Immunological signature analysis of strains of maize dwarf mosaic virus and sugarcane mosaic virus

by

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A Thesis Submitted to the
Graduate Faculty in Partial Fullfillment of the
Requirements for the Degree of
MASTER OF SCIENCE

Department: Microbiology, Immunology and Preventive Medicine
Major: Microbiology

Iowa State University
Ames, Iowa

1991

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LITERATURE REVIEW

Biology of Maize Dwarf Mosaic Virus and Sugarcane Mosaic Virus

In 1962, mosaic symptoms were observed on the leaves of corn plants (Zea mays) growing in southern Ohio. Approximately 50% of the corn plants in the 10,000 total acres, exhibited the mosaic symptoms (50). Within one year, corn grown in fourteen other counties exhibited similar symptoms (132). By 1964, Dale showed that the symptoms were caused by a virus (19) and designated the virus as MDMV-A. MDMV-A had similar host ranges and symptomolgies as SCMV. However, those viruses considered to be classic SCMV viruses did not have the ability to replicate in Johnsongrass (Sorghum halpense) and MDMV-A did. Mackenzie et. al. (74) isolated a new strain of MDMV that did not infect Johnsongrass and identified it as MDMV-B. The MDMV strains included a non-johnsongrass infecting strain, MDMV-B (74); several corn and sorghum infecting strains, MDMV-C,D,E,F (73); and an oat infecting strain, MDMV-O (76). Since the advent of this project, strains of this virus have been reclassified (114). The six strains of MDMV acquired for this project, are now classified as three distinct members of the potyvirus group of plant viruses. MDMV-O is now classified as johnsongrass mosaic virus (JGMV); MDMV-A, along with strains D,E, and F, are still classified as MDMV,

and MDMV-B is now classified as SCMV (108,114,115,117).

SCMV, JGMV, and MDMV are members of the potyvirus group of plant viruses. Potyviruses are flexuous, rod-shaped viruses that are 680-900 nm long, 11 nm wide, and contain single-stranded, positive-sense RNA encapsidated by repeating subunits of a single protein (48). Potyviruses have a capsid protein that varies between 30 and 37 K (48, 114). Most potyviruses are non-persistently transmissible by aphid vectors (75), although mites and fungi have also been identified as vectors (114). Pinwheel or cylindrical shaped cytoplasmic inclusion bodies are another characteristic of Potyviruses (48).

The various strains of SCMV, JGMV, and MDMV are pathogens of corn (Zea mays L.), sorghum (Sorghum bicolar L.), sugarcane (Saccharum officinarium L.), Johnsongrass (Sorghum halepense L. Pers.) and Sudangrass (Sorghum sudanense (Piper) Stapf) (100,134). Other host species, such as certain grasses endemic to Mississippi, are also susceptible to MDMV (98). MDMV-A and MDMV-B are the more economically significant MDMV strains, and may reduce yields by as much as 45 percent (96). Johnsongrass is the overwintering host for MDMV-A; but no overwintering host is known for MDMV-B (62).

The symptomology of SCMV, JGMV, and MDMV varies according to strain and/or host, but includes mosaic

patterns on leaves, as well as systemic necrosis, dwarfing, and necrosis of whole or large portions of leaves (referred to as the red leaf reaction) (86,88,117). Symptomology is affected by many environmental factors including light quality and quantity (105), temperature (38,54,105), and stage of plant development at time of infection (51).

Cellular functions of the host plant are altered by a infection with MDMV, SCMV, or JGMV. Virus infection blocks the translocation of host plant photosynthetic assimilates (13). The blockage results in decreases in total chlorophyll content (70) and ATP concentration. The reduced ATP concentration results in an impaired ability of the host to pump potassium into guard cells (70). The rate of photosynthesis and transpiration in infected johnsongrass and maize is reduced, while the rate of respiration increases due to an increase in the demand of metabolic materials from cellular pools (70).

MDMV, SCMV, and JGMV reduce the yield of the host plant by significantly lowering both the numbers of marketable ears and the fresh weight of the grain (38,59,61,79). Sweet corn cultivars infected with MDMV require a significantly longer time to reach the midsilk stage. Kernel fill, ear weight and diameter (13,38,79), stalk strength and diameter (61), pollen vigor (61,80), and plant height were also reduced in a variety of infected sweetcorn cultivars

(61,79). By extrapolating from yield data collected from infected Zea mays, Kingsland in 1980 (61) proposed that each 1% increase in disease incidence decreases grain yield by an average of 63 Kg/ha.

Over 23 species of aphids are reported to non-persistently transmit the various strains of SCMV, JGMV, and MDMV. The three major subfamilies include Aphidinae, Lachmninae, and Brepanosiphinae (62,122). Daniels et. al. (21) reported that the greenbug Schizaphis graminum could transmit MDMV. There is some debate as to whether SCMV and MDMV are soil transmissible (10). Evidence does exist to support seed transmission of MDMV-B (46,49).

Virus titer is affected by many types of biological conditions. As the plant grows, a maximum virus titer appears at the fourth or fifth leaf (54,118). It is interesting, however, that the presence of symptoms in the plant is a poor indicator of the virus titer in the plants. The correlation between virus concentration and visual assessment was low (r^2=0.399) (34).

Controlling MDMV, SCMV, and JGMV

The most significant measure developed to control the spread of MDMV, SCMV, and JGMV has been MDMV resistant and/or tolerant corn hybrids (104). The inbred MDMV-A resistant lines Pa405 (72), B68 (72), and Oh78 (72) have

been used to identify genes which encode for MDMV-A resistance. Genetic mapping showed that these plants carried a gene in a region near the centrosome of chromosome 6, identified as locus Rmd1, which induced resistance to MDMV-A (92,93). Pa405, when backcrossed with yM14 and K55, appears to induce resistance to MDMV-A, and the gene was identified and designated Mdm1 (77). Even with the identification of the resistance genes (97), effective control of MDMV has not occurred. Evidence exists that tolerant corn hybrids have been ineffective due to the inability to prevent MDMV, JGMV, and SCMV from replicating within cells (69). Thus, it is possible that these plants may act as a reservoir for infectious virus (75). Certain corn inbred lines that demonstrate resistance to MDMV-B are highly susceptible to MDMV-A, and corn inbred lines resistant to MDMV-A have been shown to be susceptible to MDMV-B (29,88). This suggests that the resistance response is, at least to some degree, strain specific. Additionally, the expression of certain genes that are thought to elicit the resistance response are modified by different environmental stimuli (48).

Eradicating or controlling the aphid vector populations by the use of insecticides has been examined. Control of a vector can only be achieved after considering the virus/vector relationship. The mechanism by which the

vector feeds, the persistence in transmittance, and the action of the insecticide, must all be considered for effective elimination of the vector. MDMV, SCMV, and JGMV are non-persistently transmitted. Virus adsorbs to the aphid stylet for a limited period of time. However, a single leaf probe by an aphid can transmit the virus (75). The use of a systemic insecticide would only be effective if it killed the aphid immediately, before the aphid transferred virus from an infected plant to a non-infected plant. Systemic insecticides may not be effective against other known or suspected vectors of MDMV, JGMV, and SCMV. Therefore, the use of a systemic insecticide has very limited application. Leaf oils can be applied to plants to inhibit probing by the aphids, but the use of leaf oils is an expensive method of protection for low value crops because the oil must be applied frequently (122).

Eradication of alternative hosts to control the spread of MDMV, SCMV, and JGMV is not feasible because the number of virus hosts are too numerous (99,100). Moreover, not all alternate hosts for each virus have been identified. This would further limit virus control by alternate host strategies.

Differentiation of Strains of MDMV, SCMV, and JGMV

Because identification and characterization of

pathogens that cause disease are a prerequisite to disease control, differentiation of viruses and strains is important. MDMV, SCMV, and JGMV are indistinguishable from each other when infected host plant tissue is examined under the electron or light microscope. Particle length, morphology, stability in sap, and longevity in detached leaves do not differentiate these viruses. Classically, plant virologists have attempted to clarify virus family relatedness based on biological and physical factors such as longevity in vitro, thermal inactivation point, dilution end point, sedimentation coefficients, and buoyant densities. MDMV-A and MDMV-B, which are the most economically significant strains, have the same longevity in vitro, thermal inactivation point, and dilution end point (124). MDMV-A and MDMV-B also have the same sedimentation coefficient of 170 ± 5 S (124). Buoyant densities of the two are also similar (e.g., 1.3432 and 1.3427 for MDMV-A and MDMV-B, respectively)(124). Physical appearance of the bands in sucrose gradients does vary: MDMV-B appears as a narrow band, whereas the MDMV-A band appears to be much broader (124).

The use of differential hosts to distinguish viral strains has been explored (33). Although significant differences in symptoms are seldom observed (117), strains can be occasionally differentiated provided that the host is

healthy and environmental conditions are uniform (48). Rosenkranz (99) demonstrated that grasses native to Mississippi were unable to be used as differential hosts for MDMV-A and MDMV-B because many of the grasses were susceptible to both isolates (87). With the exception of MDMV-O infecting oats, the various MDMV viruses have hosts similar to those of MDMV-A (123). MDMV-B can be distinguished from other MDMV strains by using differential hosts (52,106). Shukla et. al. (117) also classified MDMV-B as a strain of SCMV. These studies have been useful in dividing the MDMV strains into johnsongrass-infecting (such as MDMV-A) or the johnsongrass non-infecting (such as MDMV-B) groups (117).

Cross-protection has been used to define related strains of viruses (75). Cross protection is the protection of a plant from infection by a severe virus strain by prior infection of the plant with a mild strain of the same virus. In general, related strains of a virus cross-protect; unrelated virus strains do not. MDMV-A, however, did not prevent MDMV-B from multiplying when both were inoculated onto sorghum (106). This supports the placement of these two viruses in different groups upon reclassification. The inherent problem of cross-protection is that the viruses must first be classified as to their relatedness. Additionally, MDMV-A and MDMV-B both cause mosaic symptoms

and dwarfing, so it is difficult to differentiate between each virus in a host. Cytoplasmic inclusion bodies have been used to differentiate MDMV viruses (53). Although Mernaugh et. al. (78) suggested that the formation of inclusion bodies was due to a host plant response to viral infection, more recent data show that the proteinaceous inclusion body has homology to both a putative RNA-dependent RNA-polymerase or a viral encoded protease (14). In addition, Hammond et. al. (41) reported that the inclusion protein was not related serologically to the coat protein. Jensen et. al. (55) isolated a 65.8-kilodalton inclusion protein from MDMV- infected sorghum. The inclusion protein isolated from SCMV, was used as an immunogen which was administered to rabbits, and the resulting antisera did not react with MDMV-A inclusion proteins. Recent findings by Shukla et. al. (111,113,116) suggest that cross-reactivity, as defined by serological tests, may be due to mischaracterization of the antibody rather than serologic relatedness.

Recently, there have been a number of attempts to distinguish strains of MDMV based on the amino acid composition and peptide sequence of their capsid protein (108). In 1970, Gillaspie et. al. (32) purified four strains of SCMV and showed that it was possible to differentiate strains based upon their amino acid

composition. Several strains of the same potyvirus elicited similar peptide profiles when separated by using high performance liquid chromatography (110). When tryptic digests of the coat protein of four strains of SCMV (SC, BC, JG and Sabi) were analyzed, the related strains SC, BC, and Sabi had a high sequence homology (95-100%) whereas the JG strain of SCMV had a sequence homology (66%) similar to that of other potyviruses (51-62%) (110). When compared with the coat protein of members of the potyvirus group, the SCMV protein subunit was found to be comprised of approximately 289 amino acid residues for SCMV and 264 amino acid residues for MDMV (81).

Another way in which strains of MDMV have been defined is by using immunochemical analysis of peptide fragments. Shukla et. al. (114,115) analyzed overlapping peptide fragments of the coat protein and concluded that the core region of the coat protein is conserved among most potyviruses. The N-terminus region of the capsid protein contained the virus- or strain-specific sequences, and was also immunodomminant.

Nucleotide sequence analysis and hybridization of viral genomes (3) is another useful technique recently utilized in the taxonomy of potyviruses. Frenkel et. al. (30) attempted to differentiate watermelon mosaic virus-2 from soybean mosaic virus-N based on nucleotide sequences of the 3'

untranslated regions of the viral genome. They concluded that the nucleotide sequences were closely related, which suggested that each was a strain of the same virus. It was previously noted by Yu et. al. (133) that the differences in amino acid sequence, based on peptide analysis (and therefore nucleotide base changes), occurred in the N-terminal residues, thus suggesting that the 3' end of a potyvirus genome could be analyzed to discern genetic relatedness. However, the nucleic acid sequence homology throughout the entire genome is not extensive. When a computer comparison of nucleotide sequence of the genome of tobacco etch virus and tobacco vein mottling virus was performed (3), matches of the sequences occurred in only seven of more than 15 nucleotide sequence sections that were analyzed.

The use of specific polyclonal (PcAb) and monoclonal (McAb) antibodies in various serological techniques has also been applied extensively to differentiate between virus strains. In general, an antibody specific for an antigen (e.g. a virus or virus protein) binds a particular site on the antigen (epitope) with a binding constant which ranges from $k= 10^{-4}$ to 10^{-10} M (94). Thus, antibody-antigen reactions are very specific and have the potential to be useful diagnostic tools. When the antibody binds to the antigen in a solution or other medium, a precipitate may be

formed. This precipitation reaction is the basis of the microprecipitin test (128) and the Ouchterlony gel diffusion test (5,85). These methods have limited usefulness because concentrations of both the antibody and antigen must be optimal (precipitation may not occur if the antibody or antigen is in excess or is limited), or the optimal antibody-antigen concentration (the zone of equivalence) requires a large quantity of immunoreagent, which in some instances, is not practical. In addition, detection of the precipitate is subject to visual assessment and therefore variation and lack of sensitivity. False positives can occur when cellular components cause non-specific aggregation (75). Lastly, in Ouchterlony tests, rod-shaped particles may not diffuse as freely in agar as the icosahedral viruses, without the use of some modifying agent such as SDS, pyridine, pyrollidone, etc. Therefore, the use of either of these tests may not clarify strain relatedness. To circumvent these problems, researchers attempted to attach either the virus or the antibody, non-specifically to a solid phase support. These techniques include immunoblotting (both Western blotting (125) and dot immunoblot assay (24,42,66,67)), enzyme-linked immunosorbent assay (ELISA) (15,18,44,57,58,60,63,95,129), serologically specific microscopy (1,2,4,22,23,41,84,102), and radioimmunoassays (RIA) (31,43).

In the immunoblotting techniques, nitrocellulose or nylon membranes are the most common solid phases. The membranes are used to non-covalently bind the protein to which a specific antibody conjugated to an enzyme then combines. The combination produces an insoluble product, which becomes visible after the addition of substrate. A disadvantage to this technique is that there may be an inadequate transfer of the protein from the gel to nitrocellulose (125). Another problem with this technique is that non-specific binding can occur between antibody and basic viral coat protein domains which bind to RNA during encapsidation (24). Hibi and Saito (42) attempted to demonstrate that dot immunoblotting of TMV was more rapid and convenient than ELISA, but instead, found that the dot immunoblot assay was 5-10 times less sensitive than when photometric measurements were used to assay the results. In contrast, Lanham et. al. (67) performed dot immunoblot assays on various MDMV strains and reported that dot immunoblot assays were three-fold more sensitive than ELISA. Using dot immunoblot, MDMV-A, MDMV-B, MDMV-D, MDMV-E, MDMV-F, and MDMV-O were compared and the results were analyzed by regression analysis (66). MDMV-A, MDMV-B, MDMV-E, and MDMV-O were serologically distinct (66). MDMV-D and MDMV-F could not be distinguished from MDMV-A, whereas MDMV-O showed distinct differences from all the other

strains (66). Again, these data support the reclassification of MDMV viruses.

Serologically specific microscopy has been used extensively for identifying where a virus is replicating or located within the host (22) or identification of the group of viruses infecting a plant (4,23); however, it has seen limited use in strain differentiation (1,2). This technique is useful only if the antibody or antigen can be differentially labelled, as all members of a virus group are morphologically similar when observed under the electron microscope.

The ELISA technique uses either beads or various types of plastic microtitration plates for a solid phase. In 1977, Clark and Adams (18) first utilized this technique for the detection of plant viruses and reported a sensitivity to 1 ng/ml of virus. This technique can be accomplished in two ways; the direct binding of the viral particle to the plate (24), or capture of the viral antigen by antibody immobilized on the plate (e.g. dsELISA (double-sandwich enzyme-linked immunosorbent assay (18,58)). After proper incubation and blocking of the solid phase, an antibody specific for viral antigen is added (primary antibody). The primary antibody may be conjugated to an enzyme directly; or, a secondary antibody conjugated to an enzyme, but specific for the primary antibody, can be added (indirect

ELISA). Advantages of the ELISA include: i.) the test is rapid, ii.) high sensitivity, iii.) easy virus identification (18), iv.) either purified or crude extracts of virus can be used, v.) high degree of reproducibility (63,71), and vi.) this test is quantitative. Disadvantages of the ELISA can include: i.) reduction of the binding affinity of directly labelled antibody used in an ELISA (63), ii.) potential non-specific reactions in the outer wells of a microtitration plate (71), iii.) interference of endogenous host plant enzymes (71), and iv.) polystyrene plates from different sources vary in reproducibility.

Both the direct and indirect ELISA have been evaluated for the detection of plant viruses. For assaying purified virus, Lommel (71) recommended the use of the indirect ELISA, and suggested using the double sandwich (ds) ELISA for detection of virus in crude extracts. In 1978, Koenig (63) described differentiation of closely related viral antigens by ELISA. In 1988, Jones et. al. (58) developed a dsELISA that could differentiate many of the strains of MDMV. They concluded that there was a high degree of serological relatedness between MDMV-B and SCMV-A.

The radioimmunoassay (RIA) was first used for the quantitation of plant viruses by Ball in 1974 (5). The assay was performed by coating centrifuge tubes with specific antibody. Next, a specific amount of radiolabelled

antigen (virus), and an unknown amount of unlabelled antigen, were added to the coated tubes. As the unlabelled antigen competed with the labelled antigen, the amount of radioactivity bound to the tube was reduced. By using appropriate unlabelled standards, the quantity of virus in an unknown sample could be determined. Another variation utilized the basic principle of ELISA but substituted a radiolabelled primary or secondary antibody (31). Advantages to this technique include: i.) low background (Ghabrial et. al. [31]), showed a reduction of background values of 60-88% compared to gamma-globulins labelled with alkaline phosphatase), ii.) high sensitivity (31), and iii.) rapid detection. Disadvantages include limited shelf life of radiolabelled compounds, depending on the isotope used, and the necessity for expensive equipment to assess the reactions. Because the sensitivity of the RIA is even greater than the ELISA, this technique may also be efficient for analyses of strain relatedness. Hill et. al., in 1984 (43), used the RIA to effectively differentiate and determine relatedness of MDMV-B and lettuce mosaic virus (LMV), using this technique to differentiate group relatedness.

Signature Analysis

Plant viruses have been classically named and defined

by the host from which they were originally isolated and the resulting symptoms. With the advent of molecular biology techniques, plant viruses are continually being reclassified (e.g., 114). Many plant viruses cause severe economic loss, and clarifying their relatedness has become important. However, many data have been generated about plant viruses, but few attempts have been made to standardize the techniques by which these data are generated. To aid in data management and clarify viral relatedness, a method was developed for use with animal viruses, called signature analysis (83,126,130). This analysis system appears to be equally applicable to plant viruses.

Signature analysis is a technique that combines the use of a panel of monoclonal antibodies and an antigen/antibody assay (in the present research an RIA). The panel of antibodies is reacted with different viruses or virus strains and/or isolates. The binding affinity for each monoclonal antibody combination is obtained, and a computer program is used to align and statistically analyze the data.

Antibody-antigen reactions are specific. The combination of hydrogen bonds, Van der Waals, and hydrophobic interaction provide maximal binding energy (94). These attractive forces decrease in energy as the distance and variation of the ideal complementary binding area increases, resulting in an increase in the repulsive forces.

Therefore, affinity of an antibody molecule is directly affected by variations in composition and shape of an epitope (8). This principle is used in signature analysis.

If the binding of an antibody molecule to an epitope can be characterized, conserved or similar epitopes on related virus strains can be characterized by using the same antibody. The binding curve of the known antibody-antigen interaction can then be compared with that obtained using uncharacterized antigens. This is one of the basic principles involved in signature analysis. Characterized antibodies are reacted simultaneously with antigen to generate a binding curve that reflects both the frequency of the epitope and the affinity of the antibody to that epitope. This method discerns the antigenic relatedness of viruses (127).

Signature analysis provides discrimination superior to information obtained with the use of polyclonal antibody (PcAb) or monoclonal antibody (McAb) alone. Once McAbs have been prepared to a virus, it is a relatively straight-forward procedure to characterize the antibodies (using an ELISA), and select antibodies for use in signature analysis. Signature analysis is a relatively rapid assay which incorporates known techniques. Like any technique utilizing antibody, signature analysis has a great deal of specificity, because the antibody-antigen reaction can

detect differences in epitopes varying by only 2-3 amino acids (94). Signature analysis, like any RIA, can detect virus in very low concentrations. This permits the use of virus-infected host plant material which, for certain viruses, may contain low concentrations of virus. In addition, the use of sap from virus-infected tissue helps assure that the integrity of the virus, which may often be altered by purification procedures, is maintained. This assay is not limited by the shape of the virus particle, and the antibody-antigen reaction can be measured directly or can be detected by the reaction of iodinated avidin with an antibody labelled with biotin. The reagents are relatively inexpensive and many strains of a virus can be assayed at once. Additionally, iodinated avidin is available commercially, reducing the hazards that would be encounterd by having to directly label antibody with free ^{125}I. However, the use of iodinated-avidin can introduce greater error than that obtained by the use of directly labeled antiviral antibody. Although disadvantages of this technique exist, including the need for characterized McAbs, the use of expensive gamma counters, and the need for the computer statistical program, it is a very effective method of discerning strain and viral relatedness.

MATERIALS AND METHODS

Source and Maintenance of Virus Isolates

Virus isolates were obtained from Dr. Stanley Jensen, Department of Plant Pathology, University of Nebraska. The isolates, strains MDMV-A, MDMV-B (Ia188), KS-1 (Kansas State strain-1), OHIO-0, NeB2, and Minn-11, were chosen because they were not serologically cross-reactive, based on examination of cytoplasmic inclusion body proteins (55,56). Each virus isolate was propagated in Zea mays L. cv. Golden Bantam, Sorghum cv. Bugoff, and Sorghum cv. Dekalb E59+. The symptomologies of the viruses were observed on each host. Subsequently, each virus was propagated on the host which exhibited the most severe symptoms. MDMV-A and OHIO-O were propagated in Bugoff; MDMV-B was propagated in either Bugoff or Golden Bantam; and KS-1 and Minn-11 were propagated in Dekalb E59+. NeB2 was propagated in Golden Bantam.

Purification of MDMV-A and MDMV-B

Purification of either strain was performed according to Hill et al (45). Purified virus was resuspended in 0.1 M ammonium citrate (pH 6.0). Virus concentration was estimated using the extinction coefficient for tobacco etch virus, A(0.1%/280)=2.4 (89).

