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STUDIES ON BEAN MILD MOSAIC VIRUS, A NEW VIRUS OF BEAN IN MICHIGAN

by

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ABSTRACT

STUDIES ON BEAN MILD MOSAIC VIRUS,
A NEW VIRUS OF BEAN IN MICHIGAN

by

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Bean mild mosaic virus (BMMV), a spherical RNA virus of 28 nm diameter, was found for the first time affecting beans (*Phaseolus vulgaris* L.) in Michigan. The virus was identified by the immunosorbent electron microscopy (ISEM), gel double-diffusion and double antibody sandwich ELISA (DAS-ELISA) tests. Infected plants showed mosaic, mottling, vein banding, curling of the leaves,or no symptoms at all.

The virus has very narrow host range which includes, soybean (*Glycine max* L. (Merr), pea (*Pisum sativum* L.) and bean (*Phaseolus vulgaris* L.). All eighteen bean cultivars inoculated in this study were susceptible.

Bean mild mosaic virus was seed transmitted in three bean cultivars with a range of 3.3 to 5.0%. Seed infection was also demonstrated in Michigan grown bean seed lots.

Several bean cultivars exhibited more severe symptoms when BMMV and bean yellow mosaic virus (BYMV) were inoculated together compared with single infection by either viruses. This is the first report of BMMV affecting beans in Michigan and the United States.

To my parents
for their love and support

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important food crop in many parts of the world, and is widely cultivated as a protein source. Beans are particularly important as a food crop in eastern Africa, Latin America and some Asian regions.

Numerous diseases caused by different pathogens can affect bean crops and reduce yields. Virus diseases are among the most important, affecting beans worldwide. At least 70 different viruses have been reported to infect beans (Morales, 1986). Some of these viruses are of economic importance because of a direct influence on yield, others are considered as potentially damaging pathogens (Morales, 1986).

Bean common mosaic virus (BCMV) and bean yellow mosaic virus (BYMV) are included among the most economically important viruses worldwide. Other viruses have been reported to be found in mixed infections with these or other bean viruses (Waterworth, 1981).

Bean mild mosaic virus (BMMV) has been reported in bean from Central and South America and causes mild mosaic symptoms; BMMV can also produce a synergistic effect and cause severe mosaic symptoms when occurred in combination with other viruses, such as bean curly dwarf mosaic virus or cowpea mosaic virus (Waterworth _et al_, 1977). In mixed infections, BMMV could easily be overlooked because of the mild symptoms it usually produces (Waterworth _et al_, 1977). Since BMMV is a highly stable virus producing mild foliar symptoms and is seed transmitted, it could easily become distributed over most bean producing regions of the world.

The objectives of the present study were to determine the identity of a contaminant virus in greenhouse bean plantings, determine its host range, seed transmissibility, distribution in Michigan bean production areas and determine the possible synergistic interactions with bean yellow mosaic virus in different bean cultivars.

LITERATURE REVIEW

Identification of plant viruses

Various procedures are useful for identification of plant viruses, and include such things as variation in symptoms, host range, methods of transmission, physical properties, interactions with other viruses, serological tests, electron microscopy and chemical composition. Each of these procedures provides information to determine the identity of a specific virus (Walkey, 1985).

Host range studies reveal the infection capacity of the virus. Some viruses, including BYMV, have very large host ranges which may include several species and genera; other viruses have a very narrow host range, and are usually restricted to certain species.

Serological methods have been used extensively in viral characterization and determining relationships among viruses. The great value of antigen-antibody reactions is due to the specificity of reaction; an antibody will combine only with an antigen which contains groupings of amino acid sequences similar to those causing antibody formation in an animal (Ball, 1974).

The introduction of agar diffusion techniques, immunosorbent electron microscopy (ISEM) (Derrick, 1973) and enzyme linked immunosorbent assay (ELISA) (Clark and

Adams, 1977) has allowed routine testing for a number of viruses. Frequently a combination of these three techniques are used for virus identification (Ball, 1974). Agar diffusion techniques allow visual determination of the virus as a precipitation line. There are two kinds of diffusion techniques, single and double, in which one or both reactants diffuse through a liquid phase in the gel (Crowle, 1973).

