSSMV concern resistance to the virus, epidemiology, interactions with WSBMV, and serological detection in infected tissues. Further physiochemical characterization and taxonomic classification have been prevented by the difficulty in purifying the virus. The concentration of virions in infected tissue is extremely low and the long particles have a

tendency to fragment and aggregate during the purification process. Many purification protocols have been attempted but with limited or no success. In general, purifications utilizing high pH extraction buffers, such as sodium borate pH 9.0 used to extract WSBMV from infected tissue, are not effective, possibly due to the high degree of aggregation encountered with high pH buffers. Triton X-100 has been used in conjunction with the high pH buffers but has yielded only slightly better results. Virus particles apparently are completely dissociated with extremely low pH systems such as sodium citrate pH 5.0. Neutral pH buffer systems have been the most effective in this laboratory and others. We currently use an ammonium citrate pH 6.5 extraction and differential centrifugation through sucrose pads. Haufler & Fulbright (1983b; 1985) have attempted extraction in sodium phosphate or Hepes followed by polyethylene glycol 6000 (PEG) precipitation. We have not found PEG purification of WSSMV very successful and have noticed considerable protein degradation after using PEG, regardless of the buffer system used. Rather than pelleting the virus, Haufler & Fulbright trap the particles between layers of a cesium sulfate step gradient, a procedure employed in the purification of citrus tristeza. Avoidance of pelleting the viral particles results in much less fragmentation of the particles but does little to increase overall yield. Purification of the virus for protein, such as antisera production, can be adequately accomplished by pelleting the virus, but future characterization of the nucleic acid will need to employ a purification protocol such as that using

cesium sulfate step gradients to avoid less than full-length particles.

WSSMV produces a variety of distinctive cytoplasmic inclusion bodies, including cylindrical inclusions (pinwheels), laminated aggregates, virus-like rods, and complex membrane aggregates (Hooper & Wiese, 1972; Langenberg & Schroeder, 1973; Langenberg, 1985). Hooper & Wiese (1972) found these inclusions to be quite distinct from those produced by wheat streak mosaic virus (WSMV) and WSBMV based on particle morphology and occurrence within infected tissues. WSMV and WSBMV do not induce the formation of the large, complex membranous bodies found in WSSMV-infected tissues. Also, the cylindrical inclusions associated with WSMV are smaller and the pinwheel arms are more tightly wound than those produced by WSSMV. Virus-like particles are observed in conjunction with the pinwheel arms of WSSMV inclusions but not in those associated with WSMV.

The enigmatic nature of WSSMV makes the taxonomic classification of the virus extremely difficult. Many authors make reference to the potato virus Y group (Potyvirus) when reporting on WSSMV, and there are indeed several aspects of WSSMV which would make it a likely candidate for the Potyvirus classification. Potyviruses typically are long, flexuous rods 680-900 nm in length with a width around 11-14 nm (Matthews, 1982). They have a single capsid protein of molecular weight 32-36 kDa, exhibit a particle density of 1.31 grams/cm³ in cesium chloride, and have a single positive sense single-stranded RNA of molecular weight 3-3.5X10⁶ Da. Potyviruses typically produce a

range of cytoplasmic inclusions and are generally mechanically transmissible as well as nonpersistently vectored by aphids. The molecular weight of WSSMV capsid protein, the particle width, and density in cesium chloride, and the distinctive cytoplasmic inclusions associated with infection are all well within the parameters of a prospective Potyvirus. However, the extreme length of the particle, the failure to mechanically transmit the virus, and the fact that WSSMV is vectored by the Polymyxa fungus and not an insect are obvious delineations from the accepted characteristics of known Potyviruses. Elucidation of the characteristics of WSSMV nucleic acid and purification of the inclusion body protein should facilitate the future classification of the virus.

CONCLUSIONS

The endemic nature of WSBMV and WSSMV and the fungal vector Polymyxa graminis ensures the continued existence of soil-borne viral diseases in Kansas. The lack of information on the mechanism of WSBM resistance observed in WSBM-resistant varieties, and the breaking of this resistance to WSBMV when acting synergistically with WSSMV, indicate the need for further characterization of these two viruses. A definition of the genomic organization and identification of genes controlling fungus transmission of WSBMV and WSSMV would facilitate the further future control of these viruses. Progress with WSSMV depends on the successful isolation and characterization of its nucleic acid, presumed to be RNA. Attempts at extraction and cDNA cloning of WSSMV RNA have so far been unsuccessful. Some

characteristics of the virus, such as particle morphology, the molecular weight of the capsid protein, and the production of distinctive inclusion bodies in infected tissue, closely resemble those taxonomic characteristics typical of Potyviruses. However, WSSMV is dramatically different from a Potyvirus in its extreme length and its vectoring by a soil-inhabiting fungus rather than by aphid.

Much work has been done toward the molecular characterization of WSBMV and the genomic organization of the virus will soon be deduced. Current research with BNYVV and other multicomponent viruses has contributed to the formation of models to explain the known functions and structures of WSBMV RNA. The loss of resistance to WSBM in WSBMV-resistant wheats demonstrates the inadvisability of relying on a single resistance gene for virus disease control. For this reason other control strategies should be considered for WSBMV and WSSMV. Since the discovery of the WSBMV-WSSMV synergism, there currently does not exist a known wheat cultivar resistant to viral infection in Kansas.

With the recent developments in plant transformation systems, new and unique concepts in virus disease control are being attempted. These techniques involve the transformation of the host plant with virus genes to confer a protection to the virus. Powell-Abel et al. (1986) and Loesch-Fries et al. (1986 & 1987) have demonstrated that transformation and subsequent expression in the transgenic plant with a virus capsid protein gene confers a type of cross-protection to that virus. This approach is currently limited to systems where the virus

has been cloned and characterized at the molecular level and the host plant can be readily transformed and regenerated. Transformation of wheat has been unsuccessful, not because of problems with the introduction and incorporation of foreign genes, but due to the inability to regenerate plants from the single protoplasts required in the transformation protocols. Other monocots have been transformed, including corn (Fromme, 1986) and rice (Thompson, in press), thus the successful transformation of wheat should soon be accomplished (Cocking & Davey, 1987).

In preparation for the eventual ability to routinely transform wheat, WSBMV and WSSMV must be characterized at the molecular level. The capsid protein of both viruses should be isolated and sequenced as a prelude to transformation and protection experiments similar to those of Powell-Abel et al. (1986). Cell-to-cell movement genes and genes involving fungal transmission should also be identified and characterized for eventual use in plant transformation-based control schemes. Systemic infections by WSBMV and WSSMV may be inhibited through the transformation of wheat with mutant cell-to-cell movement genes. Presumably, systemic infection would be inhibited as a result of a high level of expression of the mutated gene in every cell (possibly, only expression in root cells necessary), competing for necessary sites when wild-type virus is introduced and functional cell-to-cell movement gene expressed. Similar approaches could be developed which could prevent fungus transmission of the viruses.

The finding of the sorghum-infecting WSBMV-like virus, although

of obvious concern to breeders and growers of sorghum, is exciting considering the possibilities for further characterization of WSBMV that the new virus presents. The location of genes responsible for WSBMV host range and mechanical vector transmission may be deduced through psuedorecombination studies with WSBMV and the wheat soilborne-like virus, as well as further characterization of the Furovirus group. From an economic standpoint, the wheat soilborne-like virus has the potential to induce significant damage to future sorghum and corn crops, particularly if breeders have failed to consider Polymyxa susceptibility in these plants genetic pedigree.

The history of WSBMV and WSSMV in Kansas and the recent discoveries of a WSBMV-WSSMV synergism and the wheat soilborne-like virus illustrate patterns of evolution typical of plant viruses. Soil-borne plant viruses will obviously remain a problem in Kansas and all wheat-growing areas of the United States. Current and future research on soil-borne plant viruses is necessary to maintain or expand cereal grain crop production.

REFERENCES

- ABDULLAH, R., COCKING, E. C. & THOMPSON, J. A. (1986). Efficient plant regeneration from rice protoplasts through somatic embryogenesis. Biotechnology 4, 1087-1090.
- AHLAWAT, Y. S., MAJUMDAR, A. & CHENULU, V. V. (1976). First record of wheat spindle streak mosaic in India. Plant Disease Reporter 60, 782-783.
- BARR, D. J. S. & SLYKHUIS, J. T. (1969). Zoosporic fungi associated with wheat spindle streak mosaic in Ontario. Canadian Plant Disease Survey 49, 112-113.
- BARR, D. J. S. & SLYKHUIS, J. T. (1976). Further observations on zoosporic fungi associated with wheat spindle streak mosaic virus. Canadian Plant Disease Survey 56, 77-81.
- BAR-JOSEPH, M., LOEBENSTEIN, G. & COHEN, J. (1970). Partial purification of viruslike particles associated with citrus tristeza disease. Phytopathology 60, 75-78.
- BOUZOUBAA, S., GUILLEY, H., JONARD, G., RICHARDS, K. & PUTZ, C. (1985). Nucleotide sequence analysis of RNA-3 and RNA-4 of beet necrotic yellow vein virus, isolates F2 and G1. Journal of General Virology 66, 1553-1564.
- BOUZOUBAA, S., ZIEGLER, V., BECK, D., GUILLEY, H., RICHARDS, K. & JONARD, G. (1986). Nucleotide sequence of beet necrotic yellow vein virus RNA-2. Journal of General Virology 67, 1689-1700.
- BOUZOUBAA, S., QUILLET, L., GUILLEY, H., JONARD, G. & RICHARDS, K. (1987). Nucleotide sequence of beet necrotic yellow vein virus RNA-1. Journal of General Virology 68, 615-626.
- BRAKKE, M. K. (1971). Soil-borne wheat mosaic virus. Commonwealth Mycological Institute and Association of Applied Biologists Descriptions of Plant Viruses No. 77.
- BRAKKE, M. K., ESTES, A. P. & SCHUSTER, M. (1965). Transmission of soil-borne wheat mosaic virus. Phytopathology 55, 79-86.
- BRAKKE, M. K. & ESTES, A. P. (1967). Some factors affecting vector transmission of soil-borne wheat mosaic virus from root washings and soil debris. Phytopathology 57, 905-910.
- BRAKKE, M. K. & RAO, S. A. (1967). Maintenance of soilborne wheat mosaic virus cultures by transfer through root washings. Plant Disease Reporter 51, 1005-1008.
- BRAKKE, M. K. (1977). Sedimentation coefficients of the virions of soil-borne wheat mosaic virus. Phytopathology 6, 1433-1438.
- BRAKKE, M. K., LANGENBERG, W. G. & SAMSON, R. G. (1982). Wheat spindle streak mosaic virus in Nebraska. Plant Disease 66, 958-959.
- COCKING, E. C. & DAVEY, M. R. (1987). Gene transfer in cereals. Science 236, 1259-1262.
- FELLOWS, H., SILL, W. H. & KING, C. L. (1953). The 1952 epiphytotic of a soil-borne wheat mosaic in Kansas. Plant Disease Reporter 37, 287-289.
- FROMM, M. E., TAYLOR, L. P. & WALBOT, V. (1986). Stable transformation of maize after gene transfer by electroporation. Nature 319, 791-793.

GOLDBACH, R., REZELMAN, G. & VANKAMMEN, A. (1980). Independent replication and expression of B-component RNA of cowpea mosaic. Nature 286, 297-300.

GUMPF, D. J. (1971). Purification and properties of soil-borne wheat mosaic virus. Virology 43, 588-596.

HAUFLER, K. Z. & FULBRIGHT, D. W. (1983a). Detection of wheat spindle streak mosaic virus by serologically specific electron microscopy. Plant Disease 67, 988-990.

HAUFLER, K. Z. & FULBRIGHT, D. W. (1983b). Rapid detection and purification of wheat spindle streak mosaic virus (WSSMV). Phytopathology 73, 789.

HAUFLER, K. Z. & FULBRIGHT, D. W. (1985). Purification and partial characterization of wheat spindle streak mosaic virus (WSSMV). Phytopathology 75, 1349.

HIBINO, H., TSUCHIZAKI, T. & SAITO, Y. (1974). Comparative electron microscopy of cytoplasmic inclusions induced by 9 isolates of soil-borne wheat mosaic virus. Virology 57, 510-521.

HOOPER, G. R. & WIESE, M. V. (1972). Cytoplasmic inclusions in wheat affected by wheat spindle streak mosaic. Virology 47, 664-672.

HSU, Y. H. & BRAKKE, M. K. (1985a). Properties of soilborne wheat mosaic virus isolates in Nebraska. Phytopathology 75, 661-664.

HSU, Y. H. & BRAKKE, M. K. (1985b). Cell-free translation of soil-borne wheat mosaic virus RNAs. Virology 143, 272-279.

IKATA, S. & KAWAI, I. (1937). Some experiments concerning the development of yellow mosaic disease (white streak) of wheat. Relation between the development of yellow mosaic disease of wheat and soil temperature. Journal of Plant Protection 24, 491-501.

JACKSON, A. O., BRACKER, C. E., HUBER, D. M., SCOTT, D. H. & SHANER, G. (1975). The occurrence and transmission of a disease in Indiana with properties of wheat spindle streak mosaic virus. Plant Disease Reporter 59, 790-794.

KENDALL, T. L. & LOMMEL, S. A. (1986). Characterization of wheat spindle streak mosaic virus and time course study of viral disease progression by western blot analysis. Phytopathology 76, 957.

KOEHLER, B., BEVER, W. M. & BONNETT, O. T. (1952). Soil-borne wheat mosaic. Illinois Agricultural Experiment Station Bulletin 556, 567-599.

KUCHAREK, T. A. & WALKER, J. H. (1974). The presence of and damage caused by soilborne wheat mosaic virus in Florida. Plant Disease Reporter 58, 763-765.

KUSZALA, M., ZIEGLER, V., BOUZOUBAA, S., RICHARDS, K., PUTZ, C., GUILLEY, H. & JONARD, G. (1986). Beet necrotic yellow vein virus: different isolates are serologically similar but differ in RNA composition. Annals of Applied Biology, (in press).

LANGENBERG, W. G. & SCHROEDER, H. F. (1973). Endoplasmic reticulum-derived pinwheels in wheat infected with wheat spindle streak mosaic virus. Virology 55, 218-223.

LANGENBERG, W. G. (1985). Immunoelectron microscopy of wheat spindle streak and soil-borne wheat mosaic doubly infected wheat. Journal of Ultrastructure Research 92, 72-79.