Polyclonal Antibody Preparation

Four New Zealand white rabbits were inoculated with increasing concentrations (from 0.5 mg to 2 mg) of either purified MDMV-A or MDMV-B emulsified at a 1:1 ratio in Freund's incomplete adjuvant, over a one to three month period (see Table 1). Antisera were tested periodically for immunoreactivity to the heterologous and homologous antigen by indirect ELISA (57). After three months, the antisera cross-reacted with virus isolates that were not homologous. The antisera were collected and stored at -10°C until use.

Monoclonal Antibody Preparation

Hybridomas used for this work were obtained from the collection of J.H. Hill and D.P. Durand, Department of Microbiology, Immunology and Preventive Medicine, Iowa State University. They were generated by F.E. Jones according to the procedure described by Jones (57), and had been stored at -100°C. The McAbs used in this project were as designated by Jones et al (58). Briefly, the first two letters of the antibody designation represent the virus used as an immunogen (e.g., MDMV-A (MA) or MDMV-B(MB)). The third letter designates the immunoglobulin class (IgM (M) or IgG (G)) and the numeral represents the clone number of the hybridoma. The McAb's used in these experiments include: MAG-1, MAG-2, MAG-3, MBG-1, MBG-2, MBG-3, MBG-4, MBG-5, and

Table 1. Immunization schedule for New Zealand white rabbits, immunized via intermuscular injection in a total volume of 2.0 mls, with antigen emulsified in Freund's incomplete adjuvant

Rabbit #	Date	Antigen(Ag)	Conc. of Ag
97 H, 98 H	1-14-88	Bled for assaying	none
	2-5-88	MDMV - A	0.5 mg
	2-12-88	"	1.0 mg
	2-19-88	"	1.0 mg
	2-25-88	Bled for assaying	
	3-2-88	MDMV - A	1.5 mg
	4-4-88	Bled for assaying	
	4-14-88	MDMV - A	2.0 mg
	4-21-88	Bled for assaying	
100 H, 101 H	1-14-88	Bled for assaying	none
	2-5-88	MDMV - B	0.5 mg
	2-12-88	"	1.0 mg
	2-19-88	"	1.0 mg
	2-25-88	Bled for assaying	
	4-4-88	"	
	4-21-88	"	

MBG-6. Dr. Stanley Jensen, Department of Plant Pathology, University of Nebraska, generously donated three McAbs in ascites fluid, designated as JEN-A, JEN-B, and JEN-O.

The frozen hybridomas were immediately placed into a 37°C incubator to thaw. The suspended cells were aseptically transferred to a sterile 15-ml centrifuge tube (Corning #25310) containing 49% Dulbecco's modified Eagle medium (DMEM) (57,58,127) (DMEM, Sigma #D-5648, St. Louis, MO), 40% conditioned media (DMEM that had supported the growth of the mouse myeloma cell line Sp_2O for 2-3 days, and had been collected and centrifuged at 1000 rpm), 10% horse serum [Hyclone #A-1115-L, Logan, Utah] and 1% 200 mM L-glutamine [Sigma #G-7513]), the resulting medium was called DMEM-R. The cells were sedimented by centrifugation at 225 x g in a 13-inch (33-cm) swinging bucket rotor at room temperature (22°C) for 10 min. The pellet was resuspended in approximately 10-ml of DMEM-R and cells were transferred to wells in an 8-well tissue culture plate (Corning #25810) that was incubated in an incubator under 5% CO_2 at 37°C. After several days, the medium turned orange to yellow and the cells were resuspended in DMEM-G (DMEM; Sigma D-5648) containing 10% horse serum (Gibco #230-6050 AJ, Grand Island, New York), 1% 200 mM L-glutamine (Sigma #G-7513) and 1% antibiotic (Pen-Strep [10,000 U/ml Penicillin G and 10,000 ug/ml Streptomycin]; Gibco #600-5140-PG). After

additional incubation, growth occurred throughout the entire well and the cells were resuspended in DMEM-G and transferred to 25 cm^2 T-flasks (Corning #25100).

The hybridomas were observed daily and, when the color of the medium turned orange to yellow (indicating pH change), the DMEM-G was replaced with fresh DMEM-G. For antibody production, the hybridomas were grown in DMEM-G without changing the medium for 10-12 days. The medium was then collected and the cells were removed by centrifugation as previously described, and frozen at 4°C. The cell culture supernatants of MBG-1, MAG-2, MAG-3, MBG-2, MBG-5, MAG-1, MBG-4, MBG-6, and MBG-3 (designated henceforth as flask medium) were assayed for McAb by indirect ELISA. After 4-5 weeks growth in culture, each hybridoma was cloned via the method described by Van Deusen and Whetstone (127).

Monoclonal antibodies were obtained from flask media approximately 10 days old or from ascites fluid. Ascites fluid was produced by an intraperitoneal injection of approximately 1.0 x 10^3 hybridoma cells, suspended in 0.1 M sodium phosphate buffered saline (PBS, pH 7.3) into a Balb/c mouse that had been injected interperitoneally with 0.1 ml of 2,6,10,14-tetramethylpentadecane (pristane) three to four weeks prior to the hybridoma injection. After 10-12 days, the swelling from the resulting tumor was pronounced. A sterile 18-gauge, 1 1\2-inch needle (B-D #5196, Rutherford,

New Jersey), was inserted into the peritoneal cavity to collect the ascitic fluid. The fluid was collected and centrifuged at 1000 x g for 10 min at 22°C to sediment the cells. The supernatant was decanted and assayed for specific McAb activity, then stored at -20°C.

Immunoglobulin class and subclass were determined by the protocol and reagents supplied by the MonoAb-ID EIA Kit (Zymed Laboratories Inc., #90-6550, San Francisco, CA).

Purification of Antibody

Immunoglobulin G was the only antibody class used. All McAb and PcAb tested were purified by a two-step procedure. First, 1 ml of the antibody solution was added to an Affi-gel blue CM column (Bio-Rad #153-7304 Richmond, CA). The Affi-gel blue column was prepared by homogeneously suspending the affi-gel blue in 0.1 M PBS (pH 7.3), and slowly dispensing approximately 3 ml into a plastic, disposable 5 ml syringe (B-D#5603) plugged with glass wool. The column was equilibrated with 100 ml of running solution (0.1 M KH_2PO_4 [Fisher #P382-500] and 0.02 % NaN_3 [Fisher #S227I-25]). The column bound the albumin and serum proteases, which allowed other ascitic proteins to elute in the void volume. The void volume was collected separately and the column restored by eluting the proteases and albumins with 10 ml of a higher concentration salt solution

(0.1 M KH_2PO_4, 0.15 M NaCl, and 0.02% NaN_3), pH 7.3. The column was then equilibrated with the running solution by passing approximately 25 ml of the solution through the column.

Purified antibodies were obtained by passing the affi-gel blue void volume through a Bio-Rad protein A column (#153-6153) connected to a fractionator and U.V. recorder. A protein-A column was prepared by homogeneously suspending the protein-A solution and pipeting 5 ml into a column. The column was equilibrated with 100 ml of binding solution (0.05 M Na_2HPO_4 [Fisher #S374-500], containing 0.02% NaN_3), pH 8.0, for McAbs and pH 7.3 for PcAbs. The ascitic or serum preparation was then diluted equally with binding solution and applied to the column in 3 ml amounts. The void volume was discarded, and the column was washed with 25 ml of binding solution until the resulting peak dissipated. The antibodies were then removed by adding enough elution solution (1.0 M acetic acid pH 2.0, Fisher #A490-212) to re-establish a baseline. The column was then regenerated by using 0.5 M phosphate buffer (pH 7.3). The pH of the resulting fraction was adjusted to 7.2 with 1 N NaOH, and was assayed for the presence of specific antibody by ELISA. The protein content was estimated using $E^{0.1\%} = 1.4$. Antibody was stored at -10°C until use.

Biotin-Labelling of Antibody

Biotin-labelling of antibody was performed essentially as described by Bayer et. al. (6) and described by Jones et. al. (57): Biotinyl-N-hydroxysuccinimide ester (Sigma #M-1759) was dissolved in dimethylformamide (Fisher #D119-500) at 1:100 v/v and 10:1 mole/mole biotinyl-N-hydroxysuccinimide ester to protein ratio.

Enzyme-Linked Immunosorbent Assay

ELISAs were performed essentially as outlined by Clark and Adams (18) and Jones et. al. (58). Three basic types of ELISAs were performed in these experiments. All ELISAs included a series of wash steps with wash buffer (0.1 M Tris HCl [Sigma #T3253], pH 7.5, 0.15 M NaCl [Fisher S271-500, Pittsburgh, PA] and 0.01% Tween-20 [Fisher T164-500]) after each reaction step. The wash procedure included first expelling the reaction fluids and then filling each well with wash buffer. The wash buffer was then removed by grasping the plate by its sides, and flicking the plate via a quick wrist movement. The procedure was repeated for all wells a total of three times. The final wash included filling the wells with wash buffer and allowing it to incubate at 22°C for three minutes. For each reaction step, plates were incubated for 1 h at room temperature (22°C) in a humidified chamber, with the exception of the substrate

step, where plates were incubated 45 minutes at 37°C. Unless otherwise stated, all reagents were added at 50 ul/well. Results of the different ELISAs were expressed as a P/N ratio. P/N ratios are defined as the average absorbance, at 410 nm, in the presence of antigen, divided by the average absorbance, at 410 nm, in the absence of antigen (126). The plot was then expressed as the P/N ratio vs. the antibody or antigen dilution.

Before each type of ELISA was used experimentally, the concentrations of immunoreagents used in the ELISA were optimized. Optimization involved examining the concentration of each reactant to generate a concave or sigmoidal curve when plotted as dilution vs. absorbance, at 410 nm. The concentration yielding the maximum P/N ratio was chosen for the assay.

The indirect ELISA was used to determine recognition, by McAb or PcAb, of virus antigen in the form of purified virus or in sap from virus-infected tissue. Antigen suspended in anti-protease buffer (APB, 0.05 M potassium phosphate buffer (pH 7.3) with 10 mM EDTA and 10 mM PMSF)(50 ul/well) was added to the wells of rows A-C and E-G of a Microtiter plate (Dynatech Immulon II, Dynatech # 011-010-3450) through column 11. The wells in column 12 received APB. Wells of rows D and H contained healthy host plant sap suspended in APB, equal w/v ratio, excluding the wells of

column 12. After incubation and washing, the wells were blocked with blocking buffer (wash buffer containing 3% gelatin [EIA purity grade reagent, Bio-Rad 170-6537, Richmond CA]) (200 ul/well). After incubation and washing, twofold dilutions, in blocking buffer, of either McAb or PcAb were performed in separate tubes, and 50 ul quantities were added to each well in rows A-H, excluding the wells of column 11. Blocking buffer was added to wells of column 11. After incubation and washing, anti-rabbit IgG (Sigma #R-2004) or goat anti-mouse IgG (Sigma #M-9902) conjugated with alkaline phosphatase were diluted 1:1000 in blocking buffer and added to each well. After incubation and washing, 50 ul of P-nitrophenol phosphate (Sigma # 104-105) at a concentration of 1.0 mg/ml diluted in substrate buffer (10% diethanolamine [Sigma D-8885], containing 0.01 % $MgCl_2 \cdot 6H_2O$ [Fisher M33-500]) pH 9.0, was added to each well and the plate was incubated at 37°C. After 45 minutes, the reaction was stopped with 3 N NaOH (Fisher #S318-500)(50 ul/well) stop solution. The reaction products were read at 410 nm (Dynatech plate reader #011-93-0-0500).

In the double-antibody sandwich (ds) ELISA, serial dilutions or a specific concentration of a capture antibody, diluted in coating buffer (0.05 M Na_2CO_3 [Fisher S263-500] pH 9.6), was added to the wells of rows A-H of a Microtiter plate, excluding wells in column 12, to which only coating

buffer was added. After incubation and washing, all wells were blocked as described previously. After incubation and washing, either purified virus or sap from virus-infected tissue (diluted in APB in equal w/v ratio, and strained through 2 layers of cheesecloth), was added to the wells of rows A-C and E-G, excluding the wells of column 11. Wells in rows D and H received healthy plant sap suspended in APB, equal w/v ratio. The wells of column 11 received APB without antigen. After incubation and washing, signal antibody, diluted in blocking buffer, was added to the wells of rows A-H, excluding the wells of column 10. Blocking buffer was added to the wells of column 10. Most often, the signal antibody was directly labelled with biotin. After incubation and washing, avidin-alkaline phosphatase (Sigma #A-7294) (0.25 ug/ml) was added to all wells. After incubation and washing, substrate, as described previously, was added to each well. The plates were incubated at 37°C for 45 min. The reaction was stopped by adding 3 N NaOH (50 ul/well), and the reaction products were determined spectrophotometrically at 410 nm.

A competition ELISA was also used. Purified MDMV-A, at a concentration of 5 ug/ml in APB was added to the wells of rows A-H through column 11. APB was added to the wells of column 12. The plates were incubated and washed. In the consecutive competition ELISA, serial dilutions of the

competing antibody (unlabelled) were prepared in blocking buffer in sterile plastic vials. After blocking and washing, 50-ul quantities of each dilution were added to wells of rows A-F, excluding the wells of column 11. Blocking buffer was added to the wells of rows G and H and column 11. After incubation at room temperature on a platform rocker and subsequent washing, the biotin-labelled antibody (diluted in blocking buffer) at an optimized concentration (which varied for each antibody, depending upon the efficiency of labelling or the affinity of the antibody), was added to the wells of rows A-H, excluding the wells of column 10. The wells of column 10 received blocking buffer. After incubation at room temperature for 1 h on a platform rocker and washing, avidin-alkaline phosphatase (Sigma #A-7294, 0.25 ug/ml) was added. After incubation and washing, the substrate was added as described previously. After 45 min incubation at 37°C, the reaction was stopped with 3 N NaOH (50 ul/well), and the reaction products were determined spectrophotometrically at 410 nm. In the simultaneous competition ELISA, antigen was bound to the wells of the plate as described for the consecutive ELISA. After incubation and washing, the plates were blocked with blocking buffer. Unlabelled antibody was diluted in blocking buffer in separate sterile tubes. Optimized labelled antibody was then added to each tube

containing the unlabelled antibody plus blocking buffer. This mixture of labelled and unlabelled antibody was then added to the wells of rows A-H, excluding wells in column 11. Blocking buffer was added to the wells of column 11. The plate was then incubated at room temperature on a platform rocker for 1 h. After washing, avidin-alkaline phosphatase (Sigma #A-7294, 0.25 ug/ml) was added to all wells. The plate was incubated, washed, and substrate was added as described previously. After 45 min incubation at 37°C, the reaction was stopped with 3 N NaOH (50 ul/well) and the reaction products were determined spectrophotometrically as described.

The data generated by both types of competition ELISAs were expressed as the percent maximum absorbance. The percent maximum absorbance is defined as the absorbance generated by the labelled McAb in the presence of competing antibody, divided by the absorbance of the labelled antibody with no competing antibody and multiplied by 100.

Determination of Antibody Concentration in Ascites Fluid

Ascites fluids were used as the antibody source for competition ELISAs. To determine the antibody protein concentration in ascites fluid, wells of rows A-H in two Microtiter plates were coated with 50 ul/well of mouse McAb IgG (2 ug/ml) purified from ascites fluid, except wells in

column 12 (which received 50 ul/well of 0.1 M PBS). The plates were incubated and washed. After blocking the wells, as described previously, the plates were incubated and washed. The first plate was used to generate the standard curve by performing serial dilutions of a purified, unlabelled mouse McAb (MBG-2, 2 ug/ml, 50 ul/well) in 1:1000 dilution in blocking buffer of goat anti-mouse IgG (Sigma #M-9902) conjugated with alkaline phosphatase. The anti-mouse IgG plus MBG-2 solution was added to the wells of rows A-H, excluding column 11. Wells of column 11 received blocking buffer containing a 1:1000 dilution of anti-mouse IgG conjugated with alkaline phosphatase. The plate was incubated at 22°C for 1 h and, after washing, substrate was added (as described previously) to all wells. The plates were incubated at 37°C for 45 min and the reaction products were analyzed spectrophotometrically on a Dynatech platereader (#011-93-0-0500) at 410 nm. Wells from column 11 demonstrated that the plate-adhered antibody would react with the labelled anti-mouse IgG if there were no competing antibody in solution. Wells from column 12 demonstrated that the anti-mouse antibody specifically bound to the mouse immunoglobulin that had adsorbed to the plate. Therefore, the anti-mouse IgG bound to the purified antibody in solution, before it bound to the plate-adsorbed antibody. The results were plotted as antibody concentration (mg/ml)

vs. absorbance.

The second plate, which had wells coated with mouse immunoglobulin as in the plate described above, was used to quantify the concentration of antibody in the ascites fluid. Each sample of ascites fluid was initially diluted 1:50, and then serially diluted in a 1:1000 dilution of rabbit anti-mouse IgG conjugated to alkaline phosphatase in blocking buffer, and 50 ul of each dilution was added to the wells of rows A-H, excluding wells in column 11. Wells in column 11 received 50 ul/ml of a 1:1000 dilution of the anti-mouse IgG conjugated to alkaline phosphatase in blocking buffer. Washing, substrate, and spectrophotometric analysis of the plate were performed as for the standard curve. Wells in column 11 demonstrated that the anti-mouse IgG must be present in order to generate a signal. Wells in column 12, because they did not receive competing antibody, demonstrated that the anti-mouse IgG specifically bound to the plate-adsorbed mouse antibody. The absorbances were compared to the standard curve, and the concentration of antibody in ascites fluid was interpolated from the curve.

Signature Analysis

After analyzing the results of the competition ELISAs, a panel of apparently non-competing McAb's was selected for use in the signature analysis. First, an RIA was performed

to optimize the concentrations of capture and signal antibodies. To optimize the concentration of capture antibody, 50-ul quantities of serial dilutions of the capture antibody (from ascites fluid) were adsorbed onto the wells of rows A-H of a remov-a-well Microtiter plate (Dynatech Immulon II #011-010-6302) excluding the wells of column 12. Wells of column 12 received 50 ul/well coating buffer. The plate was incubated for 1 h at 22°C in a humid chamber. After washing, the plate was blocked with 200 ul/well of modified blocking buffer (3% gelatin [Bio-Rad # 170-6537] dissolved in coating buffer). The plate was incubated for 1 h at 22°C and washed. Purified MDMV-A (5 ug/ml, 50 ul/well) in APB was added to the wells of rows A-G, excluding the wells in row H and column 11. The wells of row H contained healthy sorghum (c.v. Bugoff) plant sap in APB (50 ul/well). The plate was incubated for 1 h at 22°C. A 1:32 dilution of a biotin-labelled, combined PcAb from rabbits 97, 98, and 100 was added to the wells of rows A-H, excluding the wells in column 10. The wells of column 10 received blocking buffer. The plate was incubated for 1 h at 22°C. Avidin labelled with ^{125}I (Amersham, Arlington Heights, Il, code #IM.236, 100 uCi, 2.22 x 10^8 dpm) was then added to each well (20,000 cpm/well). After incubation on a platform rocker at 22°C for 1-2 h, the plates were washed and each well was transferred into a plastic vial. The vials

were placed into a Tracor model 1171 (Tracor analytic, Chicago, Il) gamma counter. The window setting suggested by the manufacturer for counting ^{125}I was used. The resulting cpm were calculated into a signal (sap from virus-infected tissue, wells of rows A-G) divided by noise (healthy plant sap, wells of row H) (P/N) response and were then plotted against McAb dilution. A 100-fold higher concentration than the maximum P/N ratio, was utilized for optimal capture McAb concentration.

Optimization of the signal McAb was performed as that used to optimize the capture antibody. A 1:200 dilution of MBG-1 was performed in blocking buffer, and added to wells of rows A-H, excluding wells of column 12. Wells of column 12 received coating buffer. After incubation and washing, the plate was blocked with 200 ul/well of modified blocking buffer. After incubation and washing, purified MDMV-A and sap from healthy plant tissue was added in the same manner as described above. Serial 1:2 dilutions in blocking buffer of biotin-labelled McAb were performed in separate tubes. After incubation and washing, 50 ul quantities were added to the wells of rows A-H, excluding the wells of column 10. Wells of column 10 received blocking buffer. The plate was then treated as per the protocol for capture antibody, described previously. P/N ratios of all antibody concentrations were examined, and the concentration of the

one antibody which yielded the lowest P/N ratio was selected as the standard concentration (0.3 mg/ml) for the signature assays.

To perform a signature analysis RIA, the capture antibody (MBG-1) was added to the wells (50 ul/well) of rows A-H of a Remove-a-well™ Dynatech II Immulon plate, at a 1:500 dilution in coating buffer through column 11. Coating buffer was added to the wells of column 12 (50 ul/well). After incubation and washing, the plates were blocked with modified blocking buffer (200 ul/well). The isolates MDMV-A, MDMV-B, NeB2, and Minn-11, were prepared from infected tissue by grinding the tissue in an equal w/v ratio of APB, and straining the mixture through 2 layers of cheesecloth. Non-infected sorghum and maize were prepared in the same manner. The resulting sap were collected in sterile tubes, and were serially diluted in APB buffer. After incubation and washing, 50-ul quantities of the Ag (infected tissue sap) were added to wells of rows A-C, and E-G, excluding wells in column 11. Wells in column 11 received 50 ul/well APB. Wells in rows D and H received 50 ul quantities of either the non-infected sorghum or maize. The plates were incubated overnight at 4°C with rocking. After washing, signal antibody diluted in blocking buffer (0.3 mg/ml) was added to wells of rows A-H, excluding column 10. Blocking buffer was added to the wells of column 10 (50

ul/well). The plates were incubated for 1 h at 22° C with rocking. After washing, iodinated avidin was added as described previously. Each tube containing 1 well was counted for 2 min or 10,000 cpm. To compare data from different infected plant sap preparations, the data were analyzed statistically by a signature analysis computer program, first described by Wang et. al. (134) in 1983 and modified by the ISU Statistics Department. The program generates binding profiles, represented as graphed curves (ln P/N ratio vs. dilution of antigen [log base 2]) for each antibody. These curves were collectively called signatures.

RESULTS

Growth Conditions of the Virus Isolates

Optimum growth of each virus isolate was dependent on greenhouse temperatures within the range of 70-80°F. There was some difficulty growing the virus-infected plants in the summer (Dr. Stan Jensen, personal communication). Greenhouse temperatures above 90°F appeared to cause rapid decreases in infectious virus titers.