The agar-gel double diffusion test (Ouchterlony test) was extensively used in the past, but has been replaced by more precise tests which require very small volumes of antiserum. Nonetheless, Ouchterlony tests are still used because of easy set up (Crowle, 1973) and because a visible antibody-antigen reaction is obtained (Van Regenmortel, 1982).

The recently developed ELISA is extremely sensitive and is increasingly used to detect viruses that occur in low concentrations (Clark and Adams, 1977). A virus-specific conjugate of antibody enzyme is used to indicate virus presence by the appearance of coloration of a substrate added at the end of the test. The enzyme substrate is hydrolyzed and a bright yellow color indicates a positive reaction. Frequently, a spectrophotometer is used to quantify color as absorbance at wavelength 405 nm (Clark, 1981). The double antibody sandwich ELISA (DAS-ELISA), as developed by Clark and Adams (1977) has proven to be an economical, quick and sensitive assay that requires nominal basic laboratory skills.

Enzyme linked immunosorbent assay (ELISA) has been used successfully by plant pathologists for diagnosis of virus diseases of perennial and vegetatively propagated crops such as trees and ornamentals; the test is also used to detect virus in seed (Clark, 1981; Clark and Bar-Joseph, 1984).

Immunosorbent electron microscopy (ISEM) permits visualization of virus particles which are "trapped" by the antiserum. The use of ISEM can increase by 2 - 10,000 times the number of virus particles observed per grid as compared with other techniques not using antiserum as a trapping agent. The virus is visualized by its morphology in the electron microscope. Some advantages of ISEM are that a very small amount of antiserum is used, and results are rapidly obtained. Some of the disadvantages are that ISEM involves costly equipment, is labor intensive, requires skills with the electron microscope, and is not suitable for handling a large number of samples (Milne and Lesemann, 1984).

<u>Bean mild mosaic virus</u> (BMMV)

Bean mild mosaic virus was first reported in 1977 by Waterworth <u>et al</u> as occurring in a mixed infection with curly dwarf mosaic virus in plants of several bean cultivars (<u>Phaseolus vulgaris</u> L.) obtained from El Salvador, Central America. The virus was isolated from bean plants showing symptoms resembling those caused by bean common mosaic virus or bean golden mosaic virus. Plants also exhibited mosaic, leaf rugosity, curling and extreme dwarfing. The virus has recently been reported to induce mild mosaic symptoms in beans in Colombia (Jayasinghe, 1982).

Waterworth et al (1977) and Jayasinghe (1982) indicate that BMMV has a very narrow host range which includes mainly legume species. Symptoms in bean (Phaseolus vulgaris L.) can vary from symptomless infection to barely discernible mild mosaic. Slight vein banding or roughening of the leaf surface has also been observed.

The virus is easily transmitted mechanically and usually 100% of infection is achieved. Chrysomelid beetles mainly Diabrotica sp., Ceratoma sp., Epilachma sp. and Gynandrobrotica sp. are efficient vectors of BMMV (Hobbs, 1981; Jayasinghe, 1982). No transmission has been demonstrated by other insects such as leafhopper, aphids and mites (Jayasinghe, 1982).

Seed transmission has been found to occur in a range of 1 - 4% depending on the bean cultivar (Jayasinghe, 1982).

Bean mild mosaic virus is composed of spherical particles containing RNA, 28 nm in diameter, sedimenting as a single component. It is a very stable virus in sap with a thermal inactivation point of 84^{o}C, dilution end-point around 10^{-8} and a longevity in vitro of 6 weeks at 22^{o}C

(Waterworth, 1981). Waterworth (1981) also stated that BMMV is not serologically related to 35 other spherical particle viruses, including 10 viruses usually associated with legumes.