LANGENBERG, W. G. (1986). Deterioration of several rod-shaped wheat viruses following antibody decoration. Phytopathology 76, 339-341.

LARSEN, H. J., BRAKKE, M. K. & LANGENBERG, W. G. (1985). Relationships between wheat streak mosaic virus and soilborne wheat mosaic virus infection, disease resistance, and early growth of winter wheat. Plant Disease 69, 857-862.

LISTER, R. M. & BRACKER, C. E. (1969). Defectiveness and dependence in three related strains of tobacco rattle virus. Virology 117, 416-424.

LOESCH-FRIES, L. S., JARVIS, N. P., KRAHN, K. J., NELSON, S. E. & HALL, T. C. (1985). Expression of alfalfa mosaic virus RNA cDNA transcripts *in vitro* and *in vivo*. Virology 146, 177-187.

LOESCH-FRIES, L. S., MERLO, D., ZINNEN, T., BURHOP, L., HILL, K., KRAHN, K., JARVIS, N. & HALK, E. (1987). Expression of alfalfa mosaic virus RNA4 in transgenic plants confer virus resistance. The EMBO Journal 6, 1845-1851.

LOMMEL, S. A. & WILLIS, W. G. (1984). The role of wheat spindle streak and wheat soilborne mosaic virus in an epiphytotic of resistant wheat in Kansas. Phytopathology 74, 844.

LOMMEL, S. A., WILLIS, W. G. & KENDALL, T. L. (1986). Identification of wheat spindle streak mosaic virus and its role in a new disease of winter wheat in Kansas. Plant Disease 70, 964-968.

MATTHEWS, R. E. F. (1982). Classification and nomenclature of viruses. Fourth report of the International Committee on Taxonomy of Viruses. Reprint from Intervirology 17, No. 1-3.

MCKINNEY, H. H. (1923a). Investigation of the rosette disease of wheat and its control. Journal of Agricultural Research 23, 771-800.

MCKINNEY, H. H. (1923b). A mosaic disease of winter wheat and winter rye. United States Department of Agriculture Bulletin No. 1361.

MCKINNEY, H. H. (1930). A mosaic of wheat transmissible to all cereal species in the tribe Hordeae. Journal of Agricultural Research 40, 547-556.

MCKINNEY, H. H. (1931). Differentiation of viruses causing green and yellow mosaics of wheat. Science 73, 650-651.

MCKINNEY, H. H., ECKERSON, S. H. & WEBB, R. W. (1923). The intercellular bodies associated with rosette disease and a mosaic-like leaf mottling of wheat. Journal of Agricultural Research 26, 605-608.

MERKLE, O. G. & SMITH, E. L. (1983). Inheritance of resistance to soil borne mosaic in wheat. Crop Science 23, 1075-1076.

MIYAKE, N. (1938). Mendelian inheritance of resistance against the virus disease in wheat strains. Japan Journal of Genetics 14, 239-242.

MODAWI, R. S., HEYNE, E. G., BRUNETTA, D. & WILLIS, W. G. (1982). Genetic studies of field reaction to wheat soilborne mosaic virus. Plant Disease 66, 1183-1184.

NOLT, B. L., ROMAINE, C. P., SMITH, S. H. & COLE, H. (1981). Further evidence for the association of Polymyxa graminis with the transmission of wheat spindle streak mosaic virus. Phytopathology 71, 1269-1272.

OSMAN, T. A. & BUCK, K. W. (1987). Replication of red clover

necrotic mosaic virus RNA in cowpea protoplasts: RNA 1 replicates independently of RNA 2. Journal of General Virology 68, 289-296.

PAULSEN, A. Q. (1970). Reactions of eleven Chenopodium species to seven sap-transmissible grass viruses. Phytopathology 60, 1307.

POWELL-ABEL, P., NELSON, R. S., DE, B., HOFFMAN, N., ROGERS, S. G., FRALEY, R. T. & BEACHY, R. N. (1986). Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232, 738-743.

RAO, A. S. (1968). Biology of Polymyxa graminis in relation to soil-borne wheat mosaic virus. Phytopathology 58, 1516-1521.

RAO, A. S. & BRAKKE, M. K. (1969). Relation of soil-borne wheat mosaic virus and its fungal vector, Polymyxa graminis. Phytopathology 59, 581-587.

RICHARDS, K., JONARD, G., GUILLEY, H., ZIEGLER, V. & PUTZ, C. (1985). In vitro translation of beet necrotic yellow vein virus RNA and studies of sequence homology among the RNA species using cloned cDNA probes. Journal of General Virology 66, 345-350.

ROANE, C. W., STARLING, T. M. & MCKINNEY, H. H. (1954). Observations on wheat mosaic in Virginia. Plant Disease Reporter 38, 14-18.

ROBERTS, I. M. & HARRISON, B. D. (1979). Detection of potato leafroll and potato mop-top viruses by immunosorbent electron microscopy. Annals of Applied Biology 93, 289-297.

ROBINSON, D. J., BARKER, H., HARRISON, B. D. & MAYO, M. A. (1980). Replication of RNA-1 of tomato black ring virus independently of RNA-2. Journal of General Virology 51, 317-326.

SHIRAKO, Y. & BRAKKE, M. K. (1981). Genetic function of RNA components of soil-borne wheat mosaic virus. Phytopathology 71, 862.

SHIRAKO, Y. & BRAKKE, M. K. (1984a). Two purified RNAs of soil-borne wheat mosaic virus are needed for infection. Journal of General Virology 65, 119-127.

SHIRAKO, Y. & BRAKKE, M. K. (1984b). Spontaneous deletion mutation of soil-borne wheat mosaic virus RNA II. Journal of General Virology 65, 855-858.

SHIRAKO, Y. & EHARA, Y. (1986). Comparison of the in vitro translation products of wild-type and a deletion mutant of soil-borne wheat mosaic virus. Journal of General Virology 67, 1237-1245.

SILL, W. H. (1958). A comparison of some characteristics of soil-borne wheat mosaic viruses in the great plains and elsewhere. Plant Disease Reporter 42, 912-924.

SLYKHUIS, J. T. (1960). Evidence of soil-borne mosaic of wheat in Ontario. Canadian Plant Disease Survey 40, 43.

SLYKHUIS, J. T. (1961). The causes and distribution of mosaic diseases of wheat in Canada in 1961. Canadian Plant Disease Survey 41, 330-343.

SLYKHUIS, J. T. (1964). Noteworthy and new records of grass viruses in Canada in 1964. Canadian Plant Disease Survey 44, 242-243.

SLYKHUIS, J. T. (1970). Factors determining the development of wheat spindle streak mosaic caused by a soil-borne virus in Ontario. Phytopathology 60, 319-331.

SLYKHUIS, J. T. (1974). Differentiation of transmission and

incubation temperatures for wheat spindle streak mosaic virus. Phytopathology 64, 554-557.

SLYKHUIS, J. T. (1975a). Factors critical to mechanical transmissibility of wheat spindle streak mosaic virus. Phytopathology 65, 582-584.

SLYKHUIS, J. T. (1975b). Seasonal transmission of wheat spindle streak mosaic virus. Phytopathology 65, 1133-1136.

SLYKHUIS, J. T. (1976). Wheat spindle streak mosaic virus. Commonwealth Mycological Institute and Association of Applied Biologists Descriptions of Plant Viruses No. 167.

SLYKHUIS, J. T. & BARR, D. J. S. (1978). Confirmation of Polymyxa graminis as a vector of wheat spindle streak mosaic virus. Phytopathology 68, 639-643.

SLYKHUIS, J. T. & POLAK, Z. (1971). Factors affecting manual transmission, purification, and particle lengths of wheat spindle streak mosaic virus. Phytopathology 61, 569-574.

TAMADA, T. (1975). Beet necrotic yellow vein virus. Commonwealth Mycological Institute and Association of Applied Biologists Description of Plant Viruses No. 144.

THOMPSON, J. A., ABDULLAH, R. & COCKING, E. C. (1986). Protoplast culture of rice (Oryza sativa L.) using media solidified with agarose. Plant Science, (in press).

THOUVENEL, J. C., DOLLET, M. & FAUQUET, C. (1976). Some properties of peanut clump, a newly discovered virus. Annals of Applied Biology 84, 311-320.

TSUCHIZAKI, T., HIBINO, H. & SAITO, Y. (1973). Comparisons of soil-borne wheat mosaic virus isolates from Japan and the United States. Phytopathology 63, 634-639.

TSUCHIZAKI, T., HIBINO, H. & SAITO, Y. (1975). The biological functions of short and long particles of soil-borne wheat mosaic virus. Phytopathology 65, 523-532.

USUGI, T. & SAITO, Y. (1976). Purification and serological properties of barley yellow mosaic virus and wheat yellow mosaic virus. Annals of the Phytopathological Society of Japan 42, 12-20.

USUGI, T. & SAITO, Y. (1979). Relationship between wheat yellow mosaic virus and wheat spindle streak mosaic virus. Annals of the Phytopathological Society of Japan 45, 397-400.

WADA, E. & FUKANO, H. (1937). On the difference and discrimination of wheat mosaics in Japan. Journal of the Imperial Agricultural Experiment Station 3, 93-128.

WADSWORTH, D. F. & YOUNG, H. C. (1953). A soil-borne wheat mosaic virus in Oklahoma. Plant Disease Reporter 37, 27-29.

WIESE, M. V., SAARI, E. E., CLAYTON, J. & ELLINGBOE, A. H. (1970). Occurrence of wheat streak mosaic and a new variegation disorder, wheat spindle streak, in Michigan wheat. Plant Disease Reporter 54, 635-637.

WILLIAMS, A. S., PIRONE, T. P., SLYKHUIS, J. T. & TUTT, C. R. (1975). Wheat spindle streak mosaic virus in Kentucky. Plant Disease Reporter 59, 888-889.

ZIEGLER, V., RICHARDS, K., GUILLEY, H., JONARD, G. & PUTZ, C. (1985).

Cell-free translation of beet necrotic yellow vein virus: readthrough of the coat protein cistron. Journal of General Virology 66, 2079-2087.

Fig. 1 Electron micrograph of virus particles stained with uranyl acetate from a leaf dip preparation of symptomed, WSBMV-resistant, Newton wheat found in southcentral Kansas. The long, flexuous particles and the short, stiff rods were serologically identified as WSSMV and WSBMV, respectively.

Fig. 2 Schematic of the proposed genome organization of WSBMV RNA-II (0.5L) and the 0.4 and 0.35L RNA-II deletion mutants compared to the genome organization of BYNVV RNA-IL.

Fig. 3 Electron micrographs of leaf dip preparations from corn (N28Ht) containing the WSBMV-like Furovirus particles stained with (a) anti-TMV sera and (b) anti-WSBMV sera followed by colloidal gold-labeled goat anti-rabbit IgG. The positive staining of the particles with anti-WSBMV sera indicates a serological relationship between the capsid proteins of WSBMV and the WSBMV-like Furovirus isolated from sorghum.

Fig. 4 Foliar symptoms on corn (N28Ht) mechanically inoculated with a Furovirus originally isolated from sorghum in Kansas. Distinctive symptom morphology includes elongated, chlorotic ringspots turning necrotic at the periphery and vein-limited yellow streaking.

Figure 1



Figure 2

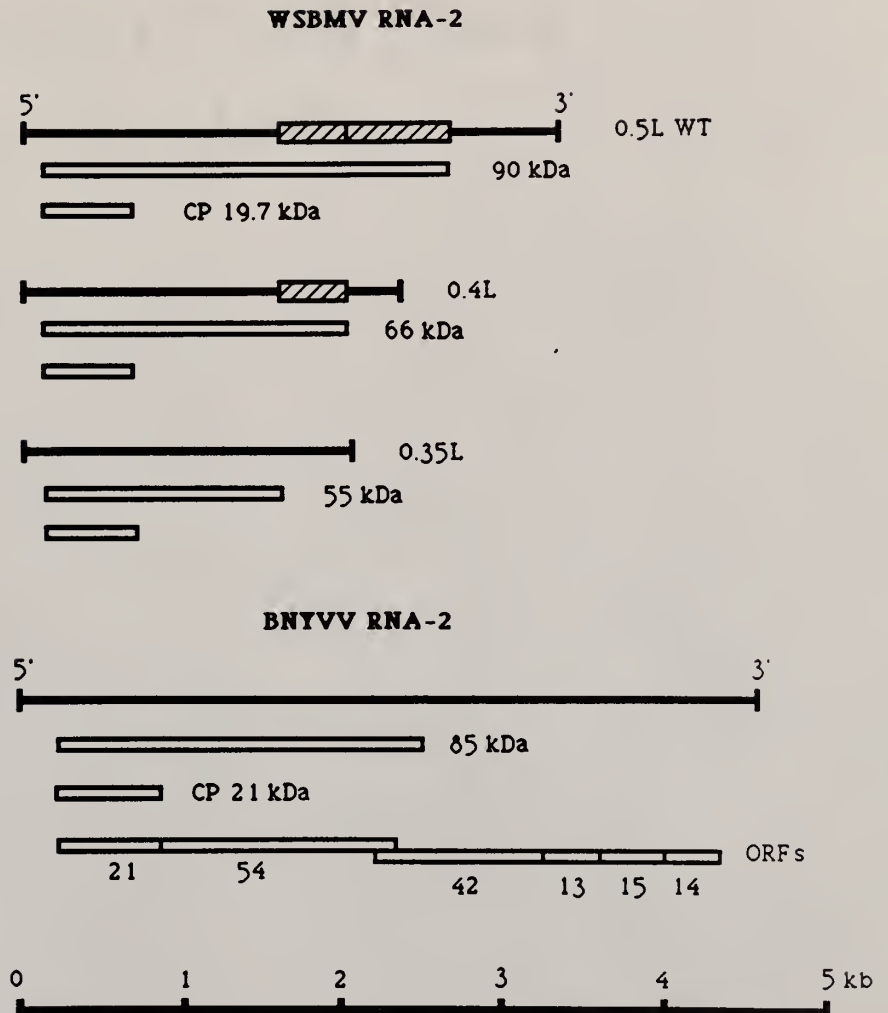
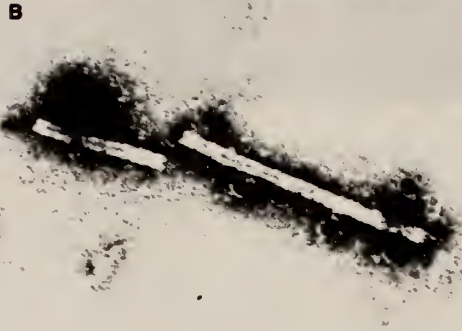


Figure 3



**IDENTIFICATION OF WHEAT SPINDLE STREAK MOSAIC VIRUS
AND ITS ROLE IN A NEW DISEASE OF WINTER WHEAT IN KANSAS**

S.A. Lommel, W.G. Willis, and T.L. Kendall

Department of Plant Pathology, Throckmorton Hall
Kansas State University, Manhattan, KS 66506

ABSTRACT

Severe mosaic, yellowing, bronzing, and stunting symptoms in patterns typical of the Polymyxa graminis-vectored wheat soilborne mosaic virus (WSEMV) appeared in early March 1984 and again in the spring of 1985 in south central Kansas. Many of the hard red winter wheat cultivars showing symptoms were considered resistant to WSEMV. Electron microscopic examination revealed WSEMV particles and other long, flexuous rods. Examination of thin sections revealed pinwheel and amorphous inclusion bodies. From these observations and with western blot analysis, it was determined that the plants were also infected with wheat spindle streak mosaic virus (WSSMV). WSEMV and WSSMV particles were observed in all symptomatic plants in a ratio of about 20:1, respectively. A comparison of wheat plants resistant to wheat soilborne mosaic (WSEM) in soils infested with WSEMV and WSEMV plus WSSMV indicated that WSEMV infected WSEM-resistant cultivars and increased to about the same levels as in WSEM-susceptible cultivars in the presence of a WSSMV infection. Environmental conditions during the springs 1984 and 1985 were optimal for wheat spindle streak mosaic (WSSM) symptom development. The sudden recognition of this disease is attributed to several new WSEM-resistant cultivars that show vivid symptoms when infected with WSEMV plus WSSMV and the conducive environmental conditions. WSSM has now been shown to be widely distributed throughout the eastern half of Kansas and, in many cases, WSSM entirely overlaps in range with WSEM.