Production of Cross-reactive PcAb

Table 2 shows the results of reactions between rabbit PcAb and purified or sap containing homologous and heterologous virus as measured by an indirect ELISA. Rabbits that were immunized with MDMV-A produced antibodies that were more cross-reactive than those immunized with MDMV-B. The last immunization, with 2.0 mg/ml MDMV-A, (dated 4-24-88, Table 2) did not increase either antibody titer or cross-reactivity. Because the amount of virus in infected sap cannot be quantified, Table 2 lists the immunoreactivity of PcAb as either recognizing the antigen (+) or not (-). Figure 1 shows the P/N ratio curves that were obtained when six different strains of MDMV were serially diluted. The PcAb generated P/N ratios greater than 20 when combined with MDMV-A, MDMV-B, NeB2, and Minn-

Table 2. Cross reactivity of PcAb against purified (P) MDMV-A and MDMV-B (5 ug/ml, 50 ul/well); and host plant sap containing virus (is) of MDMV-A, MDMV-B, OHIO-O, KS-1, Minn-11, and NeB2; at various times, as assayed by indirect ELISA

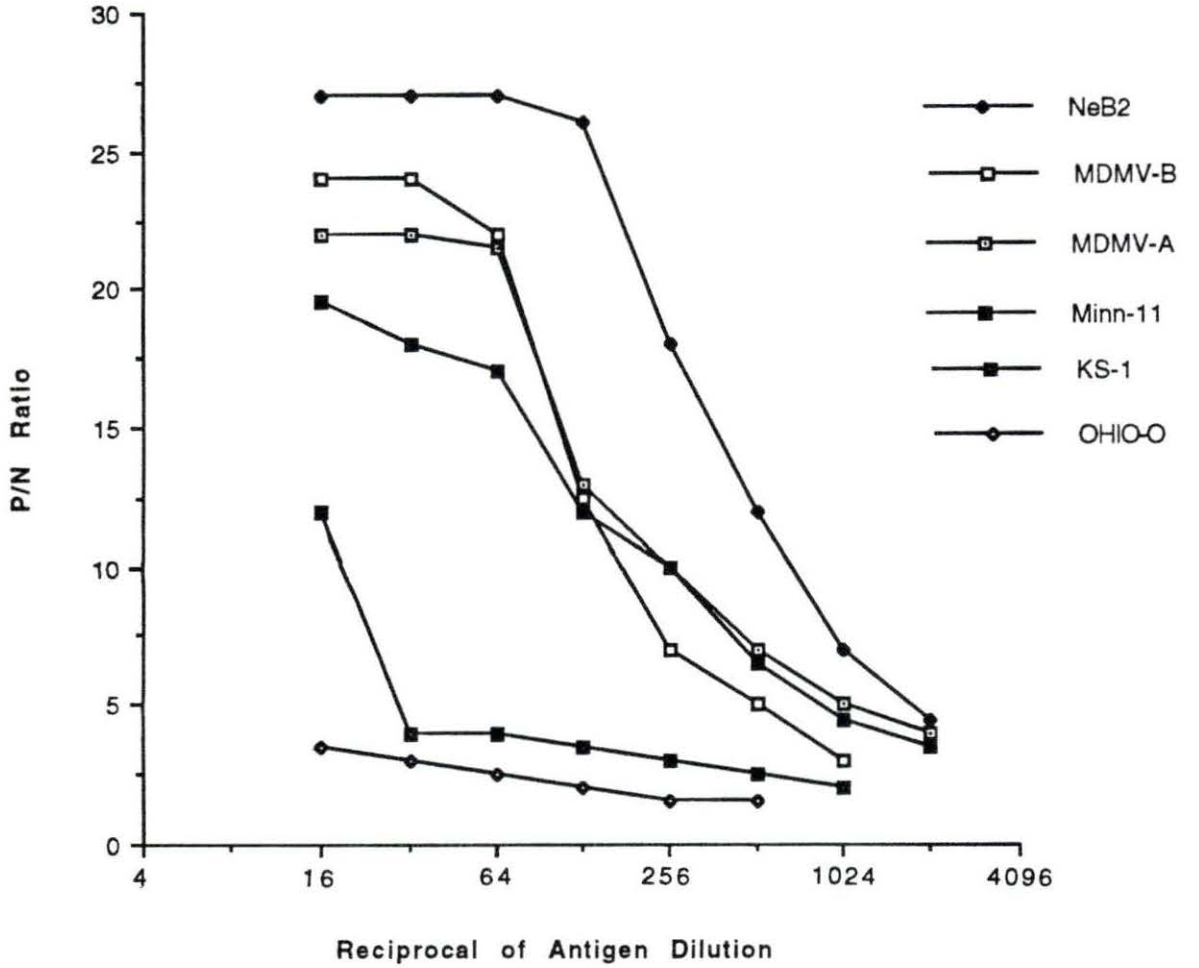
Virus (Ag)	Date	Rabbits[a] 97[b]	98	100	101
PMDMV-A	1-15-88	-	-	-	-
PMDMV-B		-	-	-	-
PMDMV-A	2-26-88	+++[c]	+++	++	-
PMDMV-B		++	++	++	+
isMDMV-A		+	+	-	-
isMDMV-B		+	+	+	+
isOHIO-O		+	+	-	-
isKS-1		+	+	+	-
isMINN-11		+	+	-	-
isNEB2		++	++	+	+
PMDMV-A	4-5-88	++++	++++	++	-
PMDMV-B		++++	+++	++	+
isMDMV-A		+	+	-	-
isMDMV-B		+	+	+	+
isOHIO-O		+	+	-	-
isKS-1		+	+	+	-
isMINN-11		+	+	-	-
isNEB2		++	+++	+	+
	4-24-88	same responses as 4-5-88			

[a]rabbits were designated according to the animal quarter protocol which assigns a consecutive number to each animal (Table 1).

[b]rabbits 97 and 98 were immunized with MDMV-A and rabbits 100 and 101 were immunized with MDMV-B.

[c]++++ Very high recognition ($O.D._{410}$ >0.75)
+++ high recognition ($O.D._{410}$ 0.50 - 0.74)
++ moderate recognition ($O.D._{410}$ 0.25 - 0.49)
+ recognition ($O.D._{410}$ 0.10 - 0.24)
- no discernable recognition ($O.D._{410}$ <0.10)

Figure 1. P/N ratios obtained from indirect ELISAs of PcAbs, reacted with serial dilutions of six MDMV isolates. The response is recorded as the P/N ratio vs. reciprocal of antigen dilution. Six virus preparations of sap from virus-infected host plant tissue (NeB2, MDMV-B, MDMV-A, Minn-11, KS-1 and OHIO-0) were serially diluted, added to the wells of an Immulon II Microtiter plate and incubated overnight on a platform rocker at 4°C. A 1:1000 dilution of PcAbs (combined from rabbits 97, 98 and 100; 50 ul/well) was added and the plates were incubated at room temperature (22°C) for 1 h. A 1:1000 dilution of anti-rabbit IgG alkaline phosphatase conjugate (50 ul/well) was added and plates were incubated at room temperature for 1 h. Substrate (1 mg/ml, 50 ul/well) was added and the plates were incubated at 37°C for 45 minutes. The reaction products were spectrophotometrically read at 410 nm.

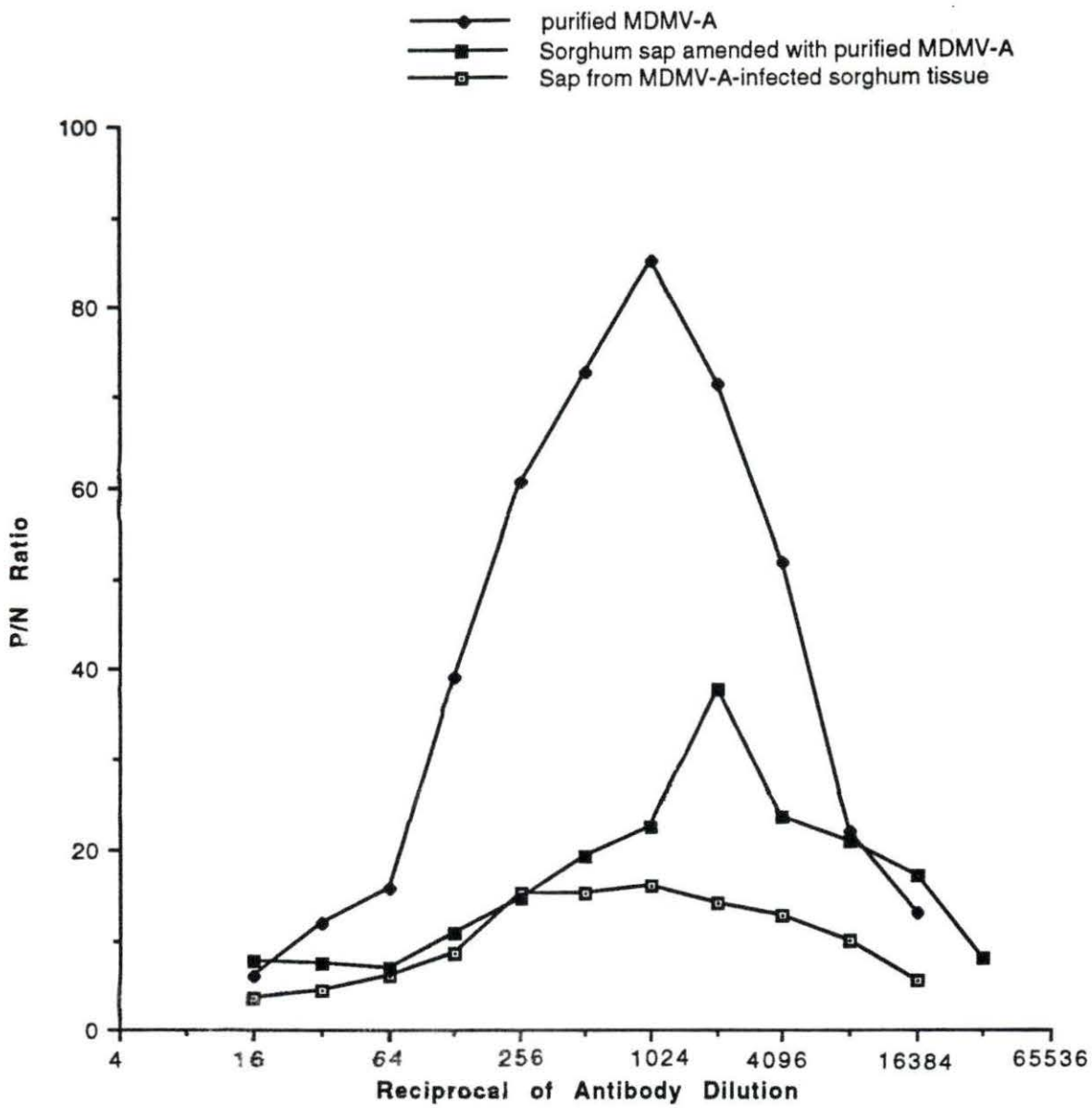

30
25
20
15
10
5
0
P/N Ratio
4
16
64
256
1024
4096
Reciprocal of Antigen Dilution
NeB2
MDMV-B
MDMV-A
Minn-11
KS-1
OHIO-O

11. The PcAb generated P/N ratios less than 12 when combined with strains KS-1 or OHIO-O. This could be due to greater recognition by the PcAb to MDMV-A, MDMV-B, NeB2 and Minn-11, or that these strains were in high enough concentration to be detected by the PcAb. KS-1 and OHIO-O were either not recognized well by the PcAb or the strains were low in concentration in the infected host tissue. Again, because the reaction is a concentration-dependent response, no attempt was made to define the PcAb's immunoreactivity for each strain of virus.

Optimization of Double Sandwich ELISA Signal and Capture PcAb

To use the PcAbs to screen McAbs for cross-reactivity, it was necessary to optimize the ELISA system. Antisera from rabbits 97, 98, and 100 were combined and purified. Figure 2 demonstrates the optimization of capture antibody using purified MDMV-A (5 ug/ml; 50 ul/well), healthy sorghum tissue amended with 5 ug/ml of purified MDMV-A (50 ul/well), and sap from MDMV-A-infected tissue. The greatest response was obtained with purified MDMV-A (P/N ratio maximum of 88). The response decreased (P/N ratio maximum of 38) when purified MDMV-A was added to healthy sorghum sap. Values obtained using sap from MDMV-A-infected sorghum tissue, were all <20, but some P/N ratios of >10 were obtained. The optimal concentration of capture antibody was either a

Figure 2. P/N ratios obtained from a dsELISA, using serial dilutions of PcAb capture antibody cocktail tested with MDMV-A. The response is recorded as the P/N ratio vs. reciprocal antibody dilution. A capture antibody comprised of PcAbs from rabbits 97, 98 and 100, were combined, serially diluted and added to the wells of a Immulon II Microtiter plate. 5 ug/ml of purified MDMV-A, healthy sorghum sap amended with 5 ug/ml of purified MDMV-A, and sap from MDMV-A-infected host tissue was added (50 ul/well). Biotin labelled PcAb pooled from the final bleedings of rabbits 97, 98, and 100 was added at a 1:2 dilution (50 ul/well). Avidin-alkaline phosphatase (5 ug/ml, 50 ul/well) was then added. After the addition of p-nitrophenyl phosphate and incubation at 37°C for 45 min, the reaction products were read spectrophotometrically at 410 nm.


purified MDMV-A
Sorghum sap amended with purified MDMV-A
Sap from MDMV-A-infected sorghum tissue
P/N Ratio
100
80
60
40
20
0
4
16
64
256
1024
4096
16384
65536
Reciprocal of Antibody Dilution

1:1024 or a 1:2048 dilution, depending upon the MDMV-A preparation used. Therefore, a 1:1000 dilution of the combined antisera was used in subsequent experiments.

To determine the optimal dilution of signal antibody to use, similar procedures were followed. Figure 3 shows the P/N ratios obtained when three MDMV-A preparations were assayed in the presence of different concentrations of signal antibody labelled with biotin. The greatest response was obtained with purified MDMV-A (P/N ratio maximum of 107). The response was decreased (P/N ratio maximum of 80) when healthy sorghum sap amended with purified MDMV-A (5 ug/ml) was used. Values obtained using sap from MDMV-A-infected host tissue, were all <60. Based on Figure 3, the dilution of 1:32 biotin labelled PcAb was chosen for subsequent use, because at that dilution a satisfactory signal was generated without expending an excess amount of antibody.

Cross-reactivity of McAb

The cross-reactivities of McAbs were first assayed by directly adsorbing the virus isolates onto the wells of Microtiter plates, and then detecting the viruses by using an indirect ELISA. Figure 4 shows the immunoreactivities of each McAb with purified MDMV-A and MDMV-B (5 ug/ml) in an indirect ELISA. Ascites fluid was used as the source of

Figure 3. P/N ratios obtained from the optimization of signal antibody prepared from combined PcAb labelled with biotin. The response is recorded as P/N ratio vs. the reciprocal antibody dilution. A dilution of PcAb (1:1000) from the final bleeding of rabbits 97, 98, and 100 was added to the wells of an Immulon II 96 well Microtiter plate. 5 ug/ml of purified MDMV-A, healthy sorghum sap amended with 5 ug/ml of purified MDMV-A, or sap from host MDMV-A-infected sorghum tissue was added (50 ul/well). Serial dilutions of biotin labelled PcAb combined from the final bleedings of rabbits 97, 98, and 100 was added to each well (50 ul/well); 50 ul/well of avidin-alkaline phosphatase was then added. After the addition of p-nitrophenyl phosphate and 37°C incubation for 45 min, the reaction products were read spectrophotometrically at 410 nm.

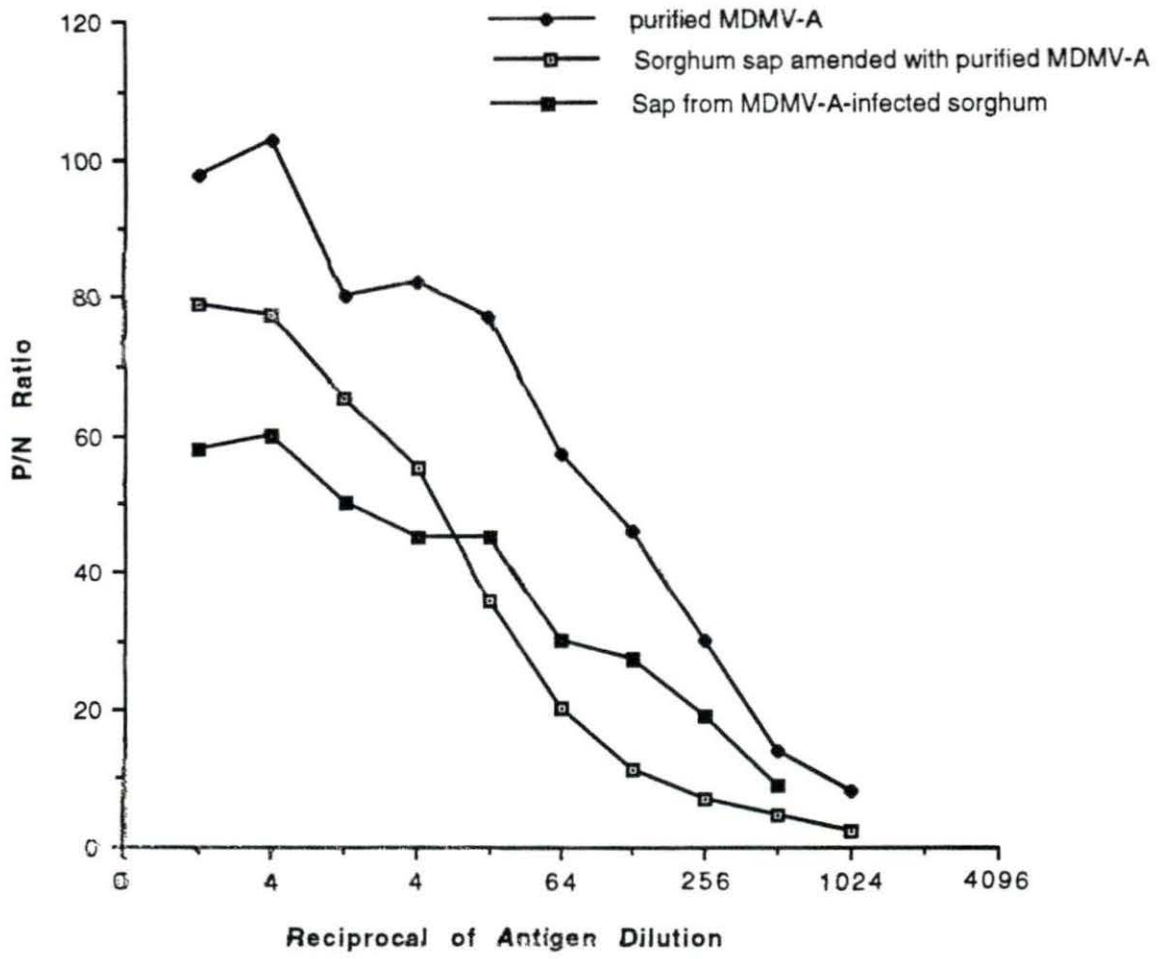
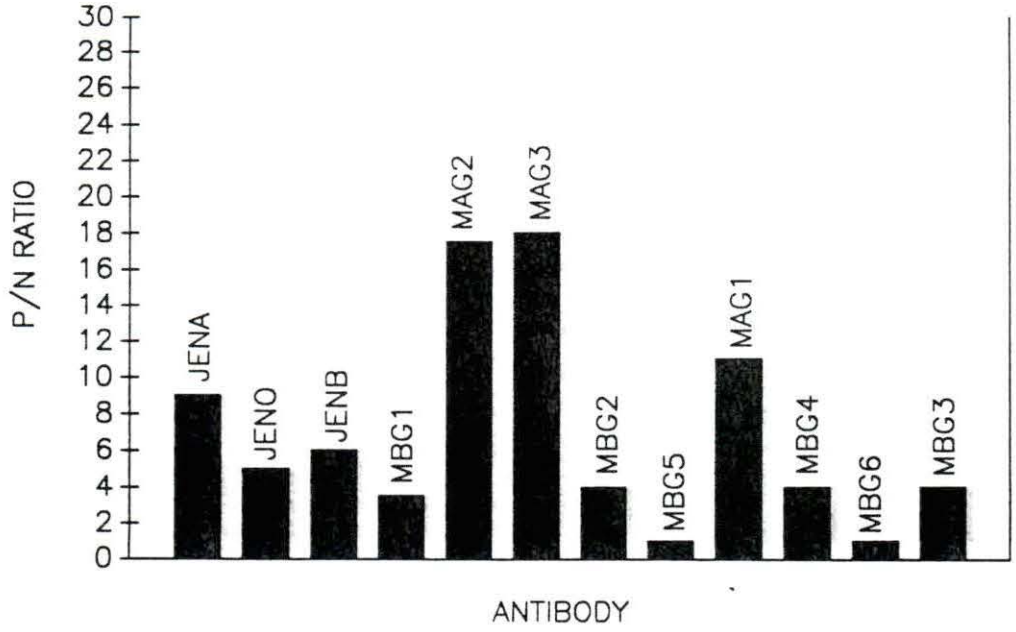

purified MDMV-A
Sorghum sap amended with purified MDMV-A
Sap from MDMV-A-infected sorghum
120
100
80
60
40
20
0
P/N Ratio
0
4
4
64
256
1024
4096
Reciprocal of Antigen Dilution

Figure 4.

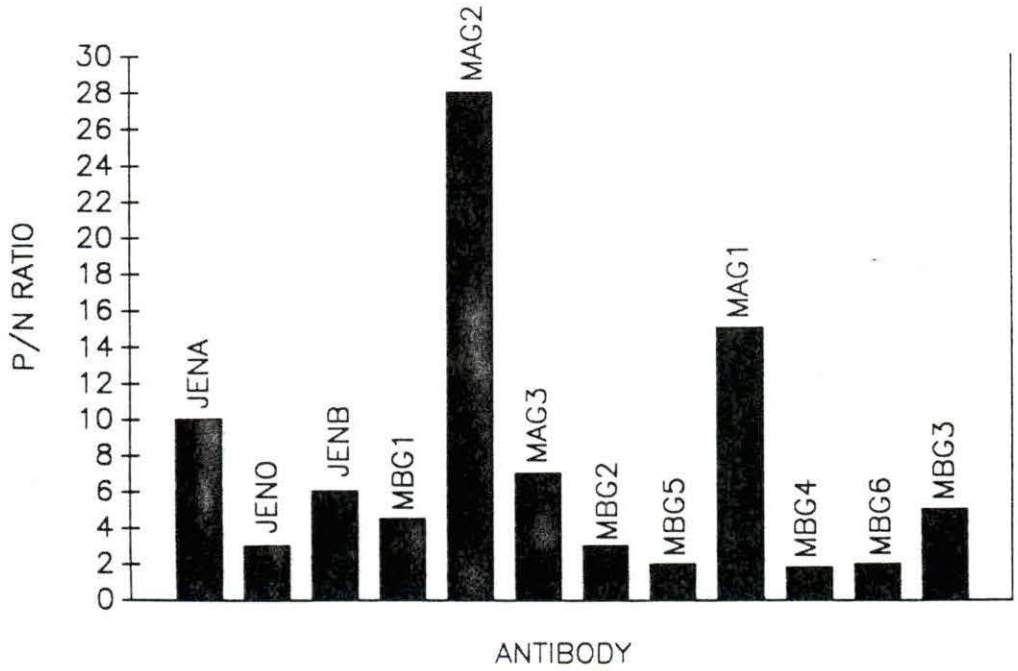
a. P/N ratios of the interactions of 12 McAbs against purified MDMV-A (5 ug/ml) as detected in an indirect ELISA. The response is recorded as the P/N ratio vs. antibody at 1:50 dilution of ascites fluid. Wells of an Immulon II 96 well Microtiter plate were coated with 5 ug/ml purified MDMV-A (50 ul/well). A 1:50 dilution of ascites fluid containing McAb (JEN-A[A], JEN-O [O], JEN-B[B], MBG-1[B1], MAG-2[A2], MAG-3[A3],MBG-2[B2], MBG-5[B5], MAG-1[A1], MBG-4[B4], MBG-6[B6], or MBG-3[B3]) were adsorbed to wells of the antigen-coated plate and the antibody-antigen combination was detected with a 1:1000 dilution of anti-mouse IgG conjugated with alkaline phosphatase (50 ul/well). After the addition of substrate and incubation at 37°C for 45 min, the reaction products were read spectrophotometrically at 410 nm.