The virus is also extremely infectious in legumes and spreads rapidly among beans in the greenhouse, often without inciting symptoms. Virus transmission was readily demonstrated by root contact, when healthy plants were grown in "infested soil" from which most of the roots of infected plants were not removed or when healthy plants were grown in "non-cleansed" infested pots (Hampton and Hancock, 1981).

Bean mild mosaic virus can occur in mixed infections with other viruses, such as bean curly dwarf mosaic virus, bean common mosaic virus, peanut stunt virus, cowpea mosaic virus and bean rugose mosaic virus (Waterworth, 1977; Hampton and Hancock, 1981).

Little is known about the economic loss in beans due to infection with BMMV. Though infected plants grew normally under greenhouse conditions, and produced healthy-looking pods, flowering and pod formation were delayed about a week (Jayasinghe, 1982). Serology has been a reliable and easy method for detecting BMMV in the field (Jayasinghe, 1982).

According to Morales (1986), BMMV is likely to be widely distributed throughout the bean-producing regions of the world because of its high infectivity, efficient vector transmission and seed transmissibility.

Seed transmission of plant viruses

Seed transmission is one of the most important means of inoculum dispersal for many plant pathogens. Seed dispersal also has the disadvantage of allowing pathogen survival for long periods, as well as allowing spread of the pathogen from one location to another. The intimate association between host and virus in the seed gives a high opportunity for infection to occur at early stages of plant development.

Only a few plant viruses have been reported to be seedborne in beans, namely bean common mosaic virus (BCMV), southern bean mosaic virus (SBMV) cucumber mosaic virus (CMV) and bean mild mosaic virus (BMMV) (Morales, 1986).

There are two means of seed transmission of viruses, in or on the seed coat, and in the embryo of the seed, the later commonly referred to "true seed transmission" (Walkey, 1985). Most viruses are found in the embryo. Bean common mosaic virus (BCMV) is a good example of true seed transmission. It has been detected internally in cotyledons and embryos but not from seed coats on beans (Ekpo and Saettler, 1974). Other viruses such as SBMV are transmitted both on the seed coat, as a contaminant, and internally in the embryo (Uyemoto and Grogan, 1977).

The incidence of seed infection with viruses varies greatly with the virus and with the host species infected.

Up to 100% of transmission has been found in seeds of individual soybean plants infected with tobacco ring spot virus (Baker and Smith, 1966). Seed transmission of BCMV can vary between 10 to 83% (Phatak, 1974). Other viruses such as BMMV have been shown to be transmitted in ranges of 1.2 to 3.6% in different Phaseolus vulgaris L. cultivars. This percentage of seed transmission was considered low compared with other beetle transmitted virus in legumes, like cowpea mosaic virus which has 10% seed transmission in cowpea Vigna unguiculata L.(Jayasinghe, 1982).

Percentage of seed transmission is influenced by stage of plant development at the time of infection, differences in strain and cultivars and environmental conditions. The rate of seed transmission may also be influenced by the presence of other viruses in mixed infection (Allen, 1983). Kuhn and Dawson (1973) found that the incidence of seed-borne southern bean mosaic virus (SBMV) was 20% in the presence of cowpea chlorotic mottle virus (CCMV) as compared with only 12% in single viral infections.

Studies with BCMV showed different levels of seed infection depending on age of host at inoculation; the highest incidence of seed transmission occurred when plants were infected at early stages (Schippers, 1963). Morales and Castano (1987) found maximum seed transmission of BCMV in plants inoculated at the primary leaf stage. Little or no infection was found on plants infected after blossoming.

Even though the optimum stage of plant development for seed infection by most viruses is prior to flowering, not all seeds of infected plants carry the virus. This is probably due to the fact that not all mega- and microspores are invaded by the virus (Shippers, 1963; Crowley, 1957).

The irregular distribution of infected seeds within a pod has also been mentioned by several researchers. Nelson (1932), suggested that viruses are restricted to certain tissues in the plant.