INTRODUCTION

Wheat soilborne mosaic virus (WSEMV) causes a major disease of hard red winter wheat in Kansas. The virus is vectored through the soil by the fungus Polymyxa graminis Led. (Rao and Brakke, 1969). Once the soil is infested with the fungus and the virus, it remains infested indefinitely (McKinney et al., 1957). Wheat soilborne mosaic (WSBM) symptoms appear early in the spring with cool and wet weather conditions with an optimal temperature of 15°C (Brakke et al., 1965). Symptoms generally disappear in the late spring as temperatures warm up. WSBM-resistant hard red winter wheat cultivars are available. The resistant cultivars do not show typical WSEMV symptoms and suffer little detectable yield loss (Campbell et al., 1975; Eversmeyer et al., 1983). WSEMV and the vector fungus are endemic throughout the eastern two-thirds of Kansas, with particularly intense infestation in the south central region. As a result, WSBM-resistant cultivars are widely sown in these regions.

Wheat spindle streak mosaic virus (WSSMV) is a long, flexuous rod (16X1,775 nm) (Haufler and Fulbright, 1983) that produces distinctive pinwheel inclusion bodies in infected tissue (Wiese and Hooper, 1971). WSSMV, like WSEMV, is vectored by P. graminis (Slykhuis and Barr, 1978). WSSMV requires temperatures below 17°C for symptom development with an optimal temperature of 10°C (Slykhuis, 1974). Slykhuis (1974) has reported symptoms of wheat spindle streak mosaic (WSSM) to include chlorotic spindle-shaped streaks, stunting, and reduced tillering.

WSSMV has been reported to occur in North America from as far north as Ontario, Canada (Slykhuis and Polack, 1969), as far south as Kentucky (Williams et al., 1975), and as far west as Nebraska (Brakke et al., 1982). There has been no report of WSSMV yet in Kansas, although J.K. Uyemoto (unpublished) made a preliminary identification in 1981 (Brakke et al., 1982).

In late February 1984, large acreages of WSEM-resistant wheats in south central Kansas developed disease symptoms that initially resembled WSEM. Yellowing turning to bronze, mosaics, stunting, and reduced tillering were observed in discrete areas within fields generally corresponding to areas of lower terrain. The initial assumption was that the symptomatic wheat was infected with WSEM. Closer inspection of the symptoms revealed spindle-shaped streaks and a bronzing of the tissue, which is generally not observed with WSEM infections.

This paper describes WSSMV in Kansas and its involvement with the ubiquitous WSEM.

MATERIALS AND METHODS

Electron microscopy. Leaf-dip preparations were made by adding one drop of leaf sap to one drop of 1% potassium phosphotungstate, pH 7.0, on a 300-mesh copper grid coated with Formvar. The drops were allowed to remain on the grid for 5-10 min before blotting off excess liquid. The preparations were then examined with a Philips EM-201 transmission electron microscope (EM).

Thin sections. Portions of field collected wheat leaves with symptoms were excised and fixed in 4% gluteraldehyde, then treated in 2% OsO_4 . The fixed tissue was dehydrated by successive washes in a series of ethanol baths. The tissue was cleared with acetone and embedded in plastic resin. Blocks were thin-sectioned with a microtome and poststained with uranyl acetate and 0.4% lead citrate. The sections were then observed with the EM.

Western blot analysis. Purified virus preparations were electrophoresed in 12% sodium dodecyl sulfate (SDS) polyacrylamide gels and electroblotted to nitrocellulose as described by Towbin et al. (1979). Proteins were fixed to the membranes by a 15-min incubation in 10% acetic acid and 25% ethanol. Membranes were incubated in virus-specific antiserum diluted with TBS:T100 (50mM Tris-HCl, pH 7.4, 200mM NaCl, and 0.1% Triton X-100). A WSSMV antiserum (Haufler and Fulbright, 1983) was provided by K.Z. Haufler, Michigan State University, East Lansing (WSSMV- MS sera) and was used at a 1:200 dilution. An antiserum (WSSMV-KS sera) manufactured from a Kansas isolate of WSSMV electrophoretically purified 36-kDa (Haufler and Fulbright, 1985) capsid protein (T.L. Kendall and S.A. Lommel, unpublished) was used at a 1:1000 dilution. WSEMV antiserum was used at a 1:3000 dilution. After antibody incubation, the membranes were rinsed in TBS:T100 and incubated for 2 hr in a 1:1000 dilution of protein A-alkaline phosphatase conjugate (Sigma, St. Louis, MO). After rinsing in TBS:T100, the membranes were developed for 30 min in the dark with naphthol AS-MX phosphate alkaline solution (Sigma) and fast violet B salt (Sigma).

Field studies. Cultivar plots were sown at two locations. A replicated cultivar test was planted at the Hesston, KS, experimental field on 17 September 1983 and harvested on 27 July 1984. This soil was naturally infested with WSEMV only. The second location, at Clearwater, KS, was naturally infested with both WSEMV and WSSMV, and contained cultivar demonstration strips. The Clearwater plot was not replicated; however, each strip was about one acre. The cultivar demonstration was planted on 29 September 1983 and harvested 3 July 1984.

Weather data. Temperature data were collected at 12-hr intervals from the airport at Wichita, KS, about 10 mi. northeast of the WSEMV plus WSSMV field plots.

RESULTS

Field observations. A pronounced yellowing, bronzing, and stunting was observed in mid-February 1984 throughout south central Kansas. Gross field symptoms included yellowing with an overall bronze appearance to the field, stunting, mosaic, and reduced tillering. The symptoms were always strongest in lower portions of the field (Fig. 1B). In late spring, after several warm days, the affected plants lost symptoms but remained stunted compared with uninfected plants.

Cultivar reactions. A hard red winter wheat demonstration trial was planted in soil infested with WSEMV plus WSSMV in September 1983 (Fig. 1A). By late February 1984, the cultivars Vona and Chisholm

were so severely affected by the two viruses that many of the plants were dead (Table 1). Newton and Arkan, two widely planted WSBM-resistant cultivars, showed significant disease symptoms in the field infested with WSBMV plus WSSMV compared with the field infested with WSBMV only. In the WSBM-only field, the two cultivars had a disease rating of 1.00 with no observable symptoms, whereas in the presence of WSSMV, they had ratings of 3.25 and 4.00, respectively (Table 1). Hawk (Agripro) and Tam 108 had the lowest disease ratings of the cultivars tested at Clearwater (Table 1). Mustang (Agripro) had the highest disease rating (4.75) of the WSBM-resistant cultivars tested. In general, the WSBM-susceptible cultivars were most severely affected by the disease, whereas the WSBM-resistant cultivars varied from severe to light symptoms.

Etiology. Field samples were collected beginning in early March 1984. Leaf-dip preparations from symptomatic wheat plants were examined for virus with the EM. All symptomatic plants contained stiff, rodlike particles of two lengths (20X280 and 140 nm) that were found to be WSBMV (Fig. 1C). In addition, all samples contained a few thin, flexuous, rodlike particles (Fig. 1C). These particles were about 13-16 nm in diameter and ranged from 100 to 1,500 nm long. In all samples examined, the number of long, flexuous rods was always less than the number of WSBMV particles. The shorter rodlike viruses were confirmed to be WSBMV by sandwich enzyme-linked immunosorbent assay (ELISA) (Lommel et al., 1982) and by western blots (Fig. 2).

Thin sections were prepared from the same field samples and observed with the EM. The infected tissue contained prominent

pinwheel inclusion bodies and areas of amorphous membranous bodies (Fig. 1D). These cytological features and the unique particle morphology suggested WSSMV.

The possibility that the long, flexuous rods were not actually wheat streak mosaic virus (WSMV) was examined. WSMV is a common virus of wheat in Kansas. The virions are long, flexuous rods about 13X700 nm, and pinwheel inclusions are produced in vivo (Shepard and Carroll, 1967). Symptomatic wheat tissue collected from fields was mechanically inoculated to N28Ht inbred corn, an excellent indicator host for WSMV (Uyemoto and Ferguson, 1980). No symptoms developed after 20 days, indicating the absence of WSMV. In addition, field-collected samples were assayed for WSMV by ELISA (Lommel *et al.*, 1982) with negative results.

The long, flexuous rods were definitively confirmed to be WSSMV by western blot analysis using a known WSSMV antiserum (WSSMV-MS). Both the WSSMV-MS and WSSMV-KS antiserum detected the 36-kDa (Haufler and Fulbright, 1985) WSSMV capsid protein (Fig. 2). There appeared to be cross-reactivity of the WSSMV-MS antiserum to WSMV capsid protein and healthy plant protein components. The WSSMV-KS antiserum manufactured to isolated WSSMV capsid protein detected only the 36-kDa WSSMV polypeptide.

Distribution. In the spring of 1984, five counties in south central, one in north central, and one in northeastern Kansas were shown to be infested with WSSMV and WSMV as assayed by the EM. By the spring of 1985, 43 counties, all in the eastern half of the state, were shown to have wheat fields infested with WSSMV and WSMV (Fig.

3).

Environmental conditions. Optimal temperatures for symptom development are 15°C for WSBMV (McKinney *et al.*, 1957) and 10°C for WSSMV (Slykhuis, 1974). At the Clearwater plots, the average daily mean temperature for February 1984 was much higher than average, above 5°C. The March temperatures were close to average (Fig. 4), and the temperatures for April and May were cooler than normal. Therefore, for a 4-mo period, the average daily mean temperatures were within the optimal range of temperatures for both WSBMV and WSSMV symptom development. Weather conditions during the spring of 1985 were not as conducive for WSBMV and WSSMV symptom development, and consequently, the disease ratings for most of the cultivars were lower in 1985.

Cultivar reaction comparisons in fields infested with WSEM only and WSEM plus WSSMV. A study was performed using several popular cultivars resistant and susceptible to WSEM that were grown in soil infested with WSBMV only and with WSBMV plus WSSMV. The cultivars tested have similar yield potential under non-WSEM conditions (Walter, 1985). In the WSEM- only plots, all cultivar reactions and yields were consistent with historic reactions (Table 1). No WSBMV was observed in many EM grid sections in leaf-dip preparations from the WSEM-resistant cultivars, except in the moderately resistant Bounty 201 in which only a few particles were detected. In contrast, many WSBMV particles were visible in preparations from the WSEM-susceptible cultivars (Table 1). No WSSMV particles were detected in any of the plants from the WSEM-only plots.

In plots with both viruses, WSBMV and WSSMV particles were easily

detected in both the cultivars susceptible and resistant to WSEM (Table 1). Numerous WSEMV particles were observed in the WSEM-resistant cultivars in about equal concentrations as the WSEM-susceptible cultivars. The disease ratings for the WSEM-resistant cultivars varied from minor (Agripro Hawk) to vivid and severe symptoms (Agripro Mustang) (Table 1). The WSEM-susceptible cultivars were all severely affected, with some killed when infected with WSEMV plus WSSMV. For all cultivars tested, the grain yields corresponded directly with the disease ratings (Table 1).

Comparisons of virus particle counts in cultivars susceptible and resistant to WSEM. A virus particle count study was performed on a WSEM-resistant cultivar (Newton) and a WSEM-susceptible cultivar (Vona) in both a field infested with WSEMV only and one field infested with WSEMV plus WSSMV. Leaf-dip preparations of Newton (WSEMV-resistant) from the WSEMV-only plot were devoid of virus particles, whereas Newton in the field infested with WSEMV plus WSSMV had an average of 20 WSEMV particles and one WSSMV particle per grid section (Table 2). Vona (WSEMV-susceptible) had 20 WSEMV virus particles per grid section in both field plots and one WSSMV particle in the doubly infested field. In essence, when Newton is coinfectd by both viruses, the concentration of WSEMV particles is equal to that of a WSEM-susceptible cultivar.

DISCUSSION

WSSMV has now been identified in Kansas. The diagnosis was

confirmed by unique particle morphology of the virus, the presence of pinwheel inclusions, and by a serological western blot analysis. The possibility that the virus was misidentified and was actually WSMV was eliminated. The presence of WSSMV along with the endemic WSEMV has resulted in a new disease situation in hard red winter wheat. This preliminary evidence suggests that the two viruses interact synergistically, resulting in a severe yield-reducing disease of wheat resistant and susceptible to WSEM.

WSEMV and WSSMV are both widely distributed throughout North America. It is evident that in Kansas, the infestations of WSEMV and WSSMV overlap over scattered regions of the eastern half of the state. All samples assayed during the 2-yr study had either WSEMV alone or WSEMV plus WSSMV. There was only a single identification in a commercial wheat field sown to Newton wheat that was infected by only WSSMV (Fig. 3).