4. b. P/N ratios of the interactions of 12 McAbs against MDMV-B (5 ug/ml) in an indirect ELISA. The response is recorded as the P/N ratio vs. antibody at 1:50 dilution of ascites fluid. Wells of an Immulon II 96 well Microtiter plate were coated with 5 ug/ml purified MDMV-B (50 ul/well). A 1:50 dilution of 12 McAb containing ascites fluids was adsorbed to wells of the antigen-coated plate and the antibody-antigen combination was detected with a 1:1000 dilution of anti-mouse IgG conjugated with alkaline phosphatase (50 ul/well). After the addition of substrate and incubation at 37°C for 45 min, the reaction products were read as above.

4. a.


P/N RATIO
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
JENA
JENO
JENB
MBG1
MAG2
MAG3
MBG2
MBG5
MAG1
MBG4
MBG6
MBG3
ANTIBODY

4. b.


P/N RATIO
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
JENA
JENO
JENB
MBG1
MAG2
MAG3
MBG2
MBG5
MAG1
MBG4
MBG6
MBG3
ANTIBODY

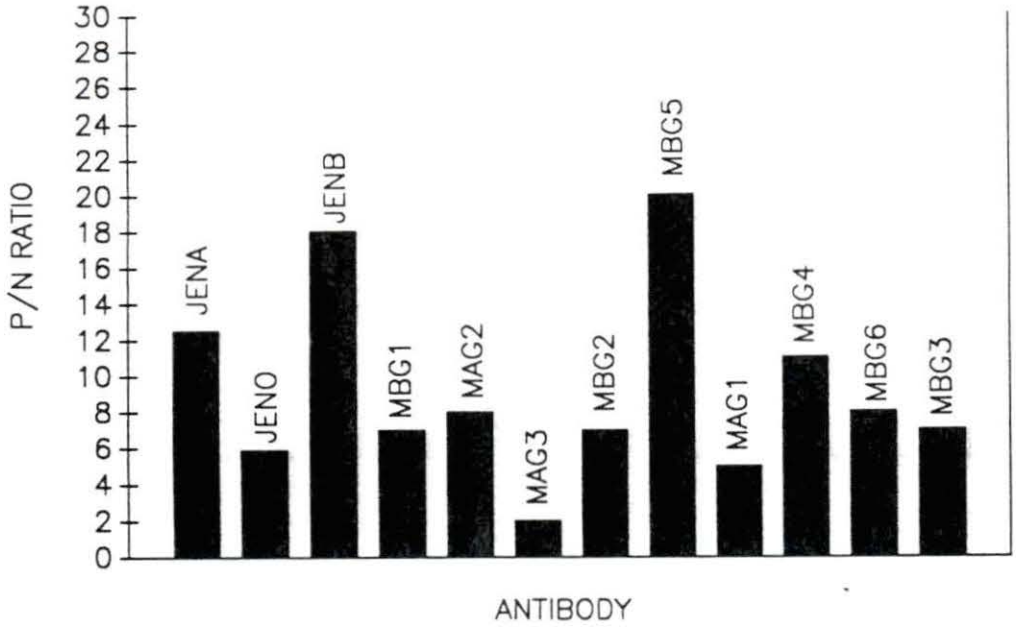
McAbs; a 1:50 dilution was chosen as the initial dilution, with 1:2 serial dilutions used thereafter. Both Figures 4 and 5 are plotted at the McAb dilution of 1:50. Inasmuch as the initial antibody concentration of each ascites fluid was unknown, the P/N ratio generated by reaction of a single McAb with the purified preparations of MDMV-A and MDMV-B cannot be directly compared to the P/N ratios of other McAbs. P/N ratios of McAbs MBG-3, MBG-6, MBG-4, MBG-1, JEN-O, JEN-B, displayed immunoreactivity, but with very low P/N ratios. JEN-A displayed a relatively high P/N ratio (P/N=9 and 10) against both MDMV-A and MDMV-B. MAG-1, MAG-2, and MAG-3 displayed high immunoreactivities (P/N ratios >10) with MDMV-A. MAG-2 generated a very high P/N ratio (almost 30) when MDMV-B was used as the antigen, and MAG-1 also yielded a high P/N ratio of about 15 with MDMV-A. In general, McAb's generated against MDMV-A yielded higher P/N ratios to both viruses, than McAbs generated against MDMV-B, as shown in Figure 5. Figure 5 also directly compares the P/N ratios generated by the McAbs in a dsELISA and indirect ELISA. Figure 4b and Figure 5b are the results of indirect ELISAs with purified MDMV-B and MDMV-A, respectively. There is no difference between Figure 4a and Figure 5b. Some of the McAbs generated a greater signal when the virus was captured by PcAb (as demonstrated by the response of JEN-A, JEN-B, MBG-5, MBG-4, and MBG-6) than when the virus was

Figure 5.

a. P/N ratios of the interaction of 12 McAbs with purified MDMV-A (5 ug/ml, 50 ul/well) captured by PcAb as detected by a dsELISA. The results are expressed as the P/N ratio vs. antibody at a 1:50 dilution. A 1:1000 dilution of PcAb combined from the final bleedings of rabbits 97, 98, and 100 were added to the wells of a 96 well Immulon II Microtiter plate and incubated for 1 h at room temperature. Purified MDMV-A (5 ug/ml) was added to the plate at 50 ul/well, and incubated for 1 h room temperature. A 1:50 dilution of 12 McAbs containing ascites fluid JEN-A[A], JEN-B[B], JEN-O[O], MBG-1[B1], MAG-2[A2], MAG-3[A3], MBG-2[B2], MBG-5[B5], MAG-1 [A1], MBG-4[B4], MBG-6[B6], and MBG-3[B3] was added to wells. A 1:1000 dilution of anti-mouse IgG conjugated to alkaline phosphatase was added (50 ul/well). After the addition of p-nitrophenyl phosphate and incubation at 37°C for 45 minutes, the reaction products were read spectrophotometrically at 410 nm.

5. b. P/N ratios of the interactions of 12 McAbs against purified MDMV-A (5 ug/ml), as detected in an indirect ELISA. The response is recorded as the P/N ratio vs. antibody at a 1:50 dilution. Wells of an Immulon II 96 well Microtiter plate were coated with 5 ug/ml purified MDMV-A (50 ul/well). A 1:50 dilution of ascites fluid containing McAb (JEN-A [A], JEN-O [O], JEN-B [B], MBG-1 [B1], MAG-2 [A2], MAG-3 [A3], MBG-2 [B2], MBG-5 [B5], MAG-1 [A1], MBG-4 [B4], MBG-6 [B6], and MBG-3 [B3]) were adsorbed to the wells of the antigen-coated plate and the antibody-antigen combination was detected with a 1:1000 dilution of anti-mouse IgG conjugated with alkaline phosphatase (50 ul/well). After the addition of substrate and incubation at 37°C for 45 min, the reaction products were read spectrophotometrically at 410 nm.

5. a.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2
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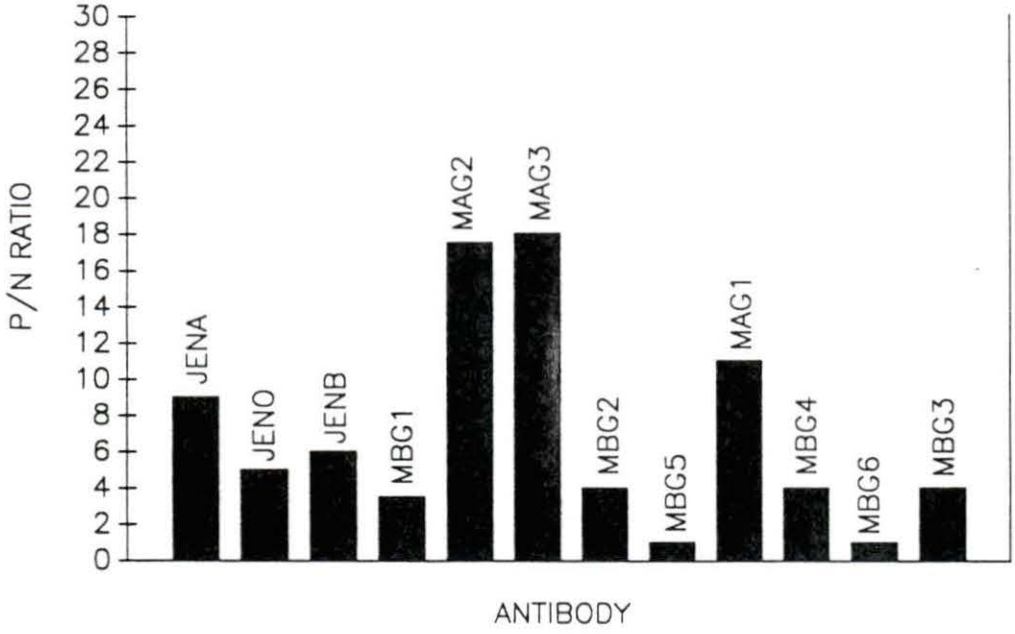
JENO
JENB
MBG1
MAG2
MAG3
MBG2
MBG5
MAG1
MBG4
MBG6
MBG3
ANTIBODY

5. b.


P/N RATIO
30
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JENO
JENB
MBG1
MAG2
MAG3
MBG2
MBG5
MAG1
MBG4
MBG6
MBG3
ANTIBODY

directly adsorbed to the wells of a microtiter plate.

Because each McAb reacted with purified MDMV-A or MDMV-B to some extent, each McAb was tested against sap from virus-infected tissue. Since the antigen concentration of sap from virus-infected tissue was unknown, the data indicate only if a reaction occurred or did not occur. Host tissue infected with isolates representing MDMV-A, MDMV-B, Minn-11, and NeB2 were recognized by all 12 McAbs (Table 3). MBG-2 and JEN-A were the only two McAbs to recognize strain KS-1. OHIO-O was only recognized by MBG-1, MAG-2, JEN-O, MAG-1, MBG-5, and MAG-3. Because KS-1 and OHIO-O were not recognized well by PcAb (Figure 1) and recognized minimally by only two McAb (Table 3), they were not analyzed by signature analysis. OHIO-O has been reclassified as johnsongrass mosaic virus, and KS-1 is a member of the MDMV group. Had the antibodies generated more than a minimal response, the data would have been useful to clarify relatedness.

Quantitation of Antibody Protein Concentration

The protein concentration of each McAb preparation was determined for the use in the competition ELISA. Without such an analysis, competition could occur due to artifacts such as protein-protein interactions induced by protein excess. To determine the protein concentration of each

Table 3. Cross-reactivity and recognition of plant sap from host tissue infected with isolates representing strains of MDMV-A, MDMV-B, OHIO-O, KS-1, Minn-11, and NeB2, by 12 McAb's, as assayed by indirect ELISA.

McAb	IgG subclass	A[a]	B	O	K	11	NeB2
MBG-1	2b	+[b]	+	+	-	+	+
MAG-2	2a	+	+	+	-	+	+
JEN-A	2a	+	+	-	+	+	+
JEN-O	NT[c]	+	+	+	-	+	+
JEN-B	NT	+	+	-	-	+	+
MAG-1	2a	+	+	+	-	+	+
MBG-4	2a	+	+	-	-	+	+
MBG-6	3	+	+	-	-	+	+
MBG-3	1	+	+	-	-	+	+
MBG-5	1	+	+	+	-	+	+
MBG-2	2a	+	+	-	+	+	+
MAG-3	2a	+	+	+	-	+	+

[a]sap from host plants infected with virus isolates representing MDMV-A (A), MDMV-B (B), OHIO-O (O), KS-1 (K), Minn-11 (11), and NeB2.

[b] = + recognition.
- no detectable recognition.

[c]not tested.

antibody preparation, a standard curve was generated by using a known amount of mouse IgG adsorbed to the wells of a Microtiter plate. Serial dilutions of an unlabelled, but quantified amount of mouse IgG in a buffer containing anti-mouse IgG conjugated with alkaline phosphatase (Figure 6), were performed. Therefore, low absorbances were generated when labelled anti-mouse IgG combined with the high concentration of mouse IgG in solution. High absorbance was obtained when the concentration of mouse IgG in solution was low, and the anti-mouse IgG combined with the plate-adsorbed mouse IgG. This generated a standard curve, from low concentrations of competing solution antibody, to high concentration of competing solution antibody. When an unknown amount of antibody in ascites fluid or flask media was added to the dilutions of labelled anti-mouse IgG, absorbances were determined which reflected the amount of labelled anti-mouse that was free to bind to the plate-adsorbed mouse IgG. Thus, the protein concentration for each competing monoclonal antibody preparation was interpolated from the standard curve by comparing absorbances. Each antibody concentration was multiplied by the dilution factor, which provided an estimate of protein concentration of the antibody in flask media or ascites fluid (Table 4). This procedure made purification of the antibody preparation unnecessary. As expected, flask medium

Figure 6. A standard curve to determine the concentration of IgG in ascites fluid or flask medium. A standard curve was generated by allowing known concentrations of an unlabelled mouse McAb to compete with known concentrations of the same mouse McAb directly adsorbed to the wells of a Microtiter plate. A mouse McAb (2 ug/ml, 50 ul/well) was adsorbed to the wells of a Microtiter plate. Serial dilutions of a mouse McAb (2 ug/ml) were performed in separate tubes containing blocking buffer with a 1:1000 dilution of goat anti-mouse IgG conjugated with alkaline phosphatase, and were added to each well at 50 ul/well. P-nitrophenyl phosphate (1 mg/ml, 50 ul/well) substrate was added to each well, and incubated for 45 at 37°C. The reaction products were read spectrophotometrically at 410 nm.

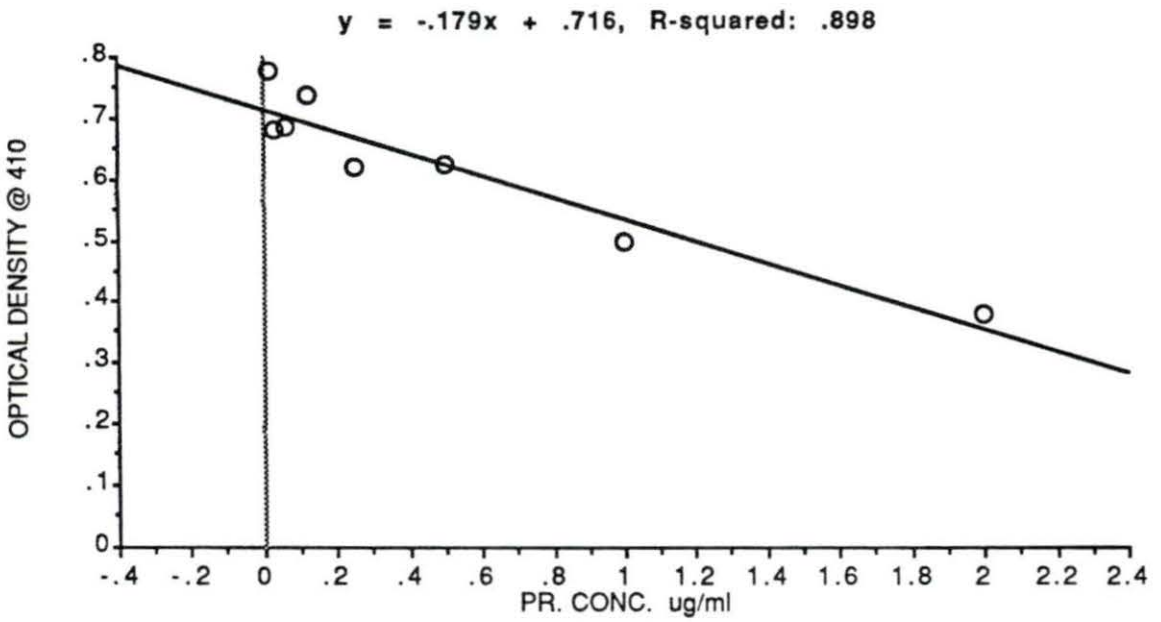

y = -.179x + .716, R-squared: .898
OPTICAL DENSITY @ 410
.8
.7
.6
.5
.4
.3
.2
.1
0
-.4 -.2 0 .2 .4 .6 .8 1 1.2 1.4 1.6 1.8 2 2.2 2.4
PR. CONC. ug/ml

Table 4. Protein concentrations of McAb-containing ascites fluid or flask media, calculated from the standard curve generated in Figure 6

Antibody	Source	Point on Standard Curve x Dilution	Protein Concentration
Jensen A	ascites	1.34 x 1600	= 2.144 mg/ml
Jensen O	ascites	1.80 x 400	= 0.72 mg/ml
Jensen B	ascites	1.82 x 400	= 0.72 mg/ml
MAG-1	ascites	1.68 X 1600	= 2.688 mg/ml
MBG-4	flask	0.58 X 100	= 5.8 ug/ml
MBG-6	ascites	1.7 x 400	= 0.68 mg/ml
MBG-2	ascites	1.19 x 3200	= 3.81 mg/ml
MBG-3	ascites	2.03 x 3200	= 6.496 mg/ml
MBG-5	ascites	1.71 x 3200	= 5.472 mg/ml
MBG-1	flask	1.28 x 10	= 12.8 ug/ml
MAG-2	ascites	2.36 x 3200	= 7.552 mg/ml
MAG-3	ascites	2.23 x 3200	= 7.136 mg/ml

had a significantly lower antibody protein concentration than ascites fluid. Ascites fluid varied in antibody protein concentration from 0.7 mg/ml for MBG-6 to 7.6 mg/ml of MAG-2, flask media varied between 6.0 to 13.0 ug/ml.

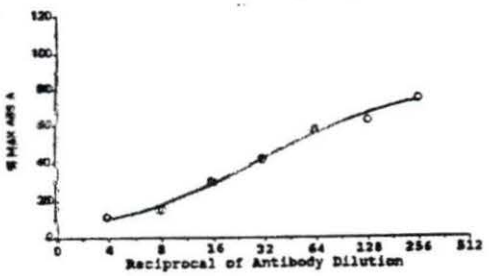
Competition ELISAs

To characterize the specific immunoreactivity of the 12 McAbs, competition ELISAs were performed. Figures 7-12 demonstrate results of simultaneous and consecutive competition ELISAs using biotin-labelled JEN-A as the labelled, competing antibody. Third degree polynomial regression analysis was used to draw the graphs. Schmajohn et. al. (103) reported that unlabelled antibody which inhibited 50% of the binding of labelled antibody could be considered as binding to similar or overlapping epitopes. In the simultaneous competition (Figure 9), MBG-6 appeared to compete with JEN-A for a similar or overlapping epitope. All other antibodies did not compete. In the consecutive competition ELISA (Figures 10,11,12), MBG-6 did not appear to compete.

JEN-O and JEN-B were eliminated from use in the signatures since the source was limited (these antibodies were provided by S. Jensen in very small quantities) and the response by each antibody was low. MBG-2 was eliminated because attempts to label it with biotin were not

Figure 7. Simultaneous competition ELISA of four different unlabelled McAbs, JEN-A (7.a), JEN-O (7.b), JEN-B (7.c), and MAG-1 (7.d), against a constant concentration (11.6 ug/ml, 50 ul/well) of biotin-labelled McAb JEN-A. Assays were performed in Immulon II 96 well Microtiter plates. Wells were coated with purified MDMV-A at 5 ug/ml, 50 ul/well. Data are mean values of two replications. Results are expressed as the percent maximum absorbance of unlabelled McAb vs. dilution of antibody.

7. a.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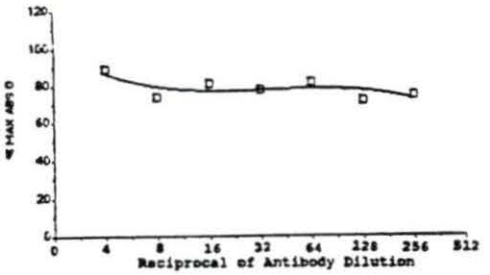
Reciprocal of Antibody Dilution

7. b.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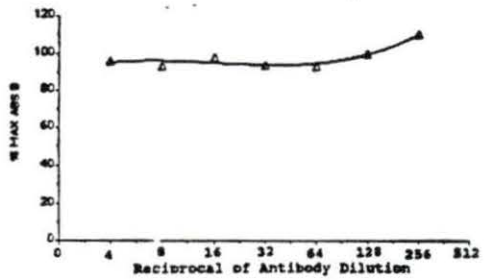
Reciprocal of Antibody Dilution

7. c.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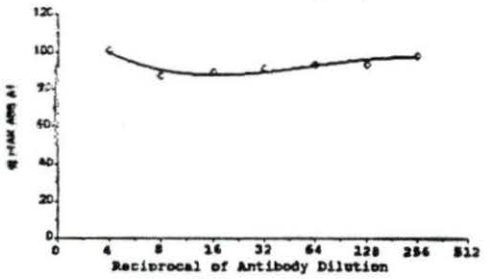
Reciprocal of Antibody Dilution

7. d.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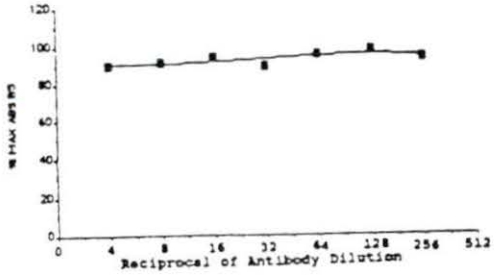
Reciprocal of Antibody Dilution

Figure 8. Simultaneous competition ELISA of four different unlabelled McAbs, MBG-5 (8.a), MBG-1 (8.b), MAG-2 (8.c), and MAG-3 (8.d), against a constant concentration (11.6 ug/ml, 50 ul/well) of biotin-labelled McAb JEN-A. Assays were performed in wells of an Immulon II 96 well microtiter plates. Wells were coated with purified MDMV-A (5 ug/ml, 50 ul/well). Data are the mean values of at least two replications. Results are expressed as percent maximum absorbance of unlabelled McAb vs. dilution of antibody.