Seed transmission is one of the most important means of survival for some viruses, especially those with a narrow host range such as BCMV and BMMV.

Mixed infection by plant viruses

Mixed plant virus infections are common in nature (Uyemoto et al, 1981). Many crops can be infected by more than one virus at the same time, as pointed out by Rochow (1972) who reported three different viruses simultaneously infecting sugar beet.

Competition between mixtures of virus strains has been observed in plant protoplasts infected with variants of cowpea chlorotic mottle virus and raspberry ringspot virus (Carr and Kim, 1983).

Plant diseases caused by double infection with two unrelated viruses may cause more severe and different symptoms than the symptoms caused by each individual virus. The virus concentrations may also be significantly altered compared to those in singly infected plants (Kassanis, 1963).

Variations of virus host range and breakdown of vector specificity can also occur as a result of mixed virus infections. Several newly discovered plant diseases have been found to be caused by virus mixtures (Clark et al, 1980; Uyemoto et al, 1981; Khan and Demski, 1982).

Bean yellow mosaic virus has been found together in beans infected with cowpea mosaic virus (CPMV) or cowpea severe mosaic virus (CPSMV) (Carr and Kim, 1983). Another example of double infection is found in soybean affected by soybean mosaic virus (SoyMV) and bean pod mottle virus (BPMV). Foliage distortion, chlorotic mottling, stunting, misshapen fruit, and necrosis have been observed in soybean fields doubly infected with both viruses; only mild mosaic symptoms are found in plants infected with the individual viruses (Lee and Ross, 1972).

MATERIALS AND METHODS

Identification of bean mild mosaic virus (BMMV) as a contaminant virus in bean yellow mosaic virus (BYMV) studies.

A host range study, to differentiate several BYMV strains included *Glycine max* L.(Merr), *Lens culinaris* Medik., *Nicotiana glutinosa* L., *N. rustica* L., *N. tabacum* L., *Phaseolus vulgaris* L., *Pisum sativum* L., *Vigna unguiculata* L., *Trifolium incarnatum* L., *Trifolium pratense* L. and *Vicia faba* L. During this study some bean and pea cultivars previously reported resistant to BYMV (severe strain) (Herrera and Sepulveda, 1986) developed mosaic symptoms. At the same time control plants inoculated with just buffer showed curling of the leaves, mosaic and mottling symptoms. These plants, as well as the resistant cultivars, were used to isolate and identify the contaminant virus.

Mechanical transmission

Control (non-inoculated) plants of Domino bean cultivar showing mosaic symptoms were used to isolate the virus. Leaf sap was prepared from these plants by grinding leaves showing symptoms 1:10 (w/v) in a cold mortar and pestle with 0.01 M phosphate buffer, pH 7.0. The primary leaves of 7-day-old bean plants were rub-inoculated with the sap solution using a sterile foam rubber sponge. The leaves were dusted with 300 mesh carborundum prior to inoculation. Plants were then maintained under greenhouse conditions with a temperature range of 25° to 35°C.

Electron microscopy

A negative staining procedure was used to examine plant tissue for virus particles with transmission electron microscopy. Leaf sap dips of bean leaves exhibiting mild mosaic symptoms were prepared by chopping a 1 cm square of infected leaf in a 30 ul drop of 2% ammonium molybdate on a glass slide. A drop of the suspension was placed on carbon-coated Parlodion-filmed grids for 1 minute. Grids were then blotted with filter paper and examined in a Philips 201 transmission electron microscope operated at 60 KV.

Serology

Identity of the virus was determined using gel double diffusion tests in agarose gel containing of 0.8% (w/v) agarose with 0.1% (w/v) NaN_3 and 0.85% (w/v) NaCl. Antisera against the following viruses were used in these tests: bean mild mosaic virus (BMMV), bean pod mottle virus (BPMV), black gram mottle virus (BGMV), cowpea chlorotic mottle virus (CCMV), cowpea mosaic virus (CPMV), cowpea severe mosaic virus (CPSMV) and southern bean mosaic virus (SBMV). Antisera to BMMV and SBMV were kindly provided by Dr. Francisco Morales, CIAT (Colombia) and the remainder by

Dr. Rose Gergerich, University of Arkansas. The different antisera were diluted 1:2 and 1:4 (v/v) in 0.85% NaCl solution (normal saline).