In the western blots, the WSSMV-MS antiserum reacted at low levels to WSEMV capsid protein. These data may mean that there are common antigenic sites between the capsid proteins of the two viruses, or more likely, they suggest that wheat plants in Michigan may have been infected by both viruses and that the antiserum may have antibodies to WSEMV.

Given the fact that WSEMV and WSSMV are vectored by the same soil-inhabiting fungus, *P. graminis*, which is persistent, it appears that WSSMV is now endemic in many regions of Kansas and will persist in the soil. For this reason, it is not surprising that WSEMV and WSSMV are found together in Kansas. WSEMV is firmly established in

the eastern two-thirds of the state, with a particularly intense and concentrated infestation in south central Kansas. WSSMV has the immediate potential to invade wherever WSEM is already established.

In 1984, wheat in four counties of Kansas was found to be infected with WSSMV. In 1985, wheat in 43 counties, all in the eastern half of the state, was confirmed to be infected with WSSMV as well as WSEM. This does not indicate that WSSMV is spreading rapidly but does reflect a more intensive effort to determine the distribution.

Most soilborne diseases spread slowly and predictably. Given that WSSMV is widely distributed throughout the state as well as in Nebraska (Brakke et al., 1982) and the fact that it is vectored by P. graminis, it seems contradictory that only recently have detectable disease symptoms been observed. We believe an explanation for these confusing observations involves the spring weather for 1984 and 1985 and the widespread planting of several new WSEM-resistant wheat cultivars.

Newton, a popular WSEM-resistant cultivar released in 1977, periodically showed mild WSEM-like symptoms (W.G. Willis, unpublished). Newton scored a disease rating of 3.25 under optimal environmental conditions for disease in a plot known to be infested with WSEM plus WSSMV (Table 1). Two other WSEM-resistant cultivars, Arkan and Mustang, which gained rapid popularity within the past 2 yr, had significantly higher disease ratings of 4.00 and 4.75, respectively (Table 1). Before 1983, Newton, which was widely grown in WSEM-infested areas under nonideal weather conditions for disease,

was probably infected with both WSBMV and WSSMV but did not show striking symptoms. In the fall of 1983, other WSBM-resistant cultivars were released commercially. The wide planting of these new cultivars, coupled with the conducive spring weather conditions, resulted in the sudden development of dramatic symptoms. These conclusions all assume that WSSMV has been present in Kansas soils for some time.

An observation of particular concern is that WSBM-resistant cultivars appear to be fully susceptible to WSBMV when coinfectd with WSSMV. The data in Table 2 illustrate that the virus concentration in Newton (WSBM- resistant) is equivalent to that in Vona (WSBM-susceptible) when grown in soil infested with WSBMV plus WSSMV. Some WSBM-resistant cultivars appear to have some levels of natural resistance to WSSMV or the WSBMV-WSSMV complex. For example, both Hawk and Mustang are WSBM-resistant, but Hawk had a very low disease rating whereas Mustang produced quite vivid symptoms. It is difficult to speculate without further information whether Hawk is resistant or tolerant to the WSBMV-WSSMV complex, because both viruses can be detected in infected tissue.

In conclusion, WSSMV is widely distributed throughout Kansas and has the potential to infest every location where WSBMV already exists. Therefore, until new cultivars become available that are resistant to WSBMV and WSSMV together, a severe disease situation has the potential to occur when spring weather conditions are conducive for symptom development.

REFERENCES

- BRASSE, M.K., ESTES, A.P. & SCHUSTER, M.L. (1965). Transmission of soil-borne wheat mosaic virus. Phytopathology 55, 79-86.
- BRASSE, M.K., LANGENBERG, W.G. & SAMSON, R.G. (1982). Wheat spindle streak mosaic virus in Nebraska. Plant Disease 66, 958-959.
- CAMPBELL, L.G., HEYNE, E.G., GRONAU, D.M. & NIBLETT, C. (1975). Effect of soilborne wheat mosaic virus on wheat yield. Plant Disease Reporter 59, 472-476.
- EVERSMAYER, M.G., WILLIS, W.G. & KRAMER, C.L. (1983). Effect of soil fumigation on occurrence and damage caused by soilborne wheat mosaic. Plant Disease 67, 1000-1002.
- HAUFLE, K.Z. & FULBRIGHT, D.W. (1983). Detection of wheat spindle streak mosaic virus by serologically specific electron microscopy. Plant Disease 67, 988-990.
- HAUFLE, K.Z. & FULBRIGHT, D.W. (1985). Purification and partial characterization of wheat spindle streak mosaic virus (WSSMV). Phytopathology 75, 1349.
- LOMEL, S.A., MCCAIN, A.H. & MORRIS, T.J. (1982). Evaluation of indirect enzyme-linked immunosorbent assay for the detection of plant viruses. Phytopathology 72, 1018-1022.
- MCKINNEY H.H., PADEN, W.R. & KOEHLER, B. (1957). Studies on chemical control and overseasoning of, and natural inoculation with the soil-borne virus of wheat and oats. Plant Disease Reporter 41, 256-266.
- RAO, A.S. & BRASSE, M.K. (1969). Relation of soil-borne wheat mosaic virus and its fungal vector, Polymyxa graminis. Phytopathology 59, 581-587.
- SHEPARD, J.F. & CARROLL, T.W. (1967). Electron microscopy of wheat streak mosaic virus particles in infected plant cells. Journal of Ultrastructure Research 21, 145-150.
- SLYKHUIS, J.T. (1974). Differentiation of transmission and incubation temperatures for wheat spindle streak mosaic virus. Phytopathology 64, 554-557.
- SLYKHUIS, J.T. & BARR, D.J.S. (1978). Confirmation of Polymyxa graminis as a vector of wheat spindle streak mosaic virus. Phytopathology 68, 639-643.
- SLYKHUIS, J.T. & POLACK, Z. (1969). Verification of wheat spindle streak mosaic virus in Ontario. Canadian Plant Disease Survey 49, 108-111.
- TOWBIN, H., STAHELEN, T. & GORDON, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proceedings of the National Academy of Science 76, 4350-4354.
- UYEMOTO, J.K. & FERGUSON, M.W. (1980). Wheat streak mosaic virus: Increased yields of purified virus from corn. Plant Disease 64, 460-462.
- WALTER, T.L. (1985). Performance tests with winter wheat varieties. Kansas Agricultural Experiment Station Progress Report 483.
- WIESE, M.V. & HOOPER, G.R. (1971). Soil transmission and electron microscopy of wheat spindle streak mosaic. Phytopathology 61, 331-332.
- WILLIAMS, A.S., PIRONE, T.P., SLYKHUIS, J.T. & TUTT, C.R. (1975).

Wheat spindle streak mosaic virus in Kentucky. Plant Disease Reporter
59, 888-889.

Fig. 1. (A) Clearwater cultivar demonstration strips on 20 March 1984. Soil was naturally infested with wheat soilborne mosaic virus (WSBMV) and wheat spindle streak mosaic virus (WSSMV). (B) Commercial field of Newton wheat (WSBM-resistant) on 27 February 1984 infested with WSBMV and WSSMV. Dark areas are locations of higher terrain and have symptomless plants. Light areas are plants showing mosaic and stunt symptoms. (C) Leaf-dip preparation of symptomatic Newton wheat collected from field shown in B. The shorter, wider rod particles are WSBMV, and the long, flexuous rod is WSSMV. (D) Thin section of symptomatic wheat leaf tissue from field shown in B. Membranous bodies and pinwheel inclusions are clearly present. Scale bars=300 nm.

Fig. 2. Western blot analysis of wheat soilborne mosaic virus (WSBMV) and wheat spindle streak mosaic virus (WSSMV) with WSBMV and WSSMV antiserum. (A) Silver-stained 12% SDS-polyacrylamide gel. (1) Protein molecular weight standard: phosphorylase B, 97.4 kDa; bovine serum albumin, 68.0 kDa; ovalbumin, 43.0 kDa; alpha-chymotrypsinogen, 25.7 kDa; and beta-lactoglobulin, 18.4 kDa. (2) Purified virus preparation of WSBMV. (3) Purified virus preparation of WSSMV. (B) Western blot of (1) WSBMV and (2) WSSMV detected with WSBMV antiserum (1:3000 dilution). (C) Western blot of (1) WSBMV and (2) WSSMV detected with WSSMV-MS antiserum (1:200 dilution). (D) Western blot of (1) WSBMV and (2) WSSMV detected with WSSMV-KS antiserum (1:1000 dilution).

Fig. 3. Distribution of wheat spindle streak mosaic virus (WSSMV) in

Kansas in spring 1985. Cross-hatched counties indicate that at least one field of infected wheat (WSSMV) within the county has been identified. All samples tested throughout the state were infected with wheat soilborne mosaic virus (WSBMV) and WSSMV except the location marked (); this was the only field identified with only a WSSMV infestation.

Fig. 4. Average mean temperatures for the spring 1984. Data collected at the Wichita, KS, airport. Each dot represents the average mean temperature for each day. Line represents average mean temperature averaged over a week.

Table 1. Disease ratings, yields, and virus content of hard red winter wheat cultivars from field plots infested with wheat soilborne mosaic virus (WSBMV) and WSBMV plus wheat spindle streak mosaic virus (WSSMV) during the 1983-1984 growing season.

Table 2. Relative concentrations of virus particles from wheat cultivars resistant and susceptible to wheat soilborne mosaic virus (WSBMV) that were grown in soils infested with WSBMV only and WSBMV plus wheat spindle streak mosaic virus (WSSMV).

Table 1

Cultivar or hybrid	Field reaction to WSBMV ^a	WSBMV-infested field plot ^b			WSBMV- and WSSMV-infested field plot ^c				
		Disease rating ^d	Yield (bu/acre)	WSBMV ^e	WSSMV	Disease rating	Yield (bu/acre)	WSBMV	WSSMV
Hawk	R	1.25	48	—	—	2.00	37	+	+
Bounty 201	MR	2.25	52	—	—	2.50	30	+	+
Newton	R	1.25	44	—	—	3.25	30	+	+
Arkan	R	1.00	47	—	—	4.00	25	+	+
Mustang	R	1.00	47	—	—	4.75	20	+	+
Tam 105	S	5.00	40	—	—	6.00	15	+	+
HW1010	MS	4.50	43	—	—	7.25	14	+	+
Vona	S	6.00	33	—	—	7.75	N.H. ^f	+	+
Chisholm	S	5.50	37	—	—	9.00	N.H.	+	+

^a R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible to WSBMV as determined by field reactions.

^b Plots located at Hesston, KS. Soil naturally infested with WSBMV only.

^c Plots located at Clearwater, KS. Soil naturally infested with WSBMV plus WSSMV.

^d Disease ratings are for mosaic, yellowing, and stunting, where 1 = no symptoms and 9 = severest symptoms.

^e Virus presence was determined by analysis of leaf-dip preparations with the electron microscope.

^f N.H. = not harvested.

Table 2

Cultivar	Field reaction to WSBMV ^a	Average no. of virus particles per grid section ^b			
		WSBMV-infested soil ^c		WSBMV- + WSSMV-infested soil ^d	
		WSBMV	WSSMV	WSBMV	WSSMV
Newton	R	0	0	20	1
Vona	S	20	0	20	1

^a R = resistant and S = susceptible.

^b Average number of morphologically similar virus particles from 50 grid sections, as examined with the electron microscope.

^c Plots located at Hesston, KS. Soil naturally infested with WSBMV only.

^d Plots located at Clearwater, KS. Soil naturally infested with WSBMV plus WSSMV.