8. a.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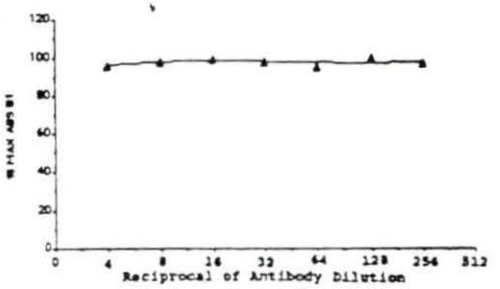
Reciprocal of Antibody Dilution

8. b.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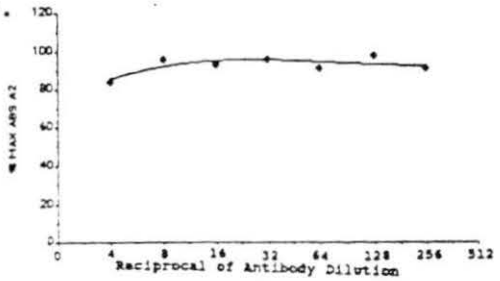
Reciprocal of Antibody Dilution

8. c.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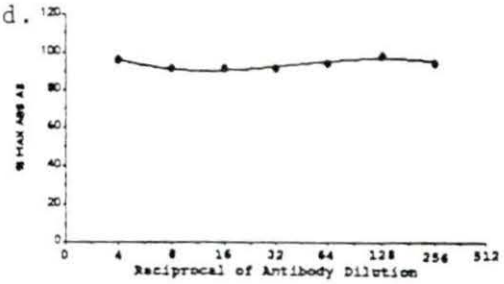
Reciprocal of Antibody Dilution

8. d.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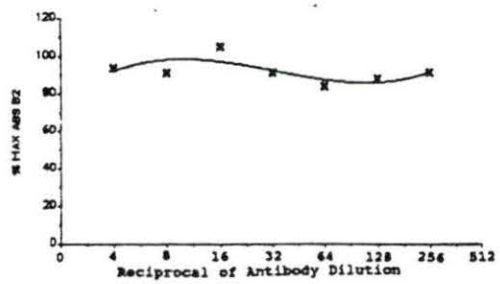
512
Reciprocal of Antibody Dilution

Figure 9. Simultaneous competition ELISA of three different unlabelled McAbs, MBG-2 (9.a), MBG-3 (9.b), and MBG-4 (9.c) and MBG-6 (9.d) against a constant concentration (11.6 ug/ml, 50 ul/well) of biotin-labelled McAb JEN-A. Assays were performed in wells of an Immulon II 96 well microtiter plates. Wells were coated with purified MDMV-A (5 ug/ml, 50 ul/well). Data are mean values of at least two replications. Results are expressed as percent maximum absorbance of unlabelled McAb vs. dilution of antibody.

9. a.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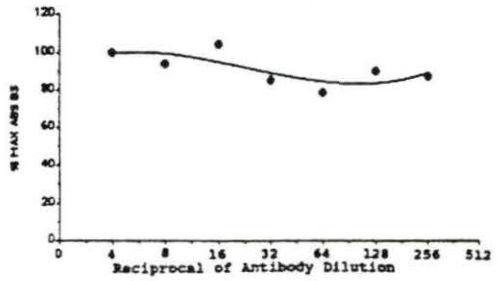
Reciprocal of Antibody Dilution

9. b.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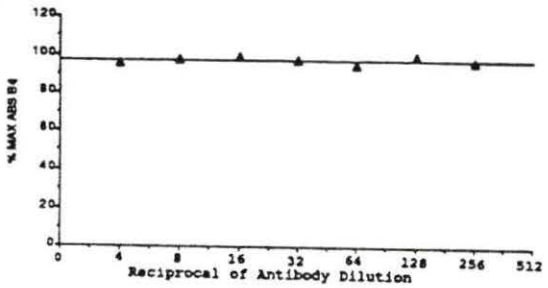
Reciprocal of Antibody Dilution

9. c.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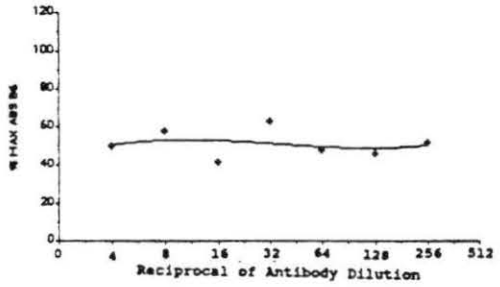
9. d.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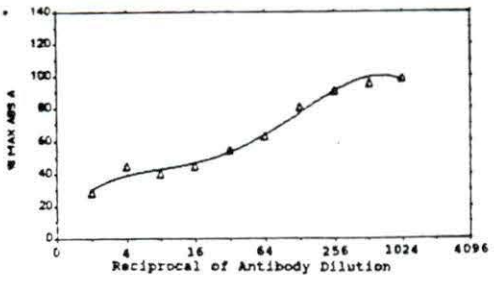
512
Reciprocal of Antibody Dilution

Figure 10. Consecutive competition ELISA of four different unlabelled McAbs, JEN-A (10.a), JEN-O (10.b), JEN-B (10.c), and MAG-1 (10.d), against a constant concentration (11.6 ug/ml, 50 ul/well) of biotin-labelled McAb JEN-A. Assays were performed in wells of an Immulon II 96 well Microtiter plates. Wells were coated with purified MDMV-A (5 ug/ml, 50 ul/well). Data are mean values of at least two replications. Results are expressed as percent maximum absorbance of unlabelled McAb vs. dilution of antibody.

10. a.



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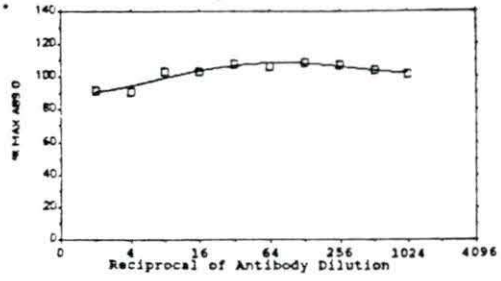

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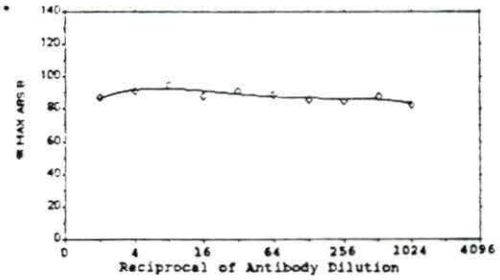

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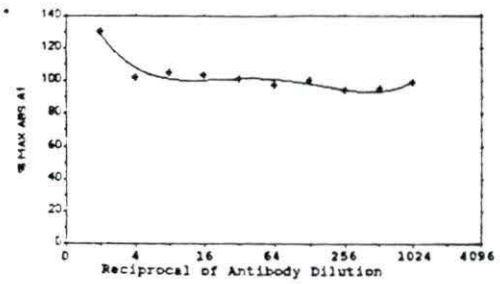
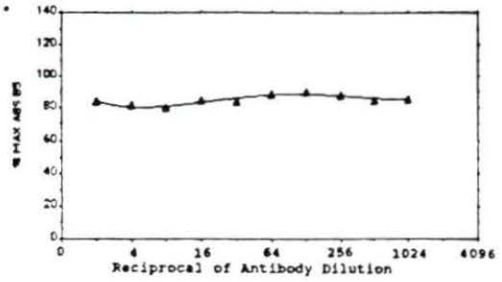


Figure 11. Consecutive competition ELISA of four different unlabelled McAbs, MBG-5 (11.a), MBG-1 (11.b), MAG-2 (11.c), and MAG-3 (11.d), against a constant concentration (11.6 ug/ml, 50 ul/well) of biotin-labelled McAb JEN-A. Assays were performed in wells of Immulon II 96 well microtiter plates. Wells were coated with purified MDMV-A (5 ug/ml, 50 ul/well). Data are mean values of at least two replications. Results are expressed as the percent maximum absorbance of unlabelled McAb vs. dilution of antibody.

11. a.



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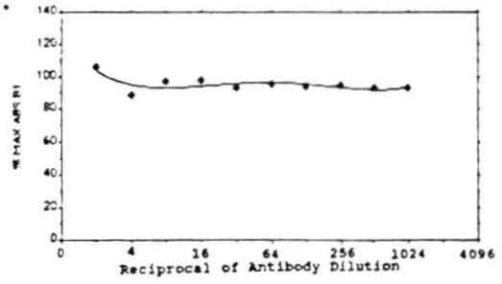

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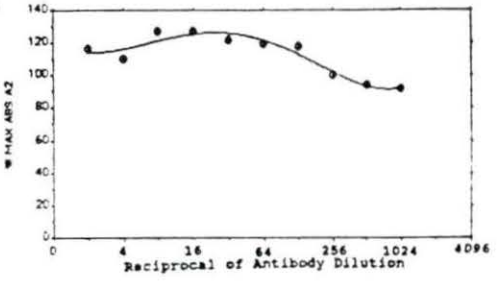


11. d.

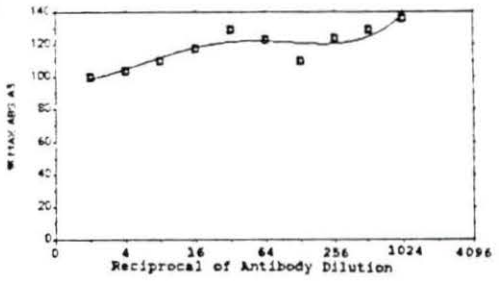
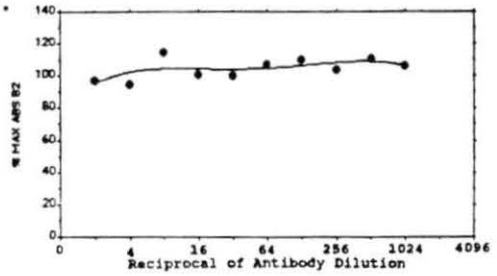
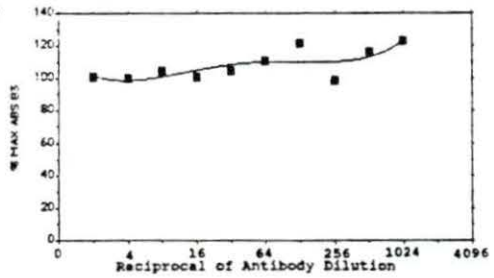


Figure 12. Consecutive competition ELISA of four different unlabelled McAbs, MBG-2 (12.a), MBG-3 (12.b), MBG-4 (12.c), and MBG-6 (12.d), against a constant concentration (11.6 ug/ml, 50 ul/well) of biotin-labelled McAb JEN-A. Assays were performed in wells of Immulon II 96 well Microtiter plates. Wells were coated with purified MDMV-A (5 ug/ml, 50 ul/well). Data are mean values of at least two replications. Results are expressed as percent maximum absorbance of unlabelled McAb vs. dilution of antibody.

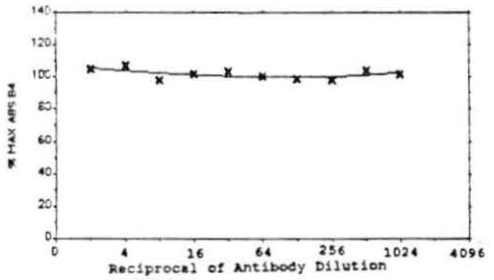
12. a.



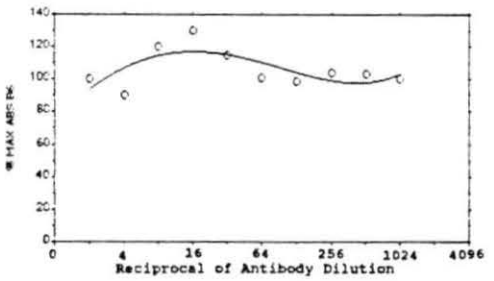
12. b.



12. c.



12. d.



successful. MBG-6 was eliminated because of the competition with JEN-A in the simultaneous competition. Therefore, 8 McAbs (MAG-1, MAG-2, MAG-3, MBG-1, MBG-3, MBG-4, MBG-5 and JEN-A) were selected for the signature analysis.

Optimization of RIA System

Based on the data that MBG-1 generated in Figures 4-12, it was chosen as the capture antibody. Ascites fluid containing MBG-1 antibody was used as the capture antibody for MDMV-A The optimal dilution of MBG-1 was 2^8 or 1:256. Biotin labelled PcAb was used as the signal antibody (1:32 dilution)(Figure 13). Therefore, a 1:200 dilution of MBG-1 ascites fluid was used as the capture antibody concentration in subsequent double sandwich RIAs.

To allow comparisons of each McAb P/N ratio in the signature analysis, each McAb was purified, labelled with biotin, and quantified. Serial dilutions of each biotin-labelled antibody were tested in a double sandwich radioimmunoassay using a 1:200 dilution of MBG-1 as capture antibody and 5 ug/ml of MDMV-A (Figure 14). Iodinated-avidin was used for detection. Table 5 summarizes the P/N ratios of the dilution curves and lists the calculated protein concentrations. MAG-3 generated the lowest response (undiluted at the midpoint) with the greatest amount of protein (0.3 mg/ml). Therefore, each biotin-labelled McAb

Figure 13. P/N ratios obtained from the optimization of dilutions of ascites fluid containing McAb, using MBG-1 as a capture antibody in a dsELISA. The results are expressed as the P/N ratio vs. MBG-1 dilution. Mouse McAb MBG-1 (B1) in ascites fluid was serially diluted and adsorbed to the wells of an Immulon II Microtiter plate (50 ul/well). Purified MDMV-A (5 ug/ml, 50 ul/well) was then added to each well. A 1:32 dilution of PcAb combined from the final bleeding of rabbits 97, 98, and 100 and labelled with biotin, was added at 50 ul/well. Avidin-alkaline phosphatase was added at a concentration of 1 ug/ml, 50 ul/well. After the addition of p-nitrophenyl phosphate and incubation at 37°C for 45 minutes, the reaction products were read spectrophotometrically at 410 nm.

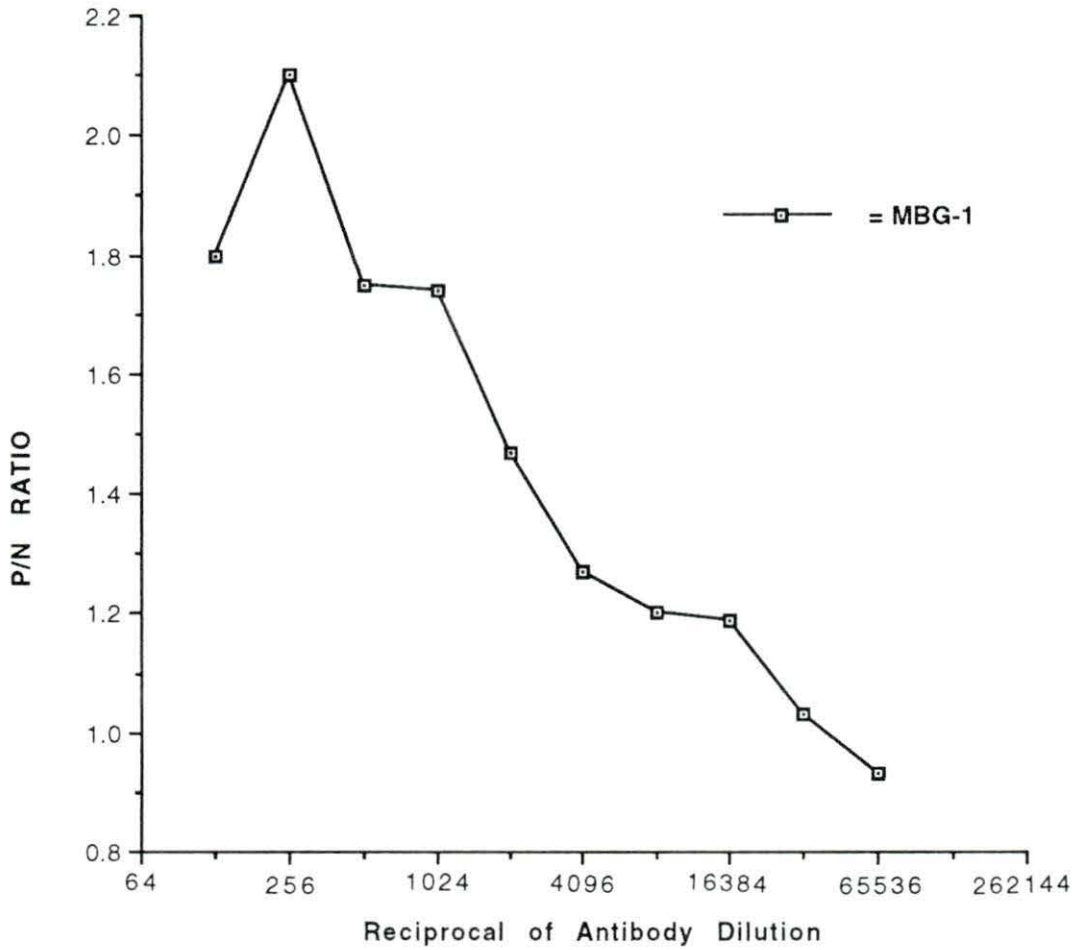
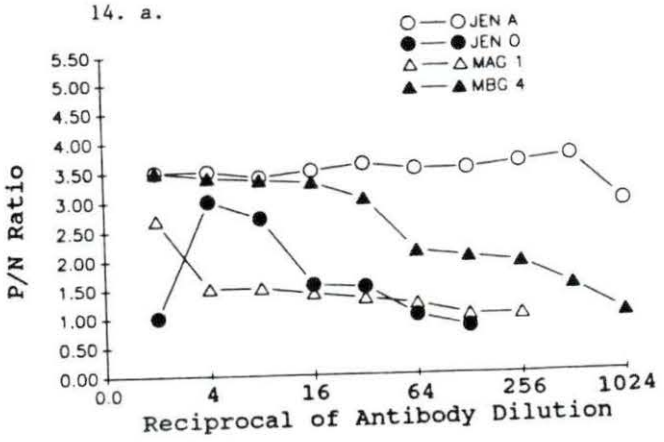

2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
P/N RATIO
= MBG-1
64
256
1024
4096
16384
65536
262144
Reciprocal of Antibody Dilution

Figure 14.a. P/N ratios obtained from 4 mouse McAbs labelled with biotin and used as signal antibodies in a dsRIA. The results are expressed as the P/N ratio vs. reciprocal antibody dilution. A 1:200 dilution of ascites fluid containing MBG-1 (50 ul/well) was adsorbed to the wells of a 96 well Immulon II remove-a-well Microtiter plate. MDMV-A (5ug/ml, 50 ul/well) was added. Serial 1:2 dilutions of McAb, JEN-A (A), JEN-O (O), MAG-2(A2), and MBG-4 (B4) labelled with biotin, were performed, and added at 50 ul/well. Avidin conjugated with ^{125}I was added to each well at 20,000 cpm. The wells were counted on a Tracor Analytic gamma counter for 2 minutes or 10,000 cpm.

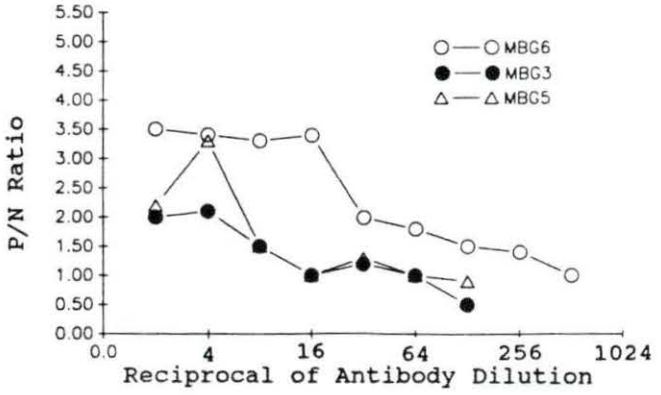
14.b. P/N ratios obtained from 3 mouse McAbs labelled with biotin and used as signal antibodies in a dsRIA. The results are expressed as the P/N ratio vs. reciprocal antibody dilution. With the exception that serial dilutions of McAb, MBG-6 (B6), MBG-3 (B3), and MBG-5 (B5) labelled with biotin, were performed, and added at 50 ul/well, the assay was completed and read as described above.

14.c. P/N ratios obtained from 3 mouse McAbs labelled with biotin and used as signal antibodies in a dsRIA. With the exception that serial 1:2 dilutions of McAb, MBG-1 (B1), MAG-2 (A2), and MAG-3 (A3) labelled with biotin, were performed, and added at 50 ul/well, the assay was completed and read as described above.

14. a.


JEN A
JEN O
MAG 1
MBG 4
P/N Ratio
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.0
4
16
64
256
1024
Reciprocal of Antibody Dilution

14. b.


MBG6
MBG3
MBG5
P/N Ratio
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.0
4
16
64
256
1024
Reciprocal of Antibody Dilution

14. c.

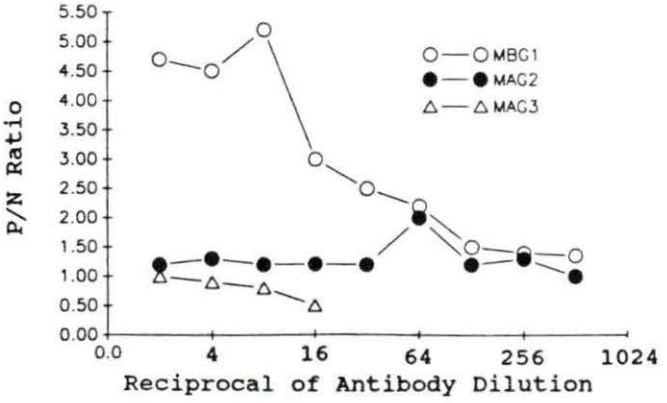

MBG1
MAG2
MAG3
P/N Ratio
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.0
4
16
64
256
1024
Reciprocal of Antibody Dilution

Table 5. Initial protein concentrations of labelled McAb, their respective concentrations at the maximum P/N ratio generated in Figure 14

McAb	dilution at highest P/N ratio	initial protein concentration mg/ml	calculated protein concentration at midpoint mg/ml
MAG-1	1:2	0.0807	0.0404
MAG-2	1:64	2.74	0.043
MBG-3	1:4	0.136	0.034
MBG-1	1:8	0.943	0.118
JEN-A	1:500	1.09	0.000218
MBG-4	1:16	0.95	0.059
MBG-5	1:4	0.236	0.059
MAG-3	UNDILUTED	0.342	0.342

was standardized to a 0.3 mg/ml concentration, to ensure that equal amounts of antibody were used for the signature analysis.

Signature Analysis

Figures 15-18 show the binding profiles of McAb's MAG-1 (Ab1), MAG-2 (Ab2), MAG-3 (Ab3), MBG-1 (Ab4), MBG-3 (Ab5), MBG-4 (Ab6), MBG-5 (Ab7) and JEN-A (Ab8) when tested with Ag1 (MDMV-A), Ag2 (MDMV-B), Ag3 (Minn-11) and Ag4 (NeB2) in a double sandwich radioimmunoassay and analyzed by the signature analysis statistical program. All eight epitopes were present on each MDMV virus isolate. The signatures shown in Figures 15 and 17 appeared to be related. Figures 16 and 18 also show relatedness to each other. However, each set of signatures suggested that each virus isolate could be differentiated by a unique antigenic signature.