Figure 1

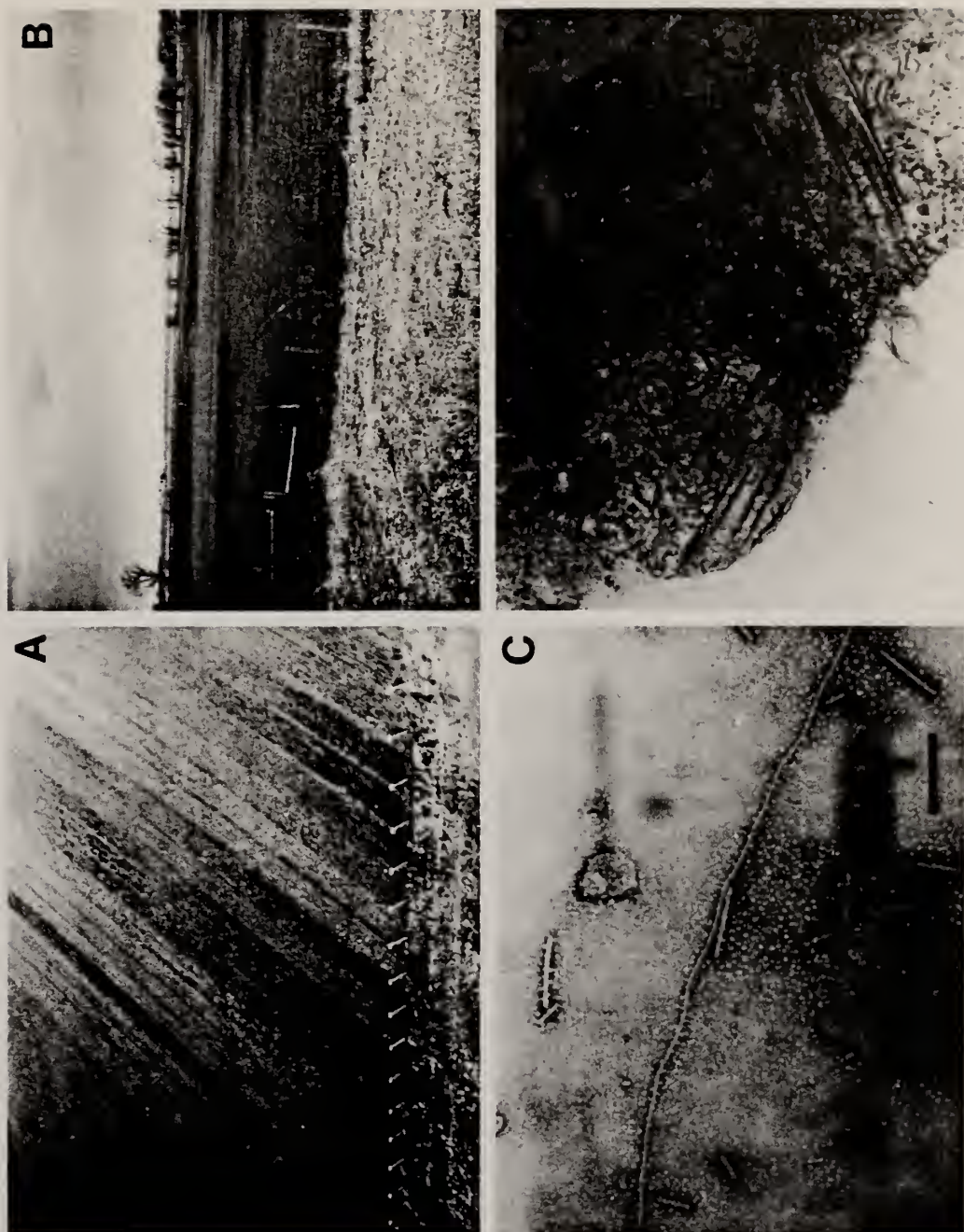


Figure 2

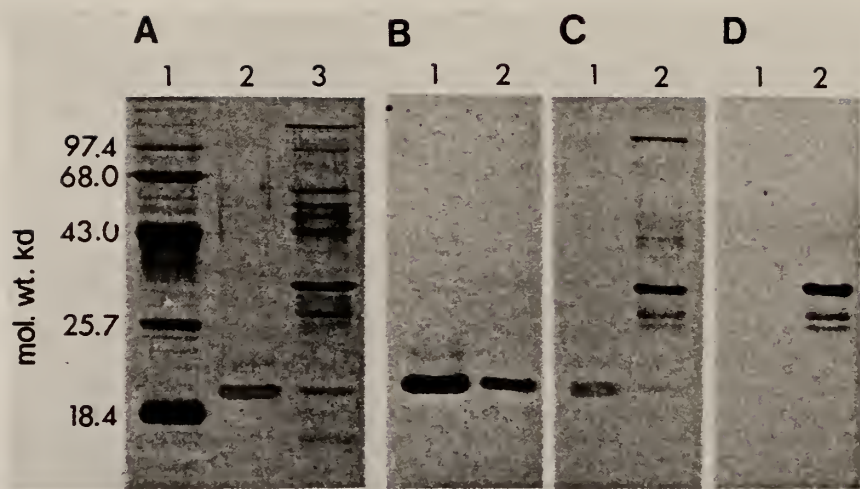


Figure 3

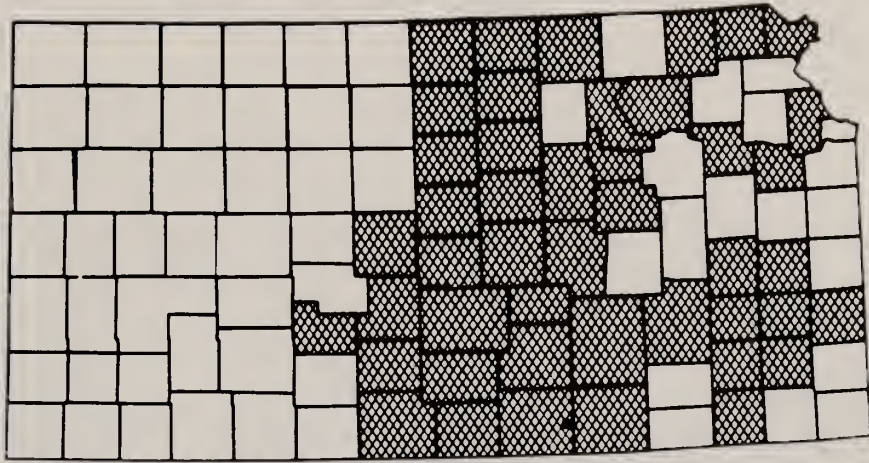
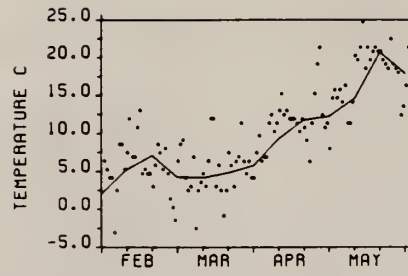


Figure 4



**MOLECULAR CHARACTERIZATION OF SORGHUM CHLOROTIC SPOT VIRUS,
A MECHANICALLY TRANSMISSIBLE FIROVIRUS**

T.L. Kendall and S.A. Lommel

Department of Plant Pathology, Throckmorton Hall
Kansas State University, Manhattan, KS 66506

SUMMARY

A virus morphologically and physicochemically similar to the type member of the Furovirus group, wheat soilborne mosaic virus (WSBMV), has been isolated from Sorghum bicolor and partially characterized at the molecular level. The virus, sorghum chlorotic spot virus (SCSV), exhibits distinct elongate chlorotic and ring spots as well as general yellowing on systemically infected leaves of sorghum and inbred Zea mays lines. In addition, SCSV is mechanically transmissible to and produces symptoms on the inoculated leaves of Nicotiana clevelandii, Chenopodium amaranticolor and C. quinoa. The virus is multicomponent and bipartite in nature with two distinct rods, 20 nm in diameter and 260 and 140 nm in length. Virions are composed of a single 20.5K capsid protein species and two nonhomologous, non-polyadenylated, genomic RNAs of 6.2kb (2.2×10^6 Da) for RNA-1 and 3.5kb (1.2×10^6 Da) for RNA-2. SCSV capsid protein is serologically related to WSBMV capsid protein as determined by western blot and immunogold cytochemical analysis. Northern blot hybridizations indicate that sequence homology exists between RNA-2 of SCSV and RNA-2 of WSBMV, presumably within the capsid protein cistrons. Unfractionated viral RNAs direct the synthesis of 150, 110, 50, 35, 25, and 20.5K polypeptides in vitro. The 110, 25, and 20.5K products are immunoprecipitated by SCSV capsid protein specific polyclonal antiserum. Comparative in vitro translation analysis with WSBMV suggest that the SCSV capsid protein cistron resides on the 5' terminus of RNA-2. A 1.8kb cDNA clone was synthesized to SCSV RNA-2. T7 transcripts from the clone directed the

synthesis of several polypeptides, none of which were immunoprecipitated by capsid protein antiserum. SCSV is similar to but distinct from WSBMV and is proposed to be a new member of the Furovirus group.

INTRODUCTION

The Furovirus (fungus-borne rod-shaped virus) group is characterized by viruses with rigid, hollow rod-shaped virions, plasmodiophoraceous fungal vectors, and divided genome (Shirako and Brakke, 1984a). Wheat soilborne mosaic virus (WSBMV) is the type member of the Furovirus group (Shirako and Brakke, 1984a) and several other plant viruses have been suggested as members, including beet necrotic yellow vein virus (BNYVV) (Tamada, 1975; Putz, 1977), potato mop-top virus (Harrison, 1974; Roberts and Harrison, 1979), Hypochoeris mosaic virus (Brunt and Stace-Smith, 1978), Nicotiana velutina virus (Randles et al., 1976), and peanut clump virus (Thouvenel et al., 1976; Thouvenel and Fauquet, 1981).

Several of these viruses have been extensively characterized at the molecular level. The BNYVV genome has been cDNA cloned and completely sequenced (Bouzoubaa et al., 1985; Bouzoubaa et al., 1986; Bouzoubaa et al., 1987) and in vitro translation studies have been used to determine the genomic organization of the virus (Richards et al., 1985; Ziegler et al., 1985). Likewise, an isolate of Indian peanut clump virus (IPCV) has been characterized (Reddy et al., 1983) and in vitro translation studies performed (Mayo and Reddy, 1985).

WSEMV has been extensively studied (Tsuchizaki et al., 1975; Brakke, 1977; Shirako and Brakke, 1984a and b; Hsu and Brakke, 1985; Shirako and Ehara, 1986) and a considerable amount of information concerning the genomic organization of the virus has been deduced without cloning and sequence data.

A comparison of physicochemical properties and genomic organization of WSEMV, BNYVV, and IPCV indicates that these viruses are indeed similar and share features consistent with characteristics of the Furovirus group. WSEMV and IPCV, in particular, are morphologically and physicochemically very similar (Shirako and Brakke, 1984a; Brakke, 1977; Tsuchizaki et al., 1973; Reddy et al., 1983; Mayo and Reddy, 1985) and these viruses have a common vector, Polymyxa graminis Led. (Estes and Brakke, 1966; Rao and Brakke, 1969; Brakke et al., 1965; Reddy et al., 1983).

Discrepancies are also apparent between the members of the Furovirus group, particularly between WSEMV and BNYVV. Although both viruses have plasmodiophoraceous fungus vectors, BNYVV is vectored by P. betae (Tamada, 1975; Fujisawa and Sugimoto, 1976) rather than P. graminis. BNYVV is composed of four discrete RNAs separately encapsidated in four distinct rod-shaped virions (Putz, 1977; Putz et al., 1983; Richards et al., 1985) and BNYVV RNA is polyadenylated (Putz et al., 1983) whereas WSEMV contains no polyadenylated sequence (Bouzoubaa et al., 1986). BNYVV and IPVC are dicotyledonous plant viruses and WSEMV is a monocotyledonous plant virus. Molecular characterization of the WSEMV genome or other WSEMV-like Furoviruses is needed to further define areas of observed discrepancies within the

Furovirus group.

We have isolated a virus from sorghum (Sorghum bicolor) that is morphologically and physicochemically indistinct from WSEMV. Sorghum chlorotic spot virus (SCSV) is proposed as the name of the virus as the dominant symptom of infection observed in sorghum is an elongated, chlorotic spot. SCSV is mechanically transmissible to several monocotyledonous and dicotyledonous hosts and shares a serological relatedness with WSEMV. Results from the characterization of SCSV indicate that the virus is much more similar in structure and function to WSEMV than to the other members of the Furovirus group. The possible use of SCSV to further define the genomic organization of WSEMV, specifically, to identify genes responsible for mechanical transmissibility, host specificity, and vector transmission, is discussed.

METHODS

Virus host range, maintenance, and purification. SCSV was mechanically inoculated to Zea mays, Sorghum bicolor, Triticum aestivum, Chenopodium quinoa, C. amaranticolor, Arachis hypogaea, and Nicotiana clevelandii. Symptomed leaves from field collected sorghum were ground in 10 mM- potassium phosphate pH 7.0 with 1% celite (w/v) and the extract inoculated to the maintenance and host range plants. Inoculated plants were maintained in the glasshouse at a constant temperature of 25°C.

Virus for purification was increased in Z. mays (N28Ht, an inbred

corn line). Virus was purified from systemically infected leaves using the method described for WSBMV (Shirako and Brakke, 1984) with the except that tissue was ground in liquid nitrogen and then allowed to thaw in extraction buffer rather than being homogenized in a blender.

Capsid protein analysis and sera production. Capsid protein was denatured by boiling for 5 minutes in 125 mM-Tris-Cl pH 6.8, 4% SDS (w/v), 20% glycerol (v/v), and 10% 2-mercaptoethanol (v/v) and electrophoresed in a 12% SDS-polyacrylamide gel using the Laemmli (1970) discontinuous buffer system. Protein bands were stained with Coomassie Brilliant Blue R after fixing in 50% methanol (v/v) and 10% acetic acid (v/v).

Denatured capsid protein for antiserum production was isolated by electrophoresis in a 12% SDS-polyacrylamide gel. The capsid protein band was visualized by incubation of the gel in cold 250 mM-KCl. The protein band was excised and protein eluted from the matrix by freezing and thawing after grinding of the gel pieces. For the initial immunization, purified protein (1 mg/ml) was emulsified with an equal volume of Freund's complete adjuvant and injected intramuscularly. Four subsequent injections were made at two week intervals with protein emulsified with Freund's incomplete adjuvant. Blood was collected at two week intervals starting three weeks after initial injection. Antiserum collected 6 weeks after the initial injection was used in the western blot and in vitro translation studies.

Protein was transferred from SDS-polyacrylamide gels to

nitrocellulose in Towbin's buffer (25 mM-Tris, 192 mM-glycine, and 20% methanol (v/v)) by electroblotting (Towbin *et al.*, 1979) for 4.5 hours at a constant current of 120 mA. Following transfer, nitrocellulose was fixed in 25% ethanol (v/v) and 10% acetic acid (v/v) and then stained with protein A-alkaline phosphatase (Leach *et al.*, 1987) with antisera to WSEMV or SCSV.

Electron microscopy Immunostaining was carried out as described by Langenberg (1985). Immunostaining was followed by colloidal gold enhancement of decoration of virus particles by specific IgG in leaf dips by treatment with goat anti-rabbit IgG-colloidal gold according to the two step method of Lin and Langenberg (1983).

RNA extraction and analysis. RNA was extracted in 50 mM-Tris-Cl pH 8.0 and 1.0% SDS (w/v) with an equal volume of phenol and incubated at 65°C for 5 minutes. Phases were separated by centrifugation and RNA was recovered from the aqueous phase by ethanol precipitation. RNA was electrophoresed under nondenaturing and denaturing conditions. Nondenaturing gels were electrophoresed in 90 mM-Tris pH 8.0, 90 mM-boric acid, and 2 mM-EDTA and denaturing gels were electrophoresed in 50 mM-MOPS pH 7.2 and 1 mM-EDTA. Denaturing gels contained 7% formaldehyde (v/v). RNA electrophoresed under denaturing conditions was incubated at 65°C for 5 minutes in four volumes of 50% deionized formamide (v/v), 6% formaldehyde (v/v), and 50 mM-MOPS pH 7.2 containing 1 mM-EDTA prior to electrophoresis.

Viral RNA was fractionated on an oligo(dT)-cellulose column equilibrated in 20 mM-Tris-Cl pH 7.6, 500 mM-NaCl, 1 mM-EDTA, and 0.1% SDS (w/v). Polyadenylated RNA was eluted from the column with 10

mM-Tris-Cl pH 7.5, 1 mM-EDTA, and 0.05% SDS (w/v). Polyadenylated and non-polyadenylated RNA fractions were recovered by ethanol precipitation and assayed by agarose gel electrophoresis. Cowpea mosaic virus (CPMV) was used as a polyadenylated positive (El Manna and Bruening, 1973) and WSBMV as a negative control (Bouzoubaa et al., 1986).

cDNA cloning of SCSV viral RNA. Purified viral RNA (10 ug) was enzymatically polyadenylated at the 3' end as described by Carrington and Morris (1984). Polyadenylation was carried out in a reaction volume of 50 ul containing 50 mM-Tris-Cl pH 7.9, 250 mM-NaCl, 10 mM-MgCl₂, 2.5 mM- MnCl₂, 500 ug/ml BSA, 10.2 units human placental ribonuclease inhibitor (BRL), and 6 units poly(A) polymerase (BRL) for 10 minutes at 37°C. The reaction was terminated by addition of EDTA to 30 mM and RNA recovered by ethanol precipitation following extraction with phenol and chloroform.

Synthesis of first strand cDNA was carried out in 50 mM-Tris-Cl pH 7.5, 75 mM-KCl, 10 mM-DTT, 3 mM-MgCl₂, 1 mM-dATP, 1 mM-dCTP, 1 mM-dGTP, 1 mM-dTTP, 1.25 mM-sodium pyrophosphate, 100 ug/ml BSA, 100 ug/ml oligo (dT₁₂₋₁₈) (Pharmacia), 15.3 units human placental ribonuclease inhibitor (BRL), and 400 units M-MLV reverse transcriptase (BRL) for 60 minutes at 37°C. The reaction was terminated by addition of EDTA to 30 mM. The DNA:RNA hybrid was extracted with phenol and chloroform and ethanol precipitated. RNA digestion and second strand synthesis was performed in a single reaction as described by Gubler and Hoffman (1983). Second strand DNA was synthesized in 20 mM-Tris-Cl pH 7.5, 5 mM-MgCl₂, 10 mM-(NH₄)₂SO₄,

100mM KCl, 1 mM-ATP, 0.5 mM-dATP, 0.5 mM-dCTP, 0.5 mM-dGTP, 0.5 mM-dTTP, 50 ug/ml BSA, 0.85 units RNase H (BRL), 19 units DNA polymerase I (BRL), and 2 units T4 DNA ligase (BRL). Reactants were incubated 60 minutes at 12°C followed by incubation at 22°C for an additional 60 minutes. Reaction was terminated with the addition of EDTA to 30 mM. Double stranded cDNA was extracted with phenol and chloroform and recovered by ethanol precipitation.

Duplex DNA was dC-tailed in 100 mM-potassium cacodylate pH 7.2, 2 mM- CoCl₂, 0.2 mM-DTT, 0.1 mM-dCTP, and 22.1 units terminal deoxynucleotidyl transferase at 37°C for 15 minutes. The reaction was terminated by addition of EDTA to 30 mM and the dC-tailed double stranded cDNA was extracted with phenol and chloroform and recovered by ethanol precipitation. The dC-tailed double stranded cDNA was then annealed to Pst-I restricted dG-tailed pBR322 (BRL) as described by Maniatis et al. (1982).

Competent E. coli RR1 cells were transformed with the plasmid as described by Maniatis et al. (1982). Transformation was by the calcium chloride method. Transformed bacteria containing the cDNA clone were selected by patching all transformed bacterial colonies on 12.5 ug/ml tetracycline plates and 100 ug/ml carbenicillin plates. Selected colonies were screened using the small scale alkaline lysis plasmid purification method (Maniatis et al., 1982).

Northern blot hybridization. Northern blots were hybridized with SCSV and WSEMV cDNA probes or nick-translated plasmid containing an SCSV cDNA insert. cDNA probes were synthesized from 2.5 ug RNA template in a 50 ul reaction containing 50 mM-Tris-Cl pH 8.1, 2 mM-DTT, 5

mM-MgCl₂, 40 mM-KCl, 250 ng DNase treated calf thymus DNA primer, 0.5 mM-dCTP, dTTP, and dGTP, 1000 units M-MLV reverse transcriptase (BRL), 25 units human placental ribonuclease inhibitor (BRL), and 0.5 mCi ³²P-dATP at 3000 Ci/mmol (NEN) for 60 minutes at 37°C. Reaction was terminated by addition of EDTA to 80 mM. The RNA was digested with the addition of 35 ul 1M NaOH and incubation for 90 minutes at 65°C. The pH was made neutral by adding 35 ul 1M HCl and the cDNA probe was extracted with phenol and chloroform. DNA was recovered by ethanol precipitation.

SCSV cDNA clones were nick translated in a 50 ul reaction containing 2 ug plasmid DNA, 50 mM-Tris-Cl pH 7.2, 10 mM-MgSO₄, 0.1 mM-DTT, 50 ug/ml BSA, 1 nmole-dATP, dCTP, dTTP, dGTP, 10 ng/ml DNase I, 0.5 mCi ³²P-dATP at 3000 Ci/mmol, 20 units DNA polymerase I (BRL), and 1-2 ug plasmid for 60 minutes at 15°C. 1 ul 10% SDS was added to terminate the reaction. DNA was extracted with phenol and chloroform and recovered by ethanol precipitation.

SCSV and WSEMV RNA was electrophoresed under denaturing conditions for 3 hours at 120 mA and transferred to GeneScreen (NEN) in 10X SSC pH 7.0 (Thomas, 1980). Membranes were prehybridized for 8 hours and hybridized overnight in 50 mM-sodium phosphate pH 7.0, 2 M-NaCl, 0.05% SDS (w/v) and 0.6 mg/ml denatured salmon sperm DNA at 65°C. Membranes were washed three times for 10 minutes each in 2X SSC pH 7.0 and 0.1% SDS (w/v) at room temperature followed by three 10 minute washes in 0.1X SSC pH 7.0 and 0.1% SDS (w/v) at 65°C.

In vitro transcription and translation. pSCS007, the largest SCSV specific cDNA clone, was selected for use in initial in vitro

transcription and translation studies. The 1,800 basepair insert was subcloned into the Pst-I site of the transcription vector, pGEM-blue (Promega). Transcripts were synthesized using the T7 and SP6 promoters in a reaction containing 40 mM-Tris-Cl pH 7.5, 6 mM-MgCl₂, 2 mM-spermidine, 10 mM-NaCl, 10 mM-DTT, 0.5 mM-ATP, 0.5 mM-CTP, 0.5 mM-GTP, 0.5 mM-UTP, 1 unit/ul human placental ribonuclease inhibitor (BRL), 5 ug linearized plasmid, and 10 units of either T7 or SP6 RNA polymerase.

Viral RNA and the RNA transcripts generated in vitro were translated in rabbit reticulocyte lysates (Green Hectares, Madison, Wisconsin). Lysate was made exogenous RNA-dependent by micrococcal nuclease treatment (Pelham and Jackson, 1976; Dougherty and Hiebert, 1980). In vitro translation was carried out as described by Dougherty and Hiebert (1980). SCSV RNA (6-8 ug) was incubated with 100 ul prepared lysate at 30°C for 1 hour. The reaction was terminated by addition of three volumes of Laemmli protein dissociation buffer (250 mM-Tris-Cl pH 6.8, 2% SDS (w/v), 4% 2-mercaptoethanol (v/v), and 1% glycerol (v/v)). The ³⁵S-labeled translation products were analyzed by discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). After electrophoresis, the gel was fixed for 30 minutes in 30% methanol (v/v), 10% trichloroacetic acid (w/v), and 10% acetic acid (v/v), then rinsed for 30 minutes in water, followed by impregnation with fluor (Fluoro-Hance, Research Products International Corp.). The gel was then dried and fluorographed at -70°C. Immunoprecipitation of in vitro translation products was carried out as described by Hiebert and Purcifull (1981) with Staphylococcus

aureus strain cowan 1 (10% solution) and polyclonal antiserum against SCSV capsid protein. Immunoprecipitated proteins were collected by repeated centrifugation washing. The immunoprecipitated proteins were analyzed by SDS-PAGE and fluorography.

RESULTS

Biological properties

SCSV was readily mechanically transmissible to Z. mays (N28Ht), C. quinoa, C. amaranticolor, and N. clelandii (Table 1). Z. mays was the only host plant inoculated which resulted in a systemic infection, even though inoculated leaves showed no symptoms. Systemic symptoms included elongated, chlorotic spots, yellowing, and a mild mosaic on noninoculated tissue. Spots sometimes contained green centers and resembled ringspots typical of infection by several dicotyledonous plant viruses. No necrosis was observed in infected tissues. Purification of SCSV from infected Z. mays resulted in 40-100 ug virus/gram tissue. Infected N. clelandii exhibited irregular necrotic lesions and a general yellowing on inoculated leaves. Purification of SCSV from infected N. clelandii resulted in 200-500 ug virus/gram tissue indicating that replication did take place in the inoculated tissue although movement out of that tissue was limited. Distinct chlorotic local lesions were produced on inoculated leaves of C. amaranticolor while inoculation of C. quinoa resulted in a series of concentric partial rings. Inoculations on S. bicolor, A. hypogaea,

and several monogenic WSBMV susceptible and resistant varieties of T. aestivum did not result in infection (Table 1).

Temperature appeared to be an important parameter in disease development and symptom expression. Infection in Z. mays was much higher when plants were maintained at a relatively constant temperature of 25°C. During the hot summer months when glasshouse temperatures frequently exceed 35°C, percent infection in N28Ht was considerably lower and often less than 10%. Infection in Chenopodium species and N. clelandii did not appear to be temperature dependent. Attempts to induce infection by planting the hosts either at the location from which SCSV was isolated or by planting into field collected soil were unsuccessful (data not shown).

Physicochemical properties

The molecular weight of the single capsid protein observed for SCSV was determined to be 20.5K by SDS-polyacrylamide gel electrophoresis (Fig. 1), similar in size to the 19.7K capsid protein of WSBMV (Fig. 1) (Shirako and Brakke, 1984). The capsid protein was associated with two rigid, rod-shaped components of discrete size. Both components measured 20nm in diameter and were either 260 or 140nm in length with hollow centers (Fig. 2). The larger virion was always present in higher concentrations than the smaller virion, as observed by electron microscopy of purified virus (Fig. 2).

Two discrete RNA species were observed after electrophoresis of phenol extracted SCSV virions (Fig. 3). The molecular weight of the

largest RNA species, RNA-1, was approximately 2.2×10^6 (6200 nucleotides) and the molecular weight of RNA-2 was approximately 1.2×10^6 (3500 nucleotides), similar to the reported 2.28×10^6 (6500 nucleotides) and 1.23×10^6 (3500 nucleotides) for RNA-1 and RNA-2, respectively, of WSEMV (Gumpf, 1971; Shirako and Brakke, 1984)). As illustrated in figure 3, the relative concentrations of RNA-1 and RNA-2 after extraction from virions was opposite that reported for WSEMV (Shirako and Brakke, 1984). The concentration of WSEMV RNA-2 is approximately 10 times greater than RNA-1 and, at times, RNA-1 is not detected. SCSV yielded a much greater amount of RNA-1 than RNA-2. In addition to RNA-1 and RNA-2, electrophoretic analysis of RNA liberated from SCSV virions consistently resulted in a diffuse band of low molecular weight nucleic acid (Fig. 3).

Unfractionated SCSV virion RNA did not bind to oligo(dT) cellulose (Fig. 4) indicating the lack of a significant polyadenylation sequence located either internally or terminally. Nonpolyadenylated WSEMV RNA did not bind to the column while polyadenylated CPMV RNA was bound (Fig. 4). As can be seen in the figure, some degradation of the RNAs occurred after passage through the column.

Serological relationships with other Furoviruses

Immunospecific electron microscopy was performed to ascertain the serological relatedness of SCSV with WSEMV. Leaf dip preparations of SCSV infected Z. mays were made in WSEMV, tobacco mosaic virus (TMV),

and barley yellow mosaic virus (BaYMV) capsid protein antisera. SCSV virions dissociated in the presence of WSBMV antiserum but not with TMV or BaYMV antisera. Several rod-shaped plant viruses are unstable in the presence of homologous and heterologous antisera (Langenberg, 1986). Although the instability to decoration can interfere with the detection of virus particles in leaf dip preparations, it can be used as a measure of serological relatedness, and therefore a serological relationship between SCSV and WSBMV was indicated. The serological relationship between SCSV and WSBMV was confirmed by colloidal gold staining of labeled goat anti- rabbit IgG (Fig. 2).

As with the immunoelectron microscopy, western blot analysis indicated a serological relatedness between the capsid proteins of WSBMV and SCSV (Fig. 1). WSBMV antiserum positively recognized SCSV capsid protein and SCSV antiserum recognized WSBMV capsid protein, however, the reactions between capsid protein and homologous antisera were much stronger than the interactions of the capsid proteins with the nonhomologous antisera, indicating that, although the capsid proteins are serologically related and undoubtedly share some common epitopes, the serological relatedness was not complete. A western dot blot assay performed with BNYVV and PMTV antisera indicated no serological relationship with SCSV (data not shown).

Hybridization studies

cDNA cloning of SCSV RNA resulted in several small (less than 500 bp) virus specific clones as well as a single 1800 bp clone, pSCS007.

The smaller clones were not characterized. However, we characterized pSCS007 as the clone potentially represented 50% of the RNA-2 genome or 25% of the RNA-1 genome.

pSCS007 was labeled with ^{32}P -dATP by nick translation and used to probe northern blots of unfractionated WSEMV and SCSV RNAs. pSCS007 hybridized exclusively to SCSV RNA-2 and not with SCSV RNA-1 or to WSEMV RNA-1, RNA-2, or the WSEMV RNA-2 deletion mutant (Fig. 5). In addition, pSCS007 did not hybridize to the diffuse band of nucleic acid in the SCSV RNA preparation (Fig. 5).

Randomly primed ^{32}P -dATP labeled cDNA probes were generated to unfractionated RNA of WSEMV and SCSV. The cDNA probes were used to determine the extent of homology at the nucleic acid level between the two viruses under high stringency conditions. Each probe was hybridized to the RNA from each virus electrophoresed through formaldehyde denaturing gels transferred to gene screen (Fig. 5). cDNA prepared from unfractionated SCSV RNA hybridized strongly with SCSV RNA-1 and RNA-2 and only slightly to WSEMV RNA-2. The SCSV cDNA probe also hybridized, again only very slightly, to the WSEMV deletion mutant of RNA-2. The SCSV cDNA probe hybridized to the diffuse band of nucleic acid found in all SCSV RNA preparations. cDNA prepared from unfractionated RNA of WSEMV hybridized strongly with WSEMV RNA-1 and RNA-2, as well as the RNA-2 deletion mutant. Interestingly, the WSEMV cDNA probe did not detect either SCSV RNA-1 or RNA-2 (Fig. 5).