Figure 15. Signature analysis of MDMV-A. Results are presented as the natural log (ln) P/N ratio vs. the dilution of Ag1 (log base 2). McAb MBG-1 (1:200 dilution of ascites fluid, 50 ul/well) was used as the capture antibody. Serial dilutions of Sorghum (cv. Bugoff) sap infected with MDMV-A (AG=1) were added to wells (50 ul/well) of a remove-a-well Immulon II Microtiter plate. After incubation overnight at 4°C with gentle rocking, 8 McAbs (0.011 mg/ml, 50 ul/well): MAG-1 (Ab=1), MAG-2 (Ab=2), MAG-3 (Ab=3), MBG-1 (Ab=4), MBG-3 (Ab=5), MBG-4 (Ab=6), MBG-5 (Ab=7), and JEN-A (Ab=8), labelled with biotin, were added. After incubation, I^{125}-avidin was added to each well at 20,000 cpm. Wells were counted on a Tracor Analytic gamma counter for 2 minutes or to 10,000 cpm.

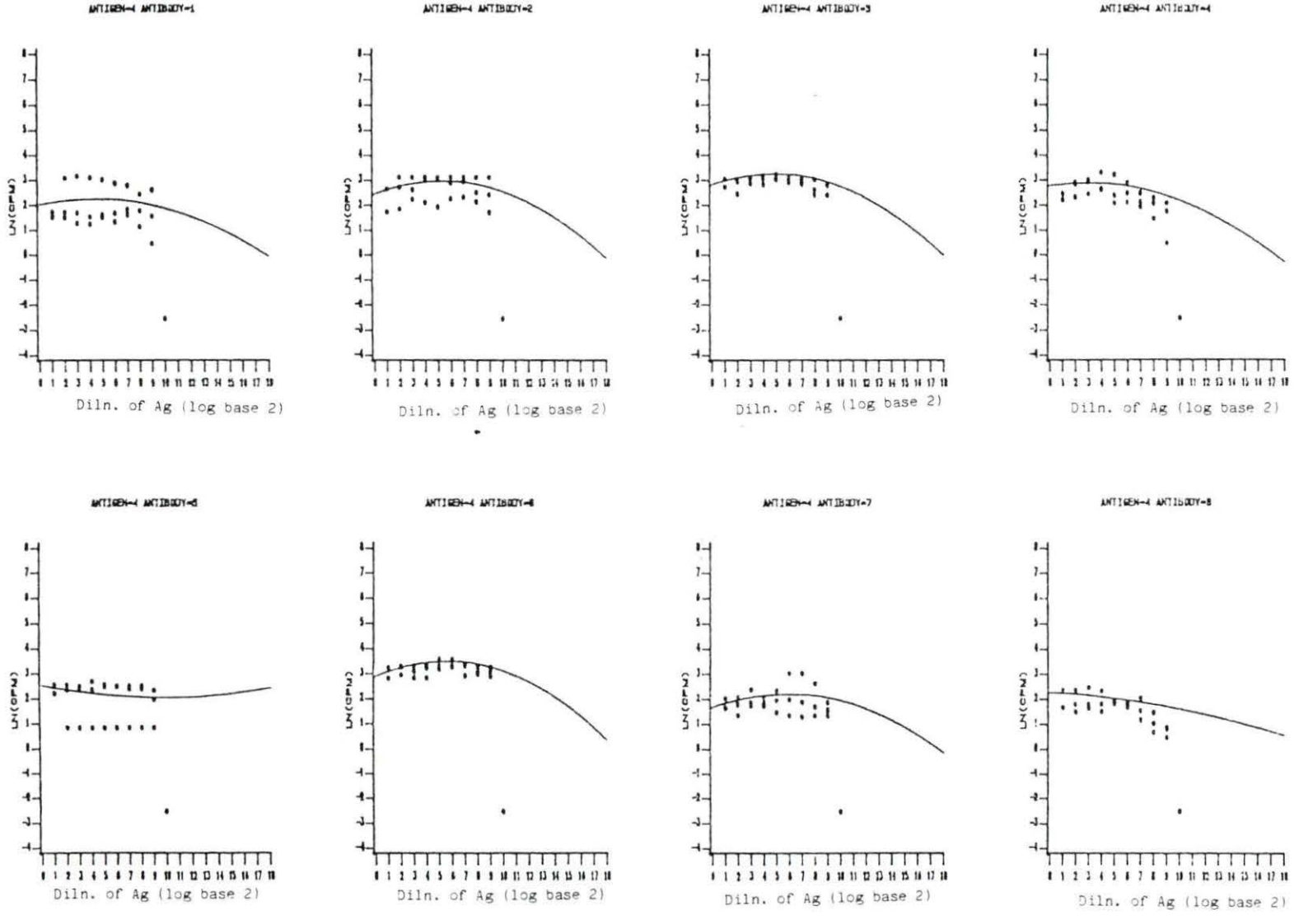
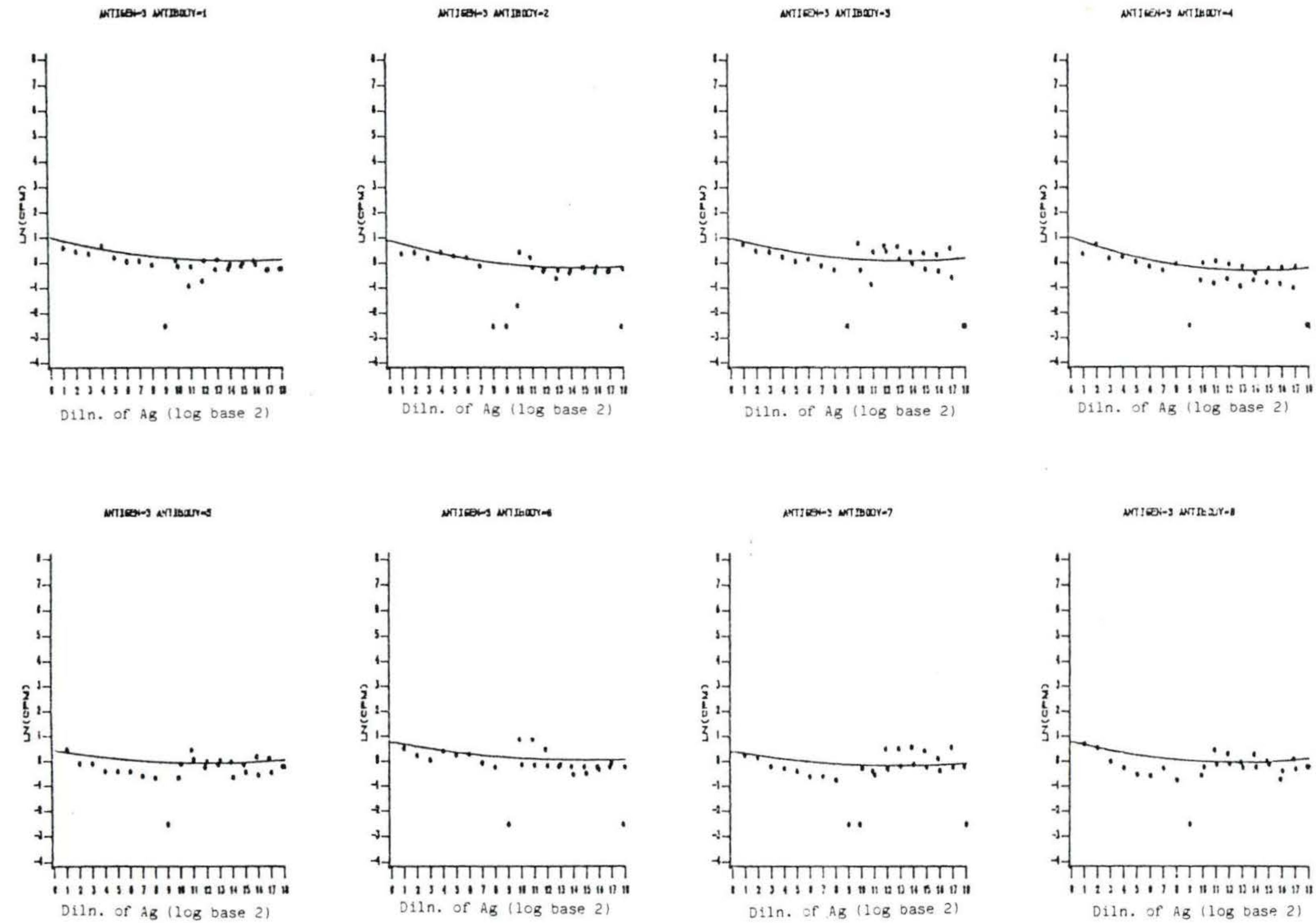

ANTIGEN=4 ANTIBODY=1
ANTIGEN=4 ANTIBODY=2
ANTIGEN=4 ANTIBODY=3
ANTIGEN=4 ANTIBODY=4
ANTIGEN=4 ANTIBODY=5
ANTIGEN=4 ANTIBODY=6
ANTIGEN=4 ANTIBODY=7
ANTIGEN=4 ANTIBODY=8
LN(CPM)
Diln. of Ag (log base 2)

Figure 16. Signature analysis of MDMV-B. Results are presented as the ln P/N ratio vs. the dilution of Ag2 (log base 2). McAb MBG-1 (1:200 dilution of ascites fluid, 50 ul/well) was used as the capture antibody. Serial dilutions of Sorghum (cv. Bugoff) sap infected with MDMV-B (AG=2) were added to wells (50 ul/well) of a remove-a-well Immulon II Microtiter plate. After incubation overnight at 4°C with gentle rocking, 8 McAbs (0.011 mg/ml, 50 ul/well): MAG-1 (Ab=1), MAG-2 (Ab=2), MAG-3 (Ab=3), MBG-1 (Ab=4), MBG-3 (Ab=5), MBG-4 (Ab=6), MBG-5 (Ab=7), and JEN-A (Ab=8) labelled with biotin, were added. After incubation, I^{125}-avidin was added to each well at 20,000 cpm. Wells were counted on a Tracor Analytic gamma counter for 2 minutes or to 10,000 cpm.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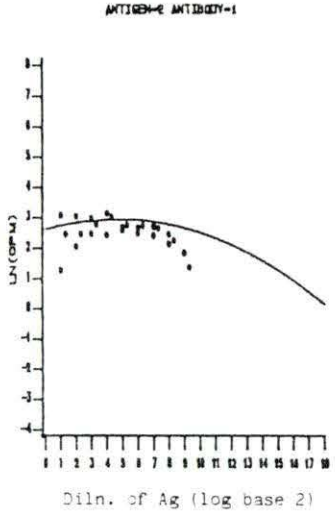
ANTIGEN=3 ANTIBODY=2
ANTIGEN=3 ANTIBODY=3
ANTIGEN=3 ANTIBODY=4
ANTIGEN=3 ANTIBODY=5
ANTIGEN=3 ANTIBODY=6
ANTIGEN=3 ANTIBODY=7
ANTIGEN=3 ANTIBODY=8
LN(CPM)
Diln. of Ag (log base 2)

Figure 17. Signature analysis of Minn-11. Results are presented as the ln P/N ratio vs. the dilution of Ag3 (log base 2). McAb MBG-1 (1:200 dilution of ascites fluid, 50 ul/well) was used as the capture antibody. Serial dilutions of Sorghum (cv. Dekalb) sap infected with Minn-11 (AG=3) were added to wells (50 ul/well)of a remove-a-well Immulon II Microtiter plate. After incubation overnight at 4°C with gentle rocking, 8 McAbs (0.011 mg/ml, 50 ul/well): MAG-1 (Ab=1), MAG-2 (Ab=2), MAG-3 (Ab=3), MBG-1 (Ab=4), MBG-3 (Ab=5), MBG-4 (Ab=6), MBG-5 (Ab=7), and JEN-A (Ab=8) labelled with biotin, were added. After incubation, I^{123}-avidin was added to each well at 20,000 cpm. Wells were counted on a Tracor Analytic gamma counter for 2 minutes or to 10,000 cpm.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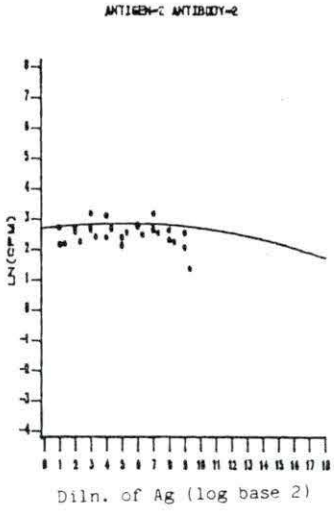
LN(CPM)
Diln. of Ag (log base 2)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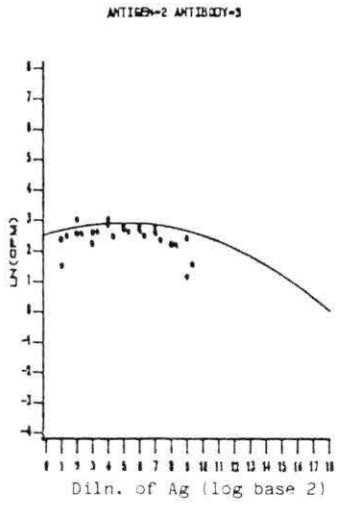
LN(CPM)
Diln. of Ag (log base 2)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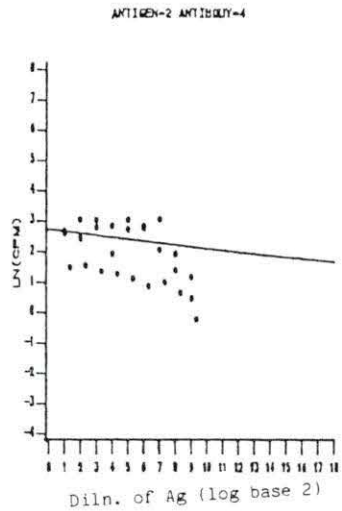
LN(CPM)
Diln. of Ag (log base 2)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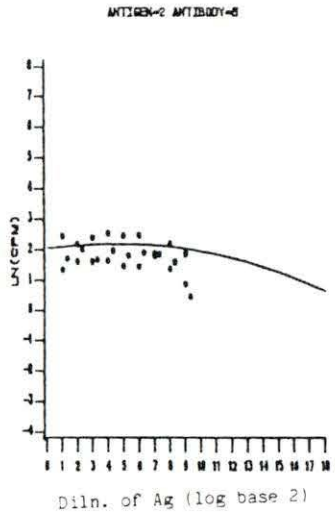
LN(CPM)
Diln. of Ag (log base 2)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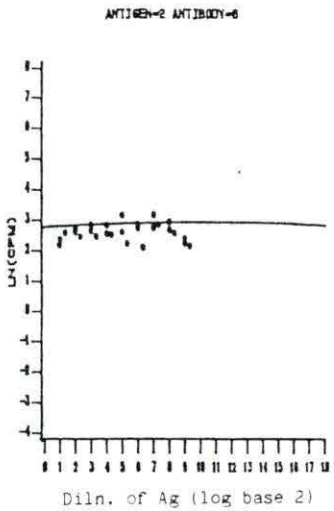
LN(CPM)
Diln. of Ag (log base 2)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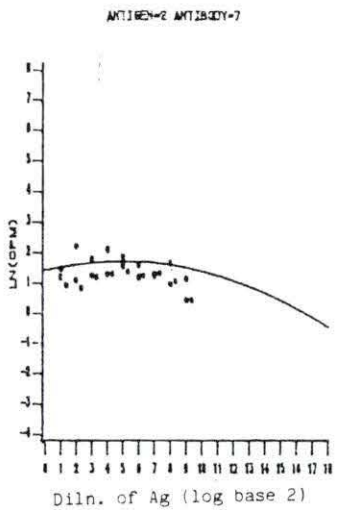
LN(CPM)
Diln. of Ag (log base 2)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LN(CPM)
Diln. of Ag (log base 2)

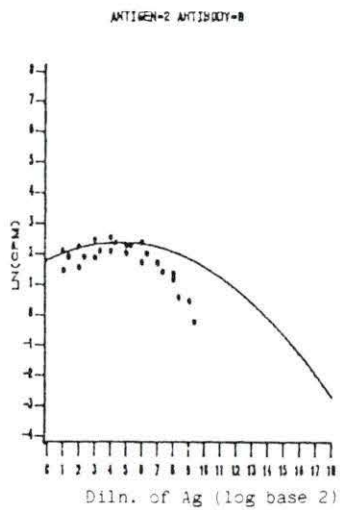
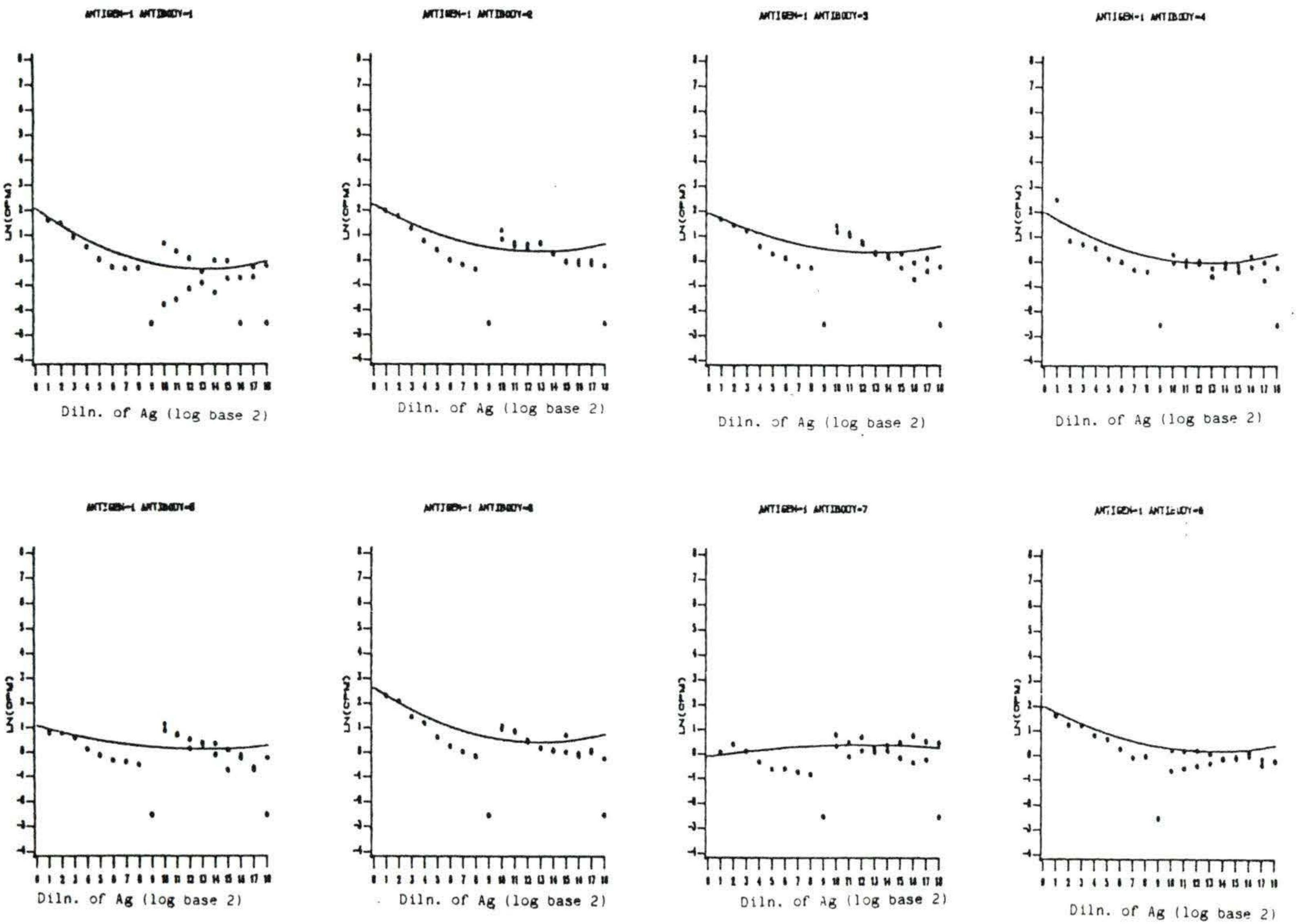

ANTIGEN=2 ANTIBODY=8
LN(CPM)
Diln. of Ag (log base 2)

Figure 18. Signature analysis of NeB2. Results are presented as the ln P/N ratio vs. the dilution of Ag4 (log base 2). McAb MBG-1 (1:200 dilution of ascites fluid, 50 ul/well) was used as the capture antibody. Serial dilutions of Zea mays L. (cv. Golden Bantam) sap infected with NeB2 (AG=4) was added to the wells (50 ul/well) of a remove-a-well Immulon II Microtiter plate. After incubation overnight at 4°C with gentle rocking, 8 McAbs (0.011 mg/ml 50 ul/well): MAG-1 (Ab=1), MAG-2 (Ab=2), MAG-3(Ab=3), MBG-1 (Ab=4), MBG-3 (Ab=5), MBG-4 (Ab=6), MBG-5 (Ab=7), and JEN-A (Ab=8) labelled with biotin, was added. After incubation, I^{125}-avidin was added to each well at 20,000 cpm. Wells were counted on a Tracor Analytic gamma counter for 2 minutes or to 10,000 cpm.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ANTIGEN=1 ANTIBODY=2
ANTIGEN=1 ANTIBODY=3
ANTIGEN=1 ANTIBODY=4
ANTIGEN=1 ANTIBODY=5
ANTIGEN=1 ANTIBODY=6
ANTIGEN=1 ANTIBODY=7
ANTIGEN=1 ANTIBODY=8
LN(CPM)
Diln. of Ag (log base 2)

DISCUSSION

Rabbits were immunized with purified MDMV-A or MDMV-B, members of the potyvirus group of plant viruses. Antisera were produced by the immunization schedule listed in Table 1. Rabbit serum samples were assayed throughout the immunization protocol for immunoreactivity with purified homologous or heterologous MDMV-A or MDMV-B; or host plant material infected with virus, by indirect ELISA (Table 2). During succeeding immunizations with increasing concentrations of antigen, the recognition of purified heterologous antigen increased. Potyviral capsid protein contains strain-specific, immunodominant sequences at the N-terminus, and strain-non-specific, homologous sequences in the core region (114). It is likely that, because antisera cross-reactivity to heterologous antigen increased as the immunization protocol proceeded, fewer immunodominant regions and therefore strain non-specific sequences of the capsid protein were recognized by the rabbit's immune system, resulting in increased recognition of the heterologous antigen. The data of Shukla et. al. (114) suggest that almost any member of the potyviruses could be used as an immunogen to generate strain non-specific, cross-reactive antisera, because all potyviruses examined contain strain non-specific regions on the coat protein. However, when the PcAb antisera made in this study were tested against potyvirus SMV no reaction was

observed (data not shown). Also, the same antisera did cross-react with potyviruses traditionally classified in SCMV subgroups (MDMV-A, MDMV-B, and MDMV-O), but that are now classified as different viruses. Therefore, the regions in the coat protein to which the antibodies reacted, must be directed at some strain-related sequence.