Translation studies

Unfractionated SCSV RNA directed the synthesis of 150, 110, 50, 35, 25, and 20.5K polypeptides in a rabbit reticulocyte in vitro translation system (Fig. 6). Immunoprecipitation of SCSV RNA translation products with SCSV capsid protein antiserum precipitated three proteins, the 20.5K capsid protein and polypeptides of 25 and 110K. Similarly, immunoprecipitation of translation products from WSBMV with WSBMV antiserum precipitated three proteins, the 19.7K capsid protein and polypeptides of 25 and 90K (Fig. 6). Immunoprecipitations of SCSV translation products with WSBMV antiserum and immunoprecipitation of WSBMV translation products with SCSV antiserum resulted in the precipitation of extremely minor amounts of the three polypeptides immunoprecipitated by homologous antisera (Fig. 6).

The positive polarity RNA transcript generated from RNA-2 specific pSCS007 directed the synthesis of two major polypeptides of molecular weight 50 and 45K, as well as several minor polypeptides (Fig. 7). Translation products of the RNA transcript were not immunoprecipitated with capsid protein antisera (data not shown). A negative polarity RNA transcript did not yield significant or definite products in vitro (data not shown).

DISCUSSION

SCSV was originally isolated from an inbred sorghum line within a breeding plot in Kansas. Interestingly, subsequent attempts at mechanical transmission to commercial sorghum hybrids proved negative

(Table 1), thus either mechanically mediated infection is dependent on the correct sorghum genotype, or more likely, sorghum can only become infected by another inoculation method, possibly by a fungal vector. Langenberg (unpublished) reported that Polymyxa graminis, the vector for WSEMV, colonizes sorghum and corn roots. Initial attempts at soil transmission by seeding plants in soil from the location where the virus was first isolated have so far proved negative, however, work in this area is being pursued. The infection pattern of SCSV in the original field is consistent with a soil-borne vector. Given that, physicochemically, SCSV is a Furovirus, we predict that it can be transmitted in nature by a plasmodiophoraceous fungus.

SCSV is readily transmitted to several inbred corn varieties by mechanical inoculation, resulting in systemic symptoms similar to those on the naturally infected sorghum. Systemic infection and symptom expression is favored at 25°C. Infection decreases significantly above 35°C or below 20°C. This is in contrast to WSEMV which causes systemic infection between 15-17°C (Brakke and Rao, 1967). In addition to systemic infection on corn, SCSV infects N. cleavelandii and limited movement of the virus through inoculated leaves is observed. Mosaics, yellowing, and high virus titre result on inoculated leaves, but symptoms and virus are not detected on non-inoculated leaves, suggesting a limitation in long distance movement through the vascular tissue. SCSV is unusual in that it infects both monocot and dicot hosts.

SCSV is composed of two rod-shaped particles of approximately 260 nm and 140 nm in length and 20 nm in diameter. Each particle is

composed of a single capsid polypeptide of 20.5K separately encapsidating two nonhomologous RNA species. RNA-1 is approximately 6200 nucleotides and RNA-2 is approximately 3500 nucleotides. SCSV particle morphology and genome size is essentially identical to WSBMV, although the observed ratio of virion components and, consequently, RNA components is the inverse of that reported for WSBMV. Electron microscopy of purified SCSV virions (Fig. 2) reveals a greater number of 260 nm particles and this is reflected in the relative concentration of RNA-1 compared to RNA-2 (Fig. 3). We can speculate that the differences observed in the concentrations of RNA-1 for SCSV and WSBMV may be related to host specificity and mechanical transmissibility of the two viruses. We have initiated experiments with pseudorecombinants composed of SCSV RNA-1 and WSBMV RNA-2 to address this question.

The nature of the diffuse band of nucleic acid has not been determined, although several possibilities exist to explain its occurrence in RNA extractions of SCSV virions. The band may mask a distinct subgenomic, satellite, or a third RNA species although the most likely explanation is degradation of RNA. As the RNA-2 specific clone, pSCS007, does not hybridize the band (Fig. 3), it would appear that the diffuse nucleic acid is entirely associated with RNA-1, being either a subgenomic of RNA-1 or the result of degradation of RNA-1.

WSBMV RNA-2 encodes capsid and inclusion body proteins and RNA-1 regulates virus concentration and infectivity (Tsuchizaki et al., 1975). A proposed map of the WSBMV genome organization (Hsu and

Brakke, 1985) locates the capsid protein gene on the 5' end of RNA-2 with the 28 and 90K polypeptides resulting from readthrough of amber stop codons located 3' to the capsid protein gene. In vitro translation (Ziegler et al., 1985) and sequence analysis (Bouzoubaa et al., 1986) of BYNVV RNA-2 identifies the 5' terminal open reading frame as the 21K capsid protein gene. The 85K polypeptide is produced by readthrough of an amber stop codon located at the 3' end of the capsid protein gene. In vitro translation of IPCV RNA-2 demonstrates that the capsid protein is coded for on RNA-2 but the location of the gene, specifically a 5' open reading frame, has not been deduced. The placement of the coat protein cistron at the 5' end of RNA-2 may be a feature common to members of the Furovirus group.

The characterization of WSEMV and BNYVV allows for speculation on the genomic organization of SCSV and particularly the organization of RNA-2. If we make the assumption that the genomic organization of SCSV is similar to that of WSEMV, BNYVV, and IPCV, specifically, that the capsid protein gene is located on RNA-2, then we can explain the relatively low amounts of capsid protein and the presumed readthrough products of 28 and 110K. RNA-2 is present in much lower concentrations compared to RNA-1 and we would, therefore, expect this to be reflected in the amount of the in vitro translation products synthesized from RNA-2. We can then speculate that the capsid protein gene is located on the 5' end of RNA-2 as the in vitro translation products synthesized from the in vitro transcript of the RNA-2 clone, a clone representative of the 3' end of RNA-2, are not immunoprecipitated by antisera to the capsid protein. Synthesis of

the RNA-2 specific clone evidently terminated 3' of the capsid protein gene and contains no homologous sequences.

Results from the northern blot hybridization experiments support the conclusion that the capsid protein cistron resides on RNA-2 and is probably located on the 5' end of RNA-2. The fact that SCSV and WSBMV capsid proteins are serologically related would suggest that sequence homology could exist within the capsid protein genes and, therefore, we might expect cross-hybridization to occur. This is observed in the hybridization of SCSV cDNA to WSBMV RNA-2 and the RNA-2 deletion mutant (Fig. 5). It is disturbing that that opposite cross-hybridization, that is WSBMV cDNA with SCSV RNA-2, is not observed, but the lack of detection can be easily explained. There is much less SCSV RNA-2 than WSBMV RNA-2 for the nonhomologous probes to hybridize, and considering the slight hybridization that did occur between SCSV cDNA and WSBMV RNA-2, it is not surprising that, under identical hybridization conditions, we did not detect hybridization of WSBMV cDNA with SCSV RNA-2. The lack of hybridization of pSCS007 to WSBMV RNA, specifically RNA-2, further suggests that the SCSV capsid protein gene is located on the 5' end of RNA-2 as the 3' end clone would cross-hybridize with WSBMV RNA-2 if the clone contained capsid protein sequences.

Results from the characterization indicate that SCSV should be classified as a Furovirus. Particle morphology, physicochemical properties, and genomic organization of SCSV are similar to members of the Furovirus group, and essentially identical to WSBMV. SCSV and WSBMV were both initially isolated from monocotyledonous hosts and are

morphologically and physicochemically indistinguishable. They are serologically related and the nucleic acids lack polyadenylation sequences. In vitro translation clearly demonstrates that the SCSV genome is organized much like the WSEMV genome. SCSV is obviously much more closely related to WSEMV than to the other Furoviruses, and is in fact quite dissimilar to BNYVV. We propose that SCSV represents an ideal system for the study of the Furoviruses in general, and WSEMV in particular. Specifically, we feel the further characterization of SCSV and future studies involving SCSV and WSEMV will further define those areas of the WSEMV genome responsible for host specificity and host resistance.

REFERENCES

- BOUZOUBAA, S., GUILLEY, H., JONARD, G., RICHARDS, K. & PUTZ, C. (1985). Nucleotide sequence analysis of RNA-3 and RNA-4 of beet necrotic yellow vein virus, isolates F2 and G1. Journal of General Virology 66, 1553-1564.
- BOUZOUBAA, S., ZIEGLER, V., BECK, D., GUILLEY, H., RICHARDS, K. & JONARD, G. (1986). Nucleotide sequence of beet necrotic yellow vein virus RNA-2. Journal of General Virology 67, 1689-1700.
- BOUZOUBAA, S., QUILLET, L., GUILLEY, H., JONARD, G. & RICHARDS, K. (1987). Nucleotide sequence of beet necrotic yellow vein virus RNA-1. Journal of General Virology 68, 615-626.
- BRAKKE, M.K., ESTES, A.P. & SCHUSTER, M.L. (1965). Transmission of soil-borne wheat mosaic virus. Phytopathology 55, 79-86.
- BRAKKE, M.K. & RAO, S.A. (1967). Maintenance of soilborne wheat mosaic virus cultures by transfer through root washings. Plant Disease Reporter 51, 1005-1008.
- BRAKKE, M.K. (1977). Sedimentation coefficients of the virions of soil-borne wheat mosaic virus. Phytopathology 67, 1433-1438.
- BRUNT, A.A. & STACE-SMITH, R. (1978). Some hosts, properties and possible affinities of a labile virus from Hypochoeris radicata (Compositae). Annals of Applied Biology 90, 205-214.
- CARRINGTON, J.C. & MORRIS, T.J. (1984). Complementary DNA cloning and analysis of carnation mottle virus RNA. Virology 139, 22-31.
- DOUGHERTY, W.G. & HIEBERT, E. (1980). Translation of potyvirus RNA in a rabbit reticulocyte lysate: cell-free translation strategy and a genetic map of the potyviral genome. Virology 104, 183-194.
- EL MANNA, M.M. & BRUENING, G. (1973). Polyadenylate sequences in the ribonucleic acids of cowpea mosaic virus. Virology 56, 198-206.
- ESTES, A.P. & BRAKKE, M.K. (1966). Correlation of Polymyxa graminis with transmission of soil-borne wheat mosaic virus. Virology 28, 772-774.
- FUJISAWA, I. & SUGIMOTO, T. (1976). Transmission of beet necrotic yellow vein virus by Polymyxa betae. Annals of the Phytopathological Society of Japan 43, 583-586.
- GUBLER, U. & HOFFMAN, B.J. (1983). A simple and very efficient method for generating cDNA libraries. Gene 25, 263-269.
- GUMPF, D.J. (1971). Purification and properties of soil-borne wheat mosaic virus. Virology 43, 588-596.
- HARRISON, B.D. (1974). Potato mop-top virus. Commonwealth Mycological Institute/Association of Applied Biologists Descriptions of Plant Viruses, no. 138.
- HIEBERT, E. & PURCIFULL, D.E. (1981). Mapping of the two coat protein genes on the middle RNA component of squash mosaic virus (Comovirus group). Virology 113, 630-636.
- HSU, Y.H. & BRAKKE, M.K. (1985). Cell-free translation of soil-borne wheat mosaic virus RNAs. Virology 143, 272-279.
- LAEMMLI, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680-685.
- LANGENBERG, W.G. (1985). Immunoelectron microscopy of wheat spindle streak and soil-borne wheat mosaic doubly infected wheat. Journal of Ultrastructure Research 92, 72-79.

LANGENBERG, W.G. (1986). Deterioration of several rod-shaped wheat viruses following antibody decoration. Phytopathology 76, 339-341.

LEACH, J.E., RAMUNDO, B.A., PEARSON, D.L. & CLAFLIN, L.E. (1987). Dot-immunobinding assay for detecting Xanthomonas campestris pv. holcicola in sorghum. Plant Disease 71, 30-33.

LIN, N.-S. & LANGENBERG, W.G. (1983). Immunohistochemical localization of barley stripe mosaic virions in infected wheat cells. Journal of Ultrastructure Research 84, 16-23.

MANIATIS, T., FRITSCH, E.F. & SAMBROOK, J. (1982). Molecular Cloning: A Laboratory Manual. New York: Cold Spring Harbor Laboratory.

MAYO, M.A. & REDDY, D.V.R. (1985). Translation products of RNA from Indian peanut clump virus. Journal of General Virology 66, 1347-1351.

PUTZ, C. (1977). Composition and structure of beet necrotic yellow vein virus. Journal of General Virology 35, 397-401.

PUTZ, C., PINCK, L., PINCK, M. & FRITSCH, C. (1983). Identification of 3' and 5' ends of beet necrotic yellow vein virus RNAs. Presence of poly A sequences. FEBS Letters 156, 41-56.

PELHAM, H.R.B. & JACKSON, R.J. (1976). An efficient mRNA-dependent translation system from reticulocyte lysate. European Journal of Biochemistry 67, 247-256.

RANDLES, J.W., HARRISON, B.D. & ROBERTS, I.M. (1976). Nicotiana velutina mosaic virus: purification, properties and affinities with other rod-shaped viruses. Annals of Applied Biology 84, 193-204.

RAO, A.S. & BRAKKE, M.K. (1969). Relation of soil-borne wheat mosaic virus and its fungal vector, Polymyxa graminis. Phytopathology 59, 581-587.

REDDY, D.V.R., RAJESHWARI, R., IIZUKA, N., LESEMAN, D.E., NOLT, B.L. & GOTO, T. (1983). The occurrence of Indian peanut clump, a soil-borne virus disease of groundnuts (Arachis hypogaea) in India. Annals of Applied Biology 102, 305-310.

RICHARDS, K., JONARD, G., GUILLEY, H., ZIEGLER, V. & PUTZ, C. (1985). In vitro translation of beet necrotic yellow vein virus RNA and studies of sequence homology among the RNA species using cloned cDNA probes. Journal of General Virology 66, 345-350.

ROBERTS, I.M. & HARRISON, B.D. (1979). Detection of potato leafroll and potato mop-top viruses by immunosorbent electron microscopy. Annals of Applied Biology 93, 289-297.

SHIRAKO, Y. & BRAKKE, M.K. (1984a). Two purified RNAs of soil-borne wheat mosaic virus are needed for infection. Journal of General Virology 65, 119-127.

SHIRAKO, Y. & BRAKKE, M.K. (1984b). Spontaneous deletion mutation of soil-borne wheat mosaic virus RNA II. Journal of General Virology 65, 855-858.

SHIRAKO, Y. & EHARA, Y. (1986). Comparison of the in vitro translation products of wild-type and a deletion mutant of soil-borne wheat mosaic virus. Journal of General Virology 67, 1237-1245.

TAMADA, T. (1975). Beet necrotic yellow vein virus. Commonwealth Mycological Institute/Association of Applied Biologists Descriptions of Plant Viruses, no. 144.

THOMAS, P.S. (1980). Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proceedings of the National Academy of Sciences, U.S.A. 77, 5201-5205.

- THOUVENEL, J.C., DOLLET, M. & FAUQUET, C. (1976). Some properties of peanut clump, a newly discovered virus. Annals of Applied Biology 84, 311-320.
- THOUVENEL, J.C. & FAUQUET, C. (1981). Further properties of peanut clump virus and studies on its natural transmission. Annals of Applied Biology 97, 99-107.
- TOWBIN, H., STAEBELIN, T. & GORDON, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proceedings of the National Academy of Sciences, U.S.A. 76, 4350-4354.
- TSUCHIZAKI, T., HIBINO, H. & SATO, Y. (1975). The biological functions of short and long particles of soil-borne wheat mosaic virus. Phytopathology 65, 523-532.
- ZIEGLER, V., RICHARDS, K., GUILLEY, H., JONARD, G. & PUTZ, C. (1985). Cell-free translation of beet necrotic yellow vein virus: readthrough of the coat protein cistron. Journal of General Virology 66, 2079-2087.

Fig. 1. SDS-polyacrylamide gel electrophoresis of SCSV and WSEMV capsid protein and western blot analysis. Lane 1, molecular weight standards; lane 2, SCSV capsid protein; lane 3, WSEMV capsid protein; lanes 4 and 5, SCSV and WSEMV capsid proteins, respectively, electroblotted to nitrocellulose and stained with SCSV capsid protein specific antiserum; lanes 6 and 7, SCSV and WSEMV capsid protein, respectively, electroblotted to nitrocellulose and stained with WSEMV capsid protein specific antiserum. The molecular weight standards used were myosin (H-chain) 200, phosphorylase B 97.4, bovine serum albumin 68, ovalbumin 43, alpha- chymotrypsinogen 25.7, beta-lactoglobulin 18.4, and lysozyme 14.3K.

Fig. 2. Electron micrographs of SCSV particles (a)purified using the sodium borate purification procedure published for WSEMV (Shirako and Brakke, 1984); (b)immunostained with WSEMV capsid protein antiserum; and (c)immunostained with TMV capsid protein antiserum. The antisera were coupled to immunogold labeled IgG to enhance visualization and stabilize the serological reaction.

Fig. 3. Agarose gel electrophoresis of unfractionated nondenatured RNA stained with ethidium bromide. Lane 1, SCSV RNA; lane 2, WSEMV RNA.

Fig. 4. Agarose gel electrophoresis of bound and unbound fractions of unfractionated viral RNA filtered through an oligo(dT) cellulose column. Lanes 1,3, and 5 contain polyadenylated RNAs which bound to

the column and were subsequently eluted. Lanes 2,4, and 6 contained the unbound nonpolyadenylated RNA fractions. CPMV RNA, lanes 1 and 2; WSEMV RNA, lanes 3 and 4; SCSV RNA, lanes 5 and 6.

Fig. 5. Northern blot hybridizations of unfractionated denatured RNA electrophoresed under denaturing conditions. Lanes 1, 3, and 5, SCSV RNA; lanes 2, 4, and 6, WSEMV RNA. Lanes 1 and 2 were hybridized to ^{32}P -labeled cDNA made to unfractionated SCSV RNA. Radioactive contamination from lane 1 is visible in the lower part of lane 2. Lanes 3 and 4 were hybridized to ^{32}P -labeled cDNA made to unfractionated WSEMV RNA. Lanes 5 and 6 were hybridized to nick-translated ^{32}P -labeled pSCS007.

Fig. 6. Fluorogram of [^{35}S]methionine-labeled translation products synthesized in vitro and directed by unfractionated viral RNAs. Lane 1, ^{14}C -labeled molecular weight standards; lane 2, translation products synthesized from SCSV RNA; lane 3, translation products synthesized from WSEMV RNA; lane 4, SCSV RNA translation products immunoprecipitated by SCSV capsid protein antiserum; lane 5, SCSV RNA translation products immunoprecipitated by WSEMV capsid protein antiserum; lane 6, WSEMV RNA translation products immunoprecipitated by SCSV capsid protein antiserum; lane 7, WSEMV RNA translation products immunoprecipitated by WSEMV capsid protein antiserum. Lanes 2 and 3 contain a contaminating 45K polypeptide associated with the translation system used.

Fig. 7. Fluorogram of [^{35}S]methionine-labeled translation products synthesized in vitro and directed by (lane 1), unfractionated WSBMV RNA; (lane 2), unfractionated SCSV RNA; and (lane 3), RNA transcript generated in vitro to the SCSV RNA-2 specific cDNA clone excised from pSCS007 and ligated into the transcription vector pGEM-blue. All lanes contain the 45K polypeptide observed as a contaminant in the translation system

Table 1

Host	Response ¹		Percent Infection ³
	Systemic ²	Inoculated	
<u>Chenopodiaceae</u>			
<u>Chenopodium amaranticolor</u>	-	L	100
<u>C. quinoa</u>	-	R	100
<u>Gramineae</u>			
<u>Sorghum bicolor</u>	-	-	-
<u>Triticum aestivum</u>	-	-	-
<u>Zea mays</u>	S,Y,M	-	15-75 ⁴
<u>Leguminosae</u>			
<u>Arachis hypogoea</u>	-	-	-
<u>Solanaceae</u>			
<u>Nicotiana clevelandii</u>	-	Y,N	100
<u>N. tabacum</u> cv. Turkish	-	-	-
<u>N. tabacum</u> cv. Turkish(NN)	-	-	-

1 Y, yellowing; N, irregular necrotic lesion; R, ringspot; L, chlorotic local lesion; S, elongated chlorotic spot; M, mild mosaic; -, no infection at 25°C

2 Systemic infection was assayed for by back inoculations of extracts from noninoculated leaves to C. amaranticolor

3 Percent infection is the percent of plants expressing either systemic or local infection after inoculation

4 Temperature dependent

Figure 1

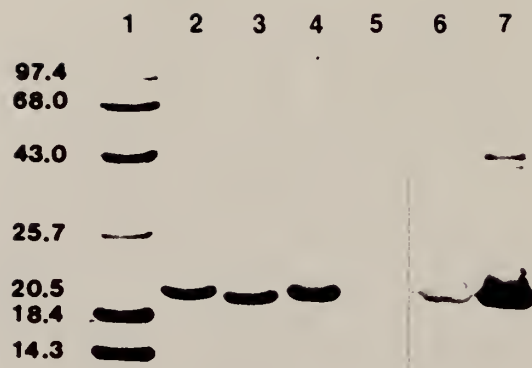


Figure 2

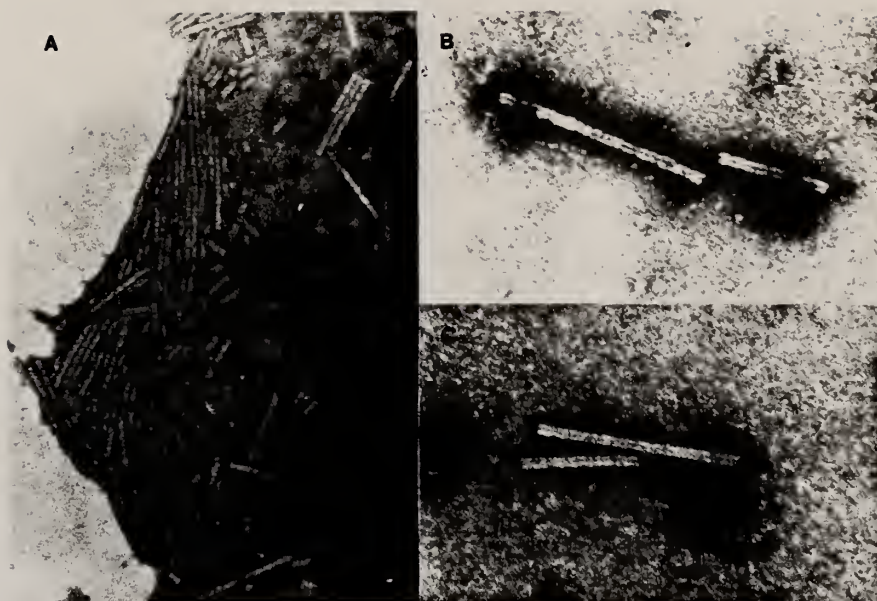


Figure 3



Figure 4

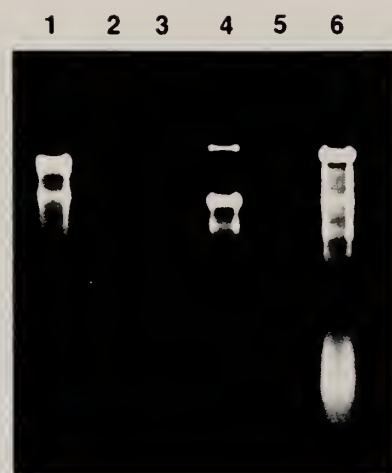


Figure 5



Figure 6

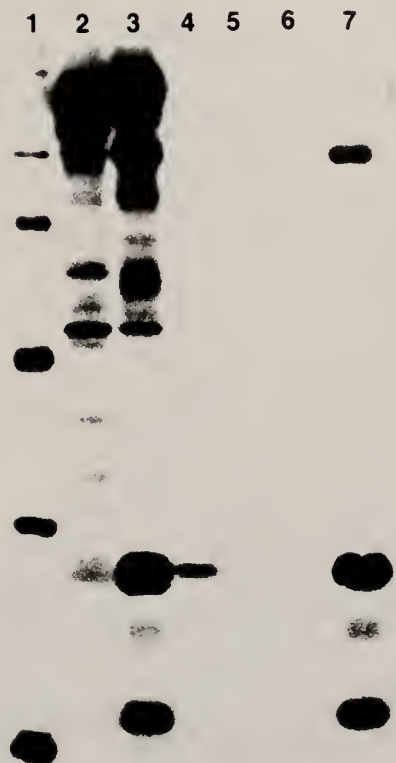
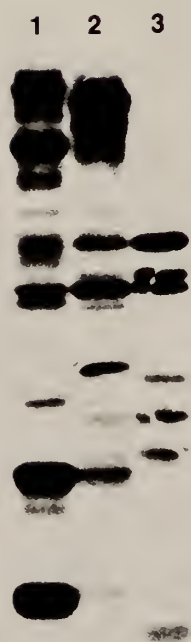


Figure 7



**ECOLOGY, EPIDEMIOLOGY, AND MOLECULAR BIOLOGY
OF FUNGUS VECTORED VIRUSES IN KANSAS**

by

TIMMY LEE KENDALL

B.S., Purdue University, 1978

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Plant Pathology

**KANSAS STATE UNIVERSITY
Manhattan, Kansas**

1988

ABSTRACT

Wheat soilborne mosaic virus (WSBMV) and wheat spindle streak mosaic virus (WSSMV) cause serious diseases of winter wheat (Triticum aestivum) in Kansas. WSBMV, the type member of the Furovirus group, is widespread and destructive. The extremely long virions of WSSMV are easily damaged during purification and, therefore, characterization has not progressed beyond elucidation of the capsid protein. WSBMV has been effectively controlled in the past through the use of a single, dominant resistance gene bred into several wheat cultivars. However, a synergistic interaction between WSBMV and WSSMV resulting in the loss of resistance to WSBMV in cultivars previously thought to be resistant has been identified. Severe mosaic, yellowing, bronzing, and stunting symptoms in patterns typical of the Polymyxa graminis vectored WSBMV were observed in many hard red winter wheat cultivars considered resistant to WSBMV. Electron microscopic examination revealed WSBMV particles as well as the long flexuous rods of WSSMV. Examination of thin sections revealed pinwheel and amorphous inclusion bodies. Western blot analysis confirmed plants were infected with both WSBMV and WSSMV. WSBMV and WSSMV particles were observed in all symptomatic plants in a ratio of 20:1, respectively, and the levels of WSBMV in resistant cultivars were equal to those observed in susceptible plants infected with WSBMV alone. The rapid spread of other soil-borne viruses and the danger

inherent in the use of single gene resistance to control WSBMV necessitates continued research on the etiology, epidemiology, vector relationships, and molecular biology of soil-borne viruses endemic in Kansas. A recently isolated Furovirus of Sorghum bicolor, sorghum chlorotic spot virus (SCSV), presents an ideal system to further define the genomic organization of the Furoviruses. SCSV is morphologically and physicochemically indistinct from WSBMV. SCSV exhibits elongate chlorotic ring spots as well as general yellowing on systemically infected leaves of sorghum and inbred Zea mays lines. In addition, SCSV is mechanically transmissible to and produces symptoms on the inoculated leaves of Nicotiana clelandii, Chenopodium amaranticolor and quinoa. Virions appear to be bipartite in nature with two rods, 20 nm in diameter and 260 and 140 nm in length, respectively. Virions are composed of a single 20.5K capsid protein species and two nonhomologous, nonpolyadenylated, genomic RNAs of 6.7kb (2×10^6 Da) for RNA-1 and 3.3kb (1.2×10^6 Da) for RNA-2. SCSV capsid protein is serologically related to WSBMV capsid protein as determined by western blot and immunogold cytochemical analysis. Northern blot hybridizations indicate that sequence homology exists between RNA-2 of SCSV and RNA-2 of WSBMV, presumably within the capsid protein cistrons. Unfractionated viral RNAs direct the synthesis of 150, 110, 50, 35, 25, and 20.5K polypeptides in vitro. The 110, 25, and 20.5K products are immunoprecipitated by SCSV capsid protein specific polyclonal antiserum. Comparative in vitro translation analysis with WSBMV suggest that the SCSV capsid protein cistron resides on the 5' terminus of RNA-2. A 1.8kb cDNA clone was synthesized to SCSV RNA-2. T7 transcripts from the clone directed the

synthesis of several polypeptides, none of which were immunoprecipitated by capsid protein antiserum. Information derived from studies with WSBMV, WSSMV, and SCSV can be used to develop future control strategies for these economically important viruses in Kansas.