Immunization protocols and selection criteria for producing PcAb or McAb must be designed to produce antibodies to defined epitopes (116,117). The polyclonal antisera may have shown a broader spectrum of reactivity (i.e. recognized other potyviruses), if immunization time had been extended, the concentration of the immunizing antigen increased, or if the preparation of the immunizing antigen was unstable during storage. Even though the immunizing antigen was purified, the purification procedure may not have removed all proteases in the preparation; these could have caused denaturation of the tertiary structure of the virus coat protein, resulting in antibodies to other than those of a native conformation.

Serial dilutions of pooled PcAb were reacted with extracts of host plant tissues infected with MDMV-A, MDMV-B, Minn-11, OHIO-O, NeB2, or KS-1, and assayed by indirect ELISA. The object of this experiment was to demonstrate specificity of PcAb for the various virus strains. As the PcAb concentration was reduced, fewer antibodies were available to combine with virus, and the resulting signal

decreased. This suggests that the PcAb recognized the various strains. The P/N ratios with the various PcAb, shown in Figure 1, are not directly comparable because the initial concentrations of antigen were unknown.

OHIO-0 and KS-1 reacted poorly with PcAbs and McAbs (Table 3). These low P/N ratios may have been due to weak antibody binding because the conserved region of the capsid protein contained few epitopes shared with the other viruses. Alternatively, virus strains KS-1 and OHIO-O may not have been in sufficient concentration in infected host plant sap to generate large P/N ratios. OHIO-O is now classified as johnsongrass mosaic virus (JGMV) (108). OHIO-O has been demonstrated to have distinct serological properties by Lanham et. al. (65,66) and Shukla et. al. (107,108,110). Because of either this distinct serological property, the concentration, or some other factor which resulted in low P/N ratios, no conclusions could be made from these results.

P/N ratios (in Figure 2 and 3) differed when PcAbs reacted with purified virus, sap from virus-infected host plant material, or healthy plant material amended with 5 ug/ml purified MDMV. Healthy sap containing 5 ug/ml purified MDMV-A did not generate the same P/N ratio as purified MDMV-A at the same concentration. This has been demonstrated previously (57). A sap component may have inhibited the antibody-antigen reaction, or proteolytic activity may have

compromised the integrity of either the antibody or the antigen. Host plant material infected with virus generated a lower P/N ratio when reacted with PcAb than when PcAb was mixed with healthy plant sap to which antigen (5 ug/ml) was added. The antigen concentration of the host plant material infected with virus was unknown. Thus, the host plant material infected with virus may have contained less antigen than healthy plant sap to which a known amount of antigen (5 ug/ml) was added.

Dilutions of ascites fluid were the source of antibody shown in Figures 4 and 5. P/N ratios were compared for each antibody using purified MDMV-A (5 ug/ml, 50 ul/well) and MDMV-B (5 ug/ml, 50 ul/well). The P/N ratios for different antibodies demonstrated different reactivities to each virus isolate. Thus, the P/N ratios reflected the degree to which the McAb combined with the antigen. In general, antibodies generated against MDMV-A recognized both purified MDMV-A and MDMV-B to a greater extent (as shown by P/N ratios) than those generated against MDMV-B. Because strain-specific sequences are thought to be located at the N-terminal, immunodominant regions of the coat protein, it is not unreasonable to expect that McAbs would bind to that region. The data obtained by using both the PcAb (Table 2) and the McAb (Figures 4 and 5) suggest that animals immunized with MDMV-A generated fewer strain-specific antibodies than those

generated by animals immunized with MDMV-B. The affinity of a McAb for its epitope, or the frequency of an epitope for the antibody, cannot be ascertained from these data. The P/N ratios may have differed because of different antibody concentrations in the different ascites fluids, or because the affinities of the McAbs for the antigen differed.

PcAb contains multiple antibodies to multiple epitopes. Figures 5a and 5b suggest that little difference existed between the PcAb and McAb when used as the capture system for antigen, although some McAbs displayed a decreased P/N ratio when the antigen was captured by PcAb. Hill et. al. (43) suggested that decreases in McAb signals, when captured with PcAb, were because both the PcAbs and McAbs were specific for the same or similar epitopes. Thus, the PcAb may mask the existence of a McAb's epitope. Also, if there is a decreased signal, the epitope to which the McAb is specific is probably present in limited numbers. It appears that both the PcAb and McAb were both directed against conserved, strain non-specific epitopes (Figures 4 and 5 and Table 2).

The P/N ratios of some of the McAbs in Figure 5a, where the antigen was directly bound to the plate, were higher than those depicted in Figure 5b, where antigen was captured onto the plate with bound PcAb. Also, some McAbs responded better to antigen captured by PcAb rather than bound directly to the plate. Therefore, in the optimal double sandwich system the

capture antibody could be either a PcAb or McAb. Nevertheless, the capture antibody should be an antibody with a unique epitope so that McAbs which recognize dissimilar epitopes, could then combine. Table 3 shows those McAbs that either react with (+) or do not react with (-) the six MDMV isolates. OHIO-0 and KS-1 did not generate a response with most of the McAbs, but it is not known whether the low response was due to lack of recognition or too low concentrations of virus. If the lack of signal was because the antibody did not recognize the strain's epitopes, then these strains would have generated useful information in the signature analysis. However, inasmuch as the PcAb responded poorly to these strains, there was no obvious control to which the McAb's response could be compared. Thus, these two strains were not chosen for the signature analysis.

Antibody concentrations in flask media and ascites fluid varied greatly (Figure 6); however, flask media contained less antibody than ascites fluid. Bayer et. al. (6) reported that the protein concentrations of ascites fluid varied from 2 to 20 mg/ml. The antibody containing ascites fluid in this experiment varied in protein concentration from 0.68 mg/ml to 7.552 mg/ml.

The results of the competition assays also demonstrated recognition of antigens by the McAbs. JEN-A was used as the labelled McAb to demonstrate competition with itself (as a

control); 11 different McAbs were used in the consecutive and simultaneous competition ELISAs. Purified MDMV-A (0.5 ug/ml) was chosen as the antigen. True competition occurs when an antibody combines with a competing antibody's epitope. However, if a competing antibody binds in close proximity, resulting in a physical obstruction and/or stearic hindrance, or a conformational change in the antigen, binding of the labelled antibody may be reduced or excluded. Also, an antigen may oscillate between two or more conformations (94). Thus, if an antibody is specific for antigen conformation #1, it may not bind to the antigen conformation #2. In the case of this panel of antibodies, competition did occur between JEN-A and MBG-6 in the simultaneous assay (Figure 8). No competition was observed between JEN-A and MBG-6 in the consecutive assay (Figure 11). MBG-6 appeared to have some affinity for the epitope, as evidenced by no competition occurring in the consecutive assay, but JEN-A had a greater affinity than that of MBG-6, because when placed simultaneously in the well, JEN-A binds. Competing antibody may have been able to overcome stearic hindrance or bind to existing similar epitopes in the consecutive assay because more time was available to replace one equilibrium of antibody-antigen interaction with another. If an unlabelled antibody combined with MDMV-A with a greater affinity than the labelled JEN-A antibody, a percent maximum absorbance

greater than 100% would be generated; this occurred in these competition experiments.

The optimal dilution of MBG-1 used as a capture McAb for the RIA was approximately 3 $\log_{10}$ lower than that of the optimal capture PcAb in the ELISA system. PcAb sera had a higher antibody protein concentration than that of MBG-1. Polyclonal antisera contain antibodies specific for many different epitopes, whereas MBG-1 ascites fluid contained antibody specific for only one epitope. Therefore, the PcAb sera contains antibody for multiple epitopes on the capsid protein, which may result in a higher P/N ratio than that obtained for MBG-1.

The dilutions of labelled antibody are listed in Table 5 and graphically shown in Figure 14. To label an antibody with biotin, biotin is covalently coupled to amino groups in an antibody molecule. Up to eight biotin groups can be coupled to an antibody molecule without substantial inactivation (94). Low initial protein concentrations (MAG-1 contained 0.0807 mg/ml) resulted in low dilution factors (the maximum P/N ratio occurred at a 1:2 dilution). High protein concentrations (JEN-A contained 1.09 mg/ml) resulted in a high dilution factor (the maximum P/N ratio occurred at a 1:500 dilution, data not shown). The difference in the signal generated by these antibodies may also have been due to either labelling efficiency or antibody affinity. Each

McAb was screened for signal ability with purified MDMV-A, and may not have a high affinity for that antigen. To select the standard protein concentration for all signal antibodies to be used in signature analysis, antibody with the highest protein concentration at the maximum P/N of the dilution curve (MAG-3) was used to determine the standard concentration.

MDMV-A (Ag1), MDMV-B (Ag2), Minn-11 (Ag3), and NeB2 (Ag4) were the four viruses analyzed by signature analysis. Eight McAbs were used to simultaneously generate binding profiles for each antigen. The collective binding profiles, or "signatures", represent the panel of McAb's relative affinity for a particular epitope and the frequency of that epitope. A signature is generated by plotting the Ln P/N ratio on the Y axis, against the dilution of antigen (log base 2) on the X axis. A statistical program (iterative least squares) aligns the unknown antigen concentration of each strain to all the antibody responses at this concentration. Therefore, it is possible to collectively compare the antibody binding profiles of each strain of virus and to align different replications of antigen at diverse concentrations to each other. Different binding profiles generated by the same antibody to each viral capsid protein reflect the relative affinity of that antibody for an epitope on the antigen and the relative frequency at which the

epitope occurs. The signatures of each virus were transferred onto transparencies, and coincidence of signatures were observed by placing one transparency over the other and physically aligning the transparencies along the X axis. Statistical quantitation of differences between the binding profiles was not generated by the set of programs used in this study. The signatures were assessed visually to determine whether they were coincident.

A comparison of the signatures of the four MDMV viruses revealed that each virus possess epitopes on their capsids that reacted with all 8 different McAbs, because P/N ratios were greater than zero for the 8 McAbs. As mentioned above, purified virus of other strains were not available. Therefore, the low response of the McAbs to virus in plant sap could not be attributed to either lack of recognizable epitopes or the lack of the appropriate concentration of virus in plant sap. If experiments would have been performed with a positive control of purified virus, the results might have indicated that these antibodies were, in fact, strain non-specific. This would have permitted the use of additional strain-specific antibodies, resulting in more complete, differential signatures.

At low dilutions of healthy plant material containing no virus (negative sap), unexpectedly high readings were observed (data not shown). The controls indicated that

iodinated-avidin bound non-specifically to one or more healthy plant sap components (perhaps endogenous biotin), but not to the plastic of the Microtiter plate. The advantage of working with iodinated avidin, used in this study, was that the hazard associated with ^{125}I-avidin is less than that of having to label immunoglobulin with free ^{125}I. Direct labelling of immunoglobulin requires the use of high levels of radioacitivity. In addition, the use of labelled avidin avoids decreases in antibody activity associated with direct iodination, and labelled avidin can be used with different classes of antibody. However, direct labelling of antibody with ^{125}-I would possibly decrease the non-specific binding to host plant material that I encountered, and therefore reduce the background.

The inherent problem with the binding profiles generated in this experiment lies in the selection of antibody. The McAb's selected for signature analysis cross-reacted with each virus. Thus, there was no strain-specific epitope, which would have been useful in clarifying strain-relatedness. These antibodies would only be useful to demonstrate that a virus isolate was a strain of SCMV, JGMV, or MDMV, but does little to decipher unique capsid epitopic composition.

Using signature analysis, the curves generated by the panel of McAbs were aligned by the computer program to an

antigen dilution of 2^{18}. The dilution series of Ab1 (MAG-1) in trial 1 appeared to increase instead of decrease. The signature program aligned the concentrations of the different virus preparations to compensate for this unusual curve, which then stretched the dilution series out to 2^{18}. The nature of the signature program was such that had the data been equal to the other two trials, the alignment of virus concentration would have occurred with less shifting in the right axis, resulting in less stretched, more definitive curves.

The curves generated by signature analysis may represent the linear portions of sigmoidal curves. However, the virus antigen concentrations may have been too low or too high to truly reflect the epitopic uniquenesses or similarities. The data generated by these antibodies suggest that each epitope was present in similar relative frequencies and that each antibody had similar relative affinities to each epitope.

The data generated in this research suggest that Ag1 (MDMV-A) and Ag3 (Minn-11) could both be strains of MDMV. This is supported by the fact that Minn-11 can infect Johnsongrass (53). Precautions, such as washing hands between the different virus rooms in the greenhouse, were taken so that there were no mixtures of virus during propagation. Thus, the similarity that exists between Ag1 and Ag3 is a real reflection of epitopic similarity. The

binding curves of Ab7 (MBG-5), and Ab1 (MAG-1) demonstrate the only readily observed differences between these strains in this study. For Ag1 and Ag3, each antibody generated curves within the same Ln P/N (0.1-2.0) ratio, suggesting similar antigenic composition. Because a statistical program is not yet available to evaluate differences between the signatures, it is not known whether the differences in the curves generated by antibodies 2, 3, 4, 5, and 8 are significant.

Based on the curves generated and presented in figures 15-18, it appears that both Ag2 (MDMV-B) and Ag4 (NeB2) are strains of SCMV. Although the binding curves of Ab2 (MAG-2), Ab5 (MBG-3), Ab6 (MBG-4), and Ab8 (JEN-A) appeared visually different, the natural log of the P/N ratios were in the same range (0.1-3.0). However, when Ag1 and Ag3 are compared with Ag2 and Ag4, the P/N ratios differ greatly, and the curves for each set of viruses are not similar. The curves generated by this panel of McAbs appear to suggest that the viruses tested are all related, but that Ag1 and Ag3 are strains of one virus and Ag2 and Ag4 are strains of another virus. This supports the data generated by analyzing serological cross reactivity of cytoplasmic inclusion bodies, where each of the isolates contained cytoplasmic inclusion bodies which did not cross react with each other (53).

Further experiments must be performed using other

corn- or johnsongrass-infecting viruses. However, the data generated in this study reinforce the classification scheme proposed by Shukla et. al. (115).

MBG-1 was used as the capture antibody in the signatures. If epitopes recognized by MBG-1 were limited to a small portion of the repeating protein subunit of the capsid protein, then MBG-1 would not have been able to be used as a signal antibody. However, MBG-1 generated a signal at least as well as the other signal antibodies, implying that the epitope was not limited to a small portion of the capsid surface.

The potential exists for signature analysis to be able to elucidate subtle antigenic differences between strains of certain viruses. Wands et. al. (130) showed that such differences can be detected in hepatitis B surface antigen. Although the data in these experiments are limited, it suggests that strain relatedness has been established. Additional experiments must be done to overcome the lack or over-abundance of antigen in the infected plant sap (as evidenced by low P/N ratios). The usefulness of the signature analysis program is limited only by the presence of antigen in such overwhelming or small amounts that a curve is not generated (as in Ag3). It is necessary, therefore, to overcome antigen concentration limitations. If the antigen concentration is too low, a method of increasing the

concentration of antigen in infected sap must be found. The volume of sap containing antigen added to each well could be increased, or the number of times a plate was coated with infected sap could be increased. However, both methods were unsuccessfully attempted in this research (data not shown).

A method must be found to block the binding of iodinated avidin to the healthy sap components. The use of purified antigen is an option which was not explored in this study because purification procedures may alter the antigenic integrity of a virus. Also, the antigen must be in sufficiently high concentration for purification, which it was not. Performing a semi-purification of sap via several ultracentrifugation steps may help reduce the high background. The easiest way to overcome excessively high concentrations of antigen in plant sap is to dilute the sap to an even greater extent than 1:250 or start with a higher dilution of sap followed by serial dilutions. If that is not effective, then the application of a smaller volume of the infected sap to the plate may be an alternative.

The signal generated by the McAbs must be increased even further. Directly iodinating the antibody may increase the signal by eliminating the non-specific binding that occurs with iodinated avidin. Signal would also increase if the system could be amplified in some way (e.g., use carbon polymers when labelling the antibody with biotin, to

eliminate stearic hindrance and make the biotin more accessible to the avidin) or use higher affinity antibodies.

Cell fusions performed in this research resulted in hybridomas that were characterized in a preliminary manner (see Appendix 1). Those hybridomas could be cloned and the resulting antibodies could then be epitopically defined by competition ELISAs. Some of these antibodies might be against strain-specific epitopes because the strain-specific N-terminus is immunodominant. If so, these antibodies could be used for signature analysis to further elucidate strain differences. Shukla et. al. (116) developed a rapid screening assay which identified virus specific PcAbs. He incorporated the use of electro-blot immunoassays where PcAb which are directed to the N-terminus region of the coat protein recognized the native protein. The same PcAb did not recognize the core protein with the N-terminus removed. The immunoassay would be very helpful when screening antibodies for their use in signature analysis.

The potential exists for detection of subtle antigenic differences between strains of virus by signature analysis. With an appropriate panel of antibodies, the relatedness of unknown field isolates could be defined. The antigenic drift of a given virus strain could be traced by the use of this technique; perhaps even its geographic origin could be determined. Virus/vector specificity studies may be enhanced

in that strain definition could be more precise. Further clarification of strain relatedness would also be useful in developing resistant strains of maize, sorghum, or sugarcane.

In conclusion, the data generated in this study support the reclassification of MDMV-A as member of the MDMV group of potyviruses and MDMV-B as a member of the SCMV group of viruses, as proposed by Shukla et. al. (109). Isolates NeB2 and Minn-11 can also be defined as a member of the SCMV group of viruses, and a member of JGMV group, respectively, based on the signatures generated in this study.

LITERATURE CITED

1. Alexander, J.D. and R. W. Toler. 1985. A technique for the differentiation of strains of individual maize dwarf mosaic virus particles in a mixed infection. Abstract no. 363. Phytopathology 75:1323.

2. Alexander, J.D. and R.W. Toler. 1986. Immuno-gold decoration as a technique for measuring serological relationships among strains of maize dwarf mosaic virus and sugarcane mosaic virus. Abstract no. 592. Phytopathology 76:1131.

3. Allison, R.F., R.E. Johnston, and W.G. Dougherty. 1986. The nucleotide sequence of the coding region of tobacco etch virus genomic RNA: Evidence for the synthesis of a single polyprotein. Virology 154:9-20.

4. Ball, E.M. and M.K. Brakke. 1968. Leaf-dip serology of electron microscopic identification of plant viruses. Virology 36:152-155.

5. Ball, E.M. 1974. Serological tests for the identification of plant viruses. The American Phytopathological Society, Inc. St. Paul, Minn. 31 pp.

6. Bayer, E.A., E. Skutolsky and M. Wilchek. 1979. The avidin-biotin complex in affinity cytochemistry. In: Methods in Enzymology, Academic Press, New York. 62:308-326.

7. Berger, D.H., L.L. Domier, G.M. Hellmann, Y. Stram, D.W. Thornbury, and T.P. Pirone. 1989. Expression in transgenic plants of a virus gene product that mediates insect transmission of potyviruses. Proc. Natl. Acad. Sci. U.S.A. 86:8402-8406.

8. Berzofsky, J.A. and A.N. Schecter. 1981. The concepts of cross-reactivity and specificity in immunology. Molecular Immuno. 18:751-763.

9. Bol, J.F. and J. Van Kan. 1988. The synthesis and possible functions of virus-induced protein in plants. Microbiol. Sci. 5:47-52.

10. Bond, W.P. and T.P. Pirone. 1970. Evidence for soil transmission of sugarcane mosaic virus. Phytopathology 60:437-440.

11. Brakke, M.K., E.M. Ball, Y.H. Hsu, and W.G. Langenberg. 1987. Wheat streak mosaic virus cylindrical inclusion body protein. J. Gen. Virol. 68:281-287.

12. Briand, J.P, Z.A. Moudallal and M.H.V. Van Regenmortel. 1982. Serological differentiation of tobamoviruses by means of monoclonal antibodies. J. Virol. Methods 5:293-301.

13. Carey, E.E. 1986. Influence of maize dwarf mosaic virus on the sugar and sorbitol contents of sweet corn kernels. Plant Disease 70:36-37.

14. Carrington, J.C. and W.G. Dougherty. 1987. Small nuclear inclusion protein encoded by plant potyvirus genome is a protease. J. Virol. 61:2540-2548.

15. Chen, L.C., D.P. Durand and J.H. Hill. 1982. Detection of pathogenic strains of soybean mosaic virus by ELISA with polystyrene plates and beads as the solid phase. Phytopathology 72:1177-1181.

16. Choe, C.W., W.S. Gardner and D.J. Gallenberg. 1986. Cytological responses of sorghum (Sorghum bicolor Moench) to infection with maize dwarf mosaic virus. Abstract no. 509. Phytopathology 76:1121.

17. Citovsky, V., D. Knorr, G. Schuster and P. Zombryski. 1990. The P30 movement protein of tobacco mosaic virus is a single-strand nucleic acid binding protein. Cell 60:637-647.

18. Clark, M.F. and A.N. Adams. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.

19. Dale, J.L. 1964. Isolation of a mechanically transmissible virus from corn in Arkansas. Plant Disease Reporter 48:661-663.

20. Dale, J.L. and L. Anzalone, Jr. 1965. Infection of sugarcane with a mechanically transmissable corn virus. Plant Disease Reporter 49:757-760.

21. Daniels, N.E., and R.W. Toler. 1969. Transmission of maize dwarf mosaic by the greenbug, Schizaphis graminum. Plant Disease Reporter 53:59-61.

22. Derrick, K.S. 1973. Detection and identification of plant viruses by serologically specific electron microscopy. Abstract. Phytopathology 63:441.

23. Derrick, K.S. 1973. Quantitative assay for plant viruses using serologically specific electron microscopy. Virology 56:652-653.

24. Dietzgen, R.G. and R.I.B. Francki. 1987. Nonspecific binding of immunoglobulin to coat proteins of certain plant viruses in immunoblots and indirect ELISA. J. Virol. Meth. 15:159-164.

25. Dougherty, W.G. and E. Hiebert. 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.

26. Dougherty, W.G., L. Willis, and R.E. Johnston. 1985. Topographic analysis of tobacco etch virus capsid protein epitopes. Virology 144:66-72.

27. Dougherty, W.G. and J.C. Carrington. 1988. Expression and function of potyviral gene products. Ann. Rev. Phytopathology 26:123-143.

28. Falk, B.W., J.H. Tsai and S.A. Lommel. 1987. Differences in levels of detection of the maize stripe virus capsid and major non-capsid protein in plant and insect hosts. J. Gen. Virol. 68:1801-1811.

29. Findley, W.R., R. Lowe, J.K. Knoke and E.J. Dollinger. 1977. Breeding corn for resistance to virus in Ohio. In: Proceedings of the International Colloquium and Workshop. Ohio ARDC, Wooster, OH. 145 pp.

30. Frenkel, M.J., C.W. Ward and D.D. Shukla. 1989. The use of 3′ non-coding nucleotide sequences in the taxonomy of potyviruses: application to watermelon mosaic virus 2 and soybean mosaic virus-N. J. Gen. Virol. 70:2775-2783.

31. Ghabrial, S. A. and R. J. Shepherd. 1980. A sensitive radioimmunosorbant assay for the detection of plant viruses. J. Gen. Virol. 48:311-317.

32. Gillaspie, A.G. and V.L. Frampton. 1970. Amino acid analysis of five strains of sugarcane mosaic virus. Abstract no. 1. Phytopathology 60:1072.

33. Gillaspie, A.G., R.G. Mock and S.S. Hearon. 1984. Status of the identification of sugarcane mosaic strains worldwide. Maize Virus Diseases Newsletter 1:66 pp.

34. Giorda, L.M. and R. W. Toler. 1986. The use of virus concentration, symptomatology, and yield as a measure of resistance to maize dwarf mosaic virus A in different sorghum cultivars. Abstract no. 125. Phytopathology 76:1072.

35. Goldberg, K.B. and M.K. Brakke. 1985. Concentration of maize chlorotic mottle virus and maize dwarf mosaic virus in corn lethal necrosis. Abstract no. 624. Phytopathology 75:1357.

36. Gordon, D.J. and L.E. Williams. 1970. The relationship of a maize virus isolate from Ohio to sugarcane mosaic virus strains and the B strain of maize dwarf mosaic virus. Abstract no. 2. Phytopathology 60:24.

37. Gough, K.H., A.A. Azad, P.J. Hanna and D.D. Shukla. 1987. Nucleotide sequence of the capsid and nuclear inclusion protein genes from johnsongrass strain of sugarcane mosaic virus ribonucleic aced. J. Gen. Virol. 68: 297-304.

38. Gregory, L.V. and J.E. Ayers. 1982. Effect of inoculation with maize dwarf mosaic virus at several growth stages on yield of sweet corn. Plant Disease Reporter 66:801-804.

39. Gugerli, P. and P. Fries. 1983. Characterization of monoclonal antibodies to potato virus Y and their use for virus detection. J. Gen. Virol. 64:2471-2477.

40. Hamilton, R.I., J.R. Edwardson, R.I.B. Francki, H.T. Hsu, R. Hull, R. Koenig and R.G. Milne. 1981. Guidelines for the identification and characterization of plant viruses. J. Gen. Virol. 54:223-241.

41. Hammond J. and R.H. Lawson. 1985. The use of antisera against cylindrical inclusion proteins to detect and identify potyviruses. Abstract no. 593. Phytopathology 75:24.

42. Hibi, T. and Y. Saito. 1985. A dot immunobinding assay for the detection of tobacco mosaic virus in infected tissues. J. Gen. Virol. 66:1191-1194.

43. Hill, E.K., J.H. Hill and D.P. Durand. 1984. Production of monoclonal antibodies to viruses in the potyvirus group: use in radioimmunoassay. J. Gen. Virol. 65: 525-532.

44. Hill, J. H., G. R. Bryant and D.P. Durand. 1981. Detection of plant viruses by using purified IgG in ELISA. J. Virol. Meth. 3:27-35.

45. Hill, J.H., R.E. Ford and H.I. Benner. 1973. Purification and partial characterization of maize dwarf mosaic virus strain B (sugarcane mosaic virus). J. Gen. Virol. 20:327-339.

46. Hill, J.H., C.A. Martinson and W.A. Russell. 1974. Seed transmission of maize dwarf mosaic and wheat streak mosaic viruses in maize and response of inbred lines. Crop Science 14:232-235.

47. Hill, J.H. 1990. Competitive binding between monoclonal antibodies to define epitope relationships or characterize immunoglobulin idiotypes. In: Serological methods for detection and identification of viral and bacterial plant pathogens. ed. R. Hampton, E. Ball, S. De Boer, APS press, St. Paul, MN. 24 pp.

48. Hollings, M. and A.A. Brunt. 1981. Potyviruses. In: Handbook of plant virus infections and comparative diagnosis. ed. E. Kurstak. New York. Elsevier/ New Holland Biomedical. 443 pp.

49. Humaydan, H.S. 1979. Studies on seed transmission of maize dwarf mosaic virus in sweet corn, sorghum, and annual grass hosts. Abstract no. 322 In: Proceedings of the Ninth International Congress of Plant Protection, Washington D.C., unpaged.

50. Janson, B.F. and C.W. Ellett. 1963. A new corn disease in Ohio. Plant Disease Reporter 47:1107-1108.

51. Jarjees, M.M. and J.K. Uyemoto. 1983. MDMV: effect of time of inoculation and symptomatology on performance of sorghum (Sorghum bicolor). Plant Disease 67:488-489.

52. Jensen, S.G., D.J. Andrews and N.E. DeVries. 1983. Reactions of pearl millet germplasm from the world detection to maize dwarf mosaic virus strains A and B. Plant Disease 67:1105-1108.

53. Jensen, S.G., B. Long-Davidson and L. Seip. 1986. Size variation among proteins induced by the sugarcane mosaic viruses in plant tissue. Phytopathology 76:528-532.

54. Jensen, S.G., M.K. Palomar, E.M. Ball and R. Samson. 1985. Factors influencing virus titer in maize dwarf mosaic virus-infected sorghum. Phytopathology 75:1132-1136.

55. Jensen, S.G., N. Van Pelt, and E.M. Ball. 1986. Serological relationships between inclusion proteins of several strains of maize dwarf mosaic virus and sugarcane mosaic virus. Abstract no. 596. Phytopathology 76:1131.

56. Jensen, S.G., N. Van Pelt and M.K. Palomar. 1985. The interaction of host and virus strain on the stability of inclusions of maize dwarf mosaic virus. Abstract no. 121. Phytopathology 75:1292.

57. Jones, F.E. 1986. Development and use of a monoclonal antibody-based enzyme immunoassay for the detection of maize dwarf mosaic virus. Thesis, M.S. Iowa State University. 1986. pp. 132.

58. Jones, F.E., J. H. Hill and D.P. Durand. 1988. Detection and differentiation of maize dwarf mosaic virus strains A and B, by the use of different class immunoglobulins in a double antibody sandwich enzyme-linked immunosorbent assay. Phytopathology 78:1118-1124.

59. Josephson, L.M., J.U. Hilty, J.M. Arnold, H.C. Kinar and J.R. Overton. 1969. Grain yield of corn reduced by maize dwarf mosaic virus infection. J. of Agron. 64:645-647.

60. Kennel, S. J. 1982. Binding of monoclonal antibodies to protein antigen in fluid phase or bound to solid supports. J. Immuno. Methods 55:1-12.

61. Kingsland, G. C. 1980. Effect of maize dwarf mosaic virus infection on yield and stalk strength of corn in the field in Southern Carolina. Plant Disease 64:271-273.

62. Knoke, J.K., L.R. Madden, and D.T. Gordon. 1983. Spread of maize dwarf mosaic virus from johnsongrass to corn. Plant Disease 67:367-370.

63. Koenig, R. 1978. ELISA in the study of homologous and heterologous reactions of plant viruses. J. Gen. Virol. 40:309-318.

64. Langenberg, W. G. 1973. Serology, physical properties, and purification of unaggregated infectious maize dwarf mosaic virus. Phytopathology 63:149-154.

65. Langham, M.A. 1986. An analysis of the strain relationships of maize dwarf mosaic virus. Thesis, PhD. Texas A & M University. pp 113.

66. Langham, M.A. and R. W. Toler. 1986. An analysis of maize dwarf mosaic virus strains by immunobinding assay. Abstract no. 604. Phytopathology 76:1132.

67. Langham, M.A., R.W. Toler, and L.W. Giorola. 1985. Detection of maize dwarf mosaic virus by immuno-blot. Abstract no. 626. Phytopathology 75:1357.

68. Lei, J.D. and G.N Agrios. 1986. Detection of virus infection and spread by immunofluorescent staining of enzyme treated leaves. Phytopathology 76:1031-1033.

69. Lei, I.D. and G.N. Agrios. 1986. Mechanisms of resistance in corn to maize dwarf mosaic virus. Phytopathology 76:1034-1040.

70. Lindsey, D.W. and R.T. Gudauskas. 1975. Effects of maize dwarf mosaic virus on water relations in corn. Phytopathology 65:434-440.

71. Lommel, S.A., A.H. McCain and T.J. Morris. 1982. Evaluation of indirect ELISA for the detection of plant viruses. Phytopathology 72:1018-1022.

72. Louie, R. 1986. Effects of genotype and inoculation protocols on resistance evaluation of maize to maize dwarf mosaic virus strains. Phytopathology 76:769-773.

73. Louie, R. and J.K. Knoke. 1975. Strains of maize dwarf mosaic virus. Plant Disease Report 59:518-522.

74. Mackenzie, D.R., C.C. Wernham and R.E. Ford. 1966. Differences in maize dwarf mosaic virus isolates of the Northeastern U.S. Plant Disease Report 50:814-818.

75. Matthews, R.E.F. 1981. Plant Virology. Academic Press, Inc. New York. pp. 274.

76. McDaniel, L.L. and D.T. Gordon. 1985. Identification of a new strain of maize dwarf mosaic virus. Plant Disease 69:602-607.

77. McMullen, M.D. and R. Louie. 1989. The linkage of molecular markers to a gene controlling the symptom response in maize to maize dwarf mosaic virus. Mol. Plant-Microbe Interact. 2:309-314.

78. Mernaugh, R.L., W.S. Gardner and K. L. Yocom. 1980. Three-dimensional structure of pinwheel inclusions as determined by analytic geometry. Virology 106:273-281.

79. Mikel, M.A., C.J. D'Arcy, A.M. Rhodes and R.E. Ford. 1981. Yield response of sweet corn to maize dwarf mosaic virus. Plant Disease 65:900-901.

80. Mikel, M.A., C.J. D'Arcy, A.M. Rhodes and R.E. Ford. 1982. Effect of maize dwarf mosaic virus infection on sweet corn pollen and silk. Phytopathology 72:428-431.

81. Moghal, S.M. and R.I.B. Francki. 1976. Towards a system for the identification and classification of potyviruses. 1. Serology and amino acid composition of six distinct viruses. Virology 73:350-362.

82. Moghal, S.M. and R.I.B. Francki. 1981. Towards a system for the identification and classification of potyviruses. II. Virus particle length, symptomatology, and cytopathology of six distinct viruses. Virology 112:210-216.

83. Monath, T.P., J.R. Wands, L.J. Hill, N.V. Brown, R.A. Marciniak, M.A. Wong, M.K. Gentry, D.S. Burke, J.A. Grant and D.W. Trent. 1986. Geographic classification of Dengue-2 virus strains by antigen signature analysis. Virology 154:313-324.

84. Nagaraj, A.N. 1965. Fluorescent antibody staining of Viral antigen in dissociated plant cells. Virology 18:329-331.

85. Ouchterlony, O. 1962. Diffusion-in-gel methods for immunological analysis II. Progr. Allergy 6:30-154.

86. Pirone, T.P. 1972. Sugarcane mosaic virus. CMI/AAB Descriptions of plant viruses. No.88

87. Presley, D.M., R.G. Henzell, R.S. Greber, D.S. Teakle and R. W. Toler. 1985. Use of a set of differential sorghum inbred lines to compare isolates of sugarcane mosaic virus from sorghum and maize in nine countries. Plant Disease 69:1046-1049.

88. Presley, D.M., R.F. Moore, and D.S. Fletcher. 1977. The inheritance of the red leaf reaction of grain sorghum to SCMV infection. Aust. J. Agric. Res. 28:853-858.

89. Purcifull, D.E. 1966. Some properties of tobacco etch virus and its alkaline degradation products. Virology 29:8-14.

90. Regenmortel, M.H.V. 1963. Serologically related plant contaminants in preparations of partially purified plant viruses. Virology 21:657-658.

91. Rest, E.B. and W.E. Splittsloesser. 1985. Three new aphid vectors of maize dwarf mosaic virus. Plant Disease 69:1100.

92. Roane, C.W., S.A. Tolin, H.S. Aycock, and P.J. Donahue. 1989. Association of Rmd1, a gene conditioning reaction to maize dwarf mosaic virus with genes conditioning endosperm color (y1) and type (su2) in maize. Phytopathology 79:1362-1368.

93. Roane, C.W., S.A. Tolin, and H.S. Aycock. 1989. Genetics of the reaction to maize dwarf mosaic virus A in several maize inbred lines. Phtyopathology 79:1364-1368.

94. Roitt, I., J. Brostoff, and D. Male. 1985. Immunology. C.V. Mosby Co. St. Louis. 251 pp.

95. Romaine, C.P., S.R. Newhart, and D. Arizola. 1981. Enzyme-linked immunosorbent assay for plant viruses in intact leaf tissue disks. Phytopathology 71:308-312.

96. Rosenkranz, E. and G.E. Scott. 1978. Effect of plant age at time of inoculation with maize dwarf mosaic virus on disease development and yield in corn. Phytopathology 68:1688-1692.

97. Rosenkranz, E. and G.E. Scott. 1984. Determination of the number of genes for resistance to maize dwarf mosaic virus strain A in five corn inbred lines. Phytopathology 74: 71-76.

98. Rosenkranz, E. 1978. Grasses native of adventive to the U.S. as new hosts of maize dwarf mosaic and sugarcane mosaic virus. Phytopathology 68:175-179.

99. Rosenkranz, E. 1980. Taxonomic distribution of native Mississippi grass species susceptible to maize dwarf mosaic and sugarcane mosaic viruses. Phytopathology 70:1056-1061.

100. Rosenkranz, E. 1981. Host range of maize dwarf mosaic virus. 152-162. In: Virus and Virus-like Diseases of Maize in the U.S. D. T. Gordon, J.K. Knoke, and G.E. Scott, eds. Southern Coop. Ser. Bull. 247. 210 pp. 24.

101. Rosenkranz, E. 1983. Susceptibility of representative native Mississippi grasses in six subfamilies to maize dwarf mosaic virus A and B and sugarcane mosaic virus B. Phytopathology 73:1314-1321.

102. Saito, Y. and Y. Iwata. 1973. Quantitative assay for plant viruses using serologically specific electron microscopy. Virology 56:652-633.

103. Schmaljohn, A.L., K.M. Kukuban and G.A. Cole. 1983. Protective monoclonal antibodies define maturational and pH dependent antigenic changes in the Sindbis virus EL glycoproteins. Virology 130: 144-154.

104. Scott, G.E. and E. Rosenkranz. 1981. Effectiveness of resistance to maize dwarf mosaic and maize chlorotic dwarf viruses in maize. Phytopathology 71:937-941.

105. Seifers, D.L. 1984. Optimum conditions for studies of maize dwarf mosaic virus strains A and B in sorghum. Plant Disease 68:1067-1069.

106. Shephard, R.J. 1965. Properties of a mosaic virus of corn and johnson grass and its relation to the sugarcane mosaic virus. Phytopathology 55:1250-1256.

107. Shukla, D.D., A.S. Inglis, N.M. McKern and K.H. Gough. 1986. Coat protein of potyviruses. 2. Amino acid sequence of coat protein of potato virus Y. Virology 152:118-125.

108. Shukla, D.D., K.H. Gough and C.W. Ward. 1987. Coat proteins of potyviruses. 3. Comparison of amino acid sequences of the coat protein of four Australian strains of sugarcane mosaic virus. Arch. Virol. 96:59-74.

109. Shukla, D.D., and C.W. Ward. 1988. Amino acid sequence homology of coat proteins as a basis for identification and classification of the potyvirus group. J. Gen. Virol. 69:2703-2710.

110. Shukla, D.D., N. M. Mckern, K.H. Gough, S.L. Tracy and S.G.Letho. 1988. Differentiation of potyviruses and their strains by high performance liquid chromatographic peptide profiling of coat proteins. J. Gen. Virol. 69:493-502.

111. Shukla, D.D., P.M. Strike, S.L. Tracy, K.H. Gough and C.W. Ward. 1988. The N and C termini of the coat proteins of potyviruses are surface located and the N terminus contains the major virus-specific epitopes. J. Gen. Virol. 69:1497-1508.

112. Shukla, D.D., J.E. Thomas, N.M. McKern, S.L. Tracy and C.W. Ward. 1988. Coat protein of potyviruses. 4. Comparison of biological properties, serological relationships, and coat protein amino acid sequences of four strains of potato virus Y. Arch. Virol. 102:207-219.

113. Shukla, D.D., G. Tribbick, T.J. Mason, D.R. Hewish, H.M. Geysen and C.W. Ward. 1989. Localization of virus-specific and group-specific epitopes of plant Potyviruses by systematic immunochemical analysis of overlapping peptide fragments. Proc. Natl. Acad. Sci. U. S. A. 86:8192-8196.

114. Shukla, D.D. and C.W. Ward. 1989. Identification and classification of potyviruses on the basis of coat protein sequence data and serology. Arch. Virol. 106:171-200.

115. Shukla, D.D. and C.W. Ward. 1989. Structure of potyvirus coat proteins and its application in the taxonomy of the potyvirus group. Adv. Virus Res. 36: 273-314.

116. Shukla, D.D., J. Jilka, M. Tosic and R.E. Ford. 1989. A novel approach to the serology of potyviruses involving affinity-purified polyclonal antibodies directed towards virus-specific N-termini of coat proteins. J. Gen. Virol. 70:13-23.

117. Shukla, D.D., Tosic, M., Ford, R.E., Jilka, J., Toler, R.W. and M.A.C. Langham. 1989. Taxonomy of potyviruses infecting maize, sorghum, and sugarcane in Australia and the United States as determined by reactivities of polyclonal antibodies directed towards virus-specific N-termini of coat proteins. Phytopathology 79:223-229.

118. Snazelle, T.E., J.B. Bancroft and A.J. Ullstrup. 1971. Purification and serology of maize dwarf mosaic and sugarcane mosaic viruses. Phytopathology 61:1059-1063.

119. Steward, M.W. and A.M. Low. 1985. The importance of antibody affinity in the performance of immunoassays for antibody. J. Immunol. Meth. 78:173-190.

120. Stryer, J.M. and T.E. Snazelle. 1989. Purification and serology of maize dwarf mosaic virus. Phytopathology 58:24-26.

121. Teakle, D.S., R.F. Moore, D.L. George and D.E. Byth. 1970. Inheritance of the necrotic and mosaic reactions in sorghum infected with a johnsongrass strain of sugarcane mosaic virus. Aust. J. Agric. Res. 21:549-556.

122. Toler, R.W. 1985. Maize dwarf mosaic, the most important virus disease of sorghum. Plant Disease 69:1011-1015.

123. Tosic, M. and R.E. Ford. 1972. Grasses differentiating sugarcane mosaic virus and maize dwarf virus. Phytopathology 62:1466-1470.

124. Tosic, M. and R.E. Ford. 1974. Physical and serological properties of maize dwarf mosaic and sugarcane mosaic virus. Phytopathology 64:312-317.

125. Towbin, H. and J. Gordon. 1984. Review Article. Immuno-blotting and dot immunobinding--- current status and outlook. J. Immunol. Meth. 72:313-340.

126. Van Deusen, R. A. 1983. Applying monoclonal antibodies to the diagnosis of animal diseases. Plant and Animal Dis. 270-274.

127. Van Deusen, R. A. and C. A. Whetstone. 1981. Practical aspects of producing and using anti-viral monoclonal antibodies as diagnostic reagents. Annu. Proc. Amer. Assn. Vet. Lab. Diag. 24:211-228.

128. Van Slogteren, D.H.M. 1955. Serological microreactions with plant viruses under paraffin oil. pages 50-55 In: Proceedings of the second conference on potato virus diseases. Lisse, Washington.

129. Voller, A., A. Bartlett, D.E. Bidwell, M.F. Clark and A.N. Adams. 1976. The detection of viruses by ELISA. J. Gen. Virol. 33:165-167.

130. Wands, J.R., M.A. Wong, J. Shorey, R. D. Brown, R.A. Marciniak and K.J. Isselbacher. 1984. Use of monoclonal antibodies in the study of viral hepatitis. Proc. Nat. Acad. Sci. U.S.A 81:2237-2241.

131. Wang, A.W., J.R. Wands, R. Marcinial, R.D. Brown and J. Shorey. 1983. An exploratory analysis of radioimmunoassays data: establishing subtypes of the Hepatitis-B virus. J. Amer. Stat. Assoc.

132. Williams, L.E. and L. J. Alexander. 1965. Maize dwarf mosaic--- a new corn disease. Phytopathology 55:802-804.

133. Yu, M.H., M.J. Frenkel, N.M. McKern, D.D. Shukla, P.M. Strike and C.W. Ward. 1989. Coat protein of potyviruses 6. Amino acid sequences suggest watermelon mosaic virus and soybean mosaic virus-N are strains of the same potyvirus. Arch. Virol. 105:55-64.

134. Zummo, N. and D.T. Gordon. 1971. Comparative study of five mosaic virus isolates infecting corn, johnsonsgrass, and sorghum in the U.S. Phytopathology 61:389-394.

ACKNOWLEDGEMENTS

I would like to thank my husband, Michael, for all his love, encouragement, and support throughout my program of study. Additional thanks to my family members who stood by my decision with undying loyalty. I would also like to thank the Department of Microbiology, the many professors and graduate students, too numerous to name individually, but especially that of my major professor Dr. Donald Durand, and my committee members, Dr. Paul Hartman, Dr. John Hill, Dr. Robert Andrews, and Dr. Richard Van Deusen. A very special thanks to Dr. Van Deusen for his continued faith, enthusiasm, support and guidance, throughout my graduate experience.

APPENDIX 1

The following are results generated by hybridomas immunized and created as per the methods described in materials and methods. MDMV-B (5 ug) was used as the immunogen in the mouse. MDMV-A or MDMV-B (5 ug/ml, 50 ul/well) were directly adsorbed onto the wells of a Microtiter plate. Plate media was used as the antibody source. Anti-mouse IgG conjugated to alkaline phosphatase was used as the signal.

Name of Ab	MDMV-A	MDMV-B	Name of Ab	MDMV-A	MDMV-B
51-E	+	+	1-5D	-	+
52-C	-	+	1-5G	-	+
54-D	-	+	1-6B	+	+
54-G	-	+	1-6G	-	+
55-E	-	+	1-7B	-	+
56-C	+	+	1-7C	-	+
510-C	-	+	1-7D	+	+
510-B	-	+	1-7E	-	+
510-F	-	+	17-F	+	+
4-10	-	+	1-8B	+	+
4-11C	-	+	1-11E	-	+
4-2c	-	+	1-11G	-	+
4-3G	+	+	3-9G	-	+
4-4C	-	+	3-2C	-	+
4-5F	-	+	3-2D	-	+
4-6	-	+	3-5B	-	+
4-6D	-	+	3-8B	-	+
4-6E	+	+	3-8E	+	+
4-6G	+	+	2-2B	-	+
4-7G	-	+	2-2C	+	+
4-8G	-	+	2-4D	+	+
4-8F	-	+	2-5D	-	+
4-9B	+	+	2-5F	+	+
1-2	-	+	2-6D	-	+
1-E	+	+	2-9B	-	+
1-3C	-	+	2-7D	+	+
2-7E	-	+	2-7G	-	+
2-8E	-	+	2-8E	-	+
2-8F	-	+	2-9C	-	+
2-9G	+	+	2-10C	-	+
2-10E	+	+	2-10F	-	+