Virus-containing sap was obtained from non-inoculated Domino bean plants showing mild mosaic symptoms. One gram of infected leaves was ground with a sterile mortar and pestle in 10 ml of 0.01 M phosphate buffer and aliquots placed in the different wells. Positive controls consisted of sap from infected leaves with the known virus pathogen, while negative controls consisted of sap from healthy bean leaves.

Immunosorbent electron microscopy (ISEM)

Antiserum for BMMV was obtained from Dr. Francisco Morales (CIAT). A further modification of the Derrick technique (1973) as modified by Milne and Luisoni (1977) and Haufler and Fulbright (1983) was used for ISEM. Freshly prepared carbon-coated Parlodion-filmed 300 mesh grids were floated on 30 ul of a 1:500 dilution of BMMV-antiserum in 0.06 M Na_2HPO_4 - KH_2PO_4 buffer at pH 7.0 (ISEM buffer) to coat grids with specific antiserum. Drops of antiserum were placed on parafilm placed in a petri dish containing moistened filter paper. Grids were incubated at 37^{o}C for 3 hrs, then rinsed twice for 10 min in ISEM buffer. Grids were then briefly blotted with filter paper and floated in 30 ul drops of plant extract obtained by grinding 0.5 - 1 gr of infected bean leaves in 3 - 5 ml of ISEM buffer. Grids were incubated overnight at 4^{o}C. After incubation grids were briefly blotted with filter paper and negatively stained with 2% ammonium molybdate, pH 7.0. To enhance visualization of virus particles, most grids were coated a second time (decorated) with antiserum. For decoration, grids incubated overnight were blotted with filter paper and then floated on a 30 ul drop of a 1:500 dilution of antiserum for 1 - 3 hrs at 4^{o}C. Grids were then blotted with filter paper and negatively stained with 2% ammonium molybdate.

All grids were examined in a Philips 201 transmission electron microscope (TEM) operated at 60 KV.

Mechanical inoculation and host range

Virus inoculum was prepared by grinding systemically infected *Phaseolus vulgaris* L. 'Black Turtle Soup' leaves 1:10 (w/v) with cold 0.01 M phosphate buffer, pH 7.0 in a cold sterilized mortar. Sap was rub inoculated with sterile foam rubber sponges onto leaves previously dusted with 300 mesh carborundum powder. Control plants were inoculated with buffer instead of sap. The host range included the following species: *Chenopodium amaranticolor* Coste & Reyn, *Chenopodium quinoa* Willd., *Glycine max* (L.) Merr cultivars Corsoy, Hobbit and Hodgson 78, *Gomphrena globosa* L., *Lens culinaris* Medik. cultivars Araucana INIA and Tekoa, *Nicotiana glutinosa* L., *N. rustica* L., *N. tabacum* L. cultivar Xanthi, *Phaseolus lunatus* L. cultivar

Henderson Bush, _Pisum sativum_ L. cultivars Alaska, Burpee Sugar Snap and Early Perfection, _Trifolium incarnatum_ L., _Trifolium pratense_ L., _Vicia faba_ L. cultivar Minor and _Vigna unguiculata_ L. cultivar Blackeye. The following _Phaseolus vulgaris_ L. cultivars were also used: Amanda, Black Turtle Soup, Blanco INIA, Blue Lake Stringless, common cranberry bean (cultivar not known), Domino, Great Northern 31, Great Northern 123, Isabella, Michelite 62, Montcalm, Olathe, Orfeo INIA, Pinto UI 114, Redkloud, Stringless Green Refugee, Top Crop and Tortola INIA. All plants were grown from seeds in 15 cm diameter sterile clay pots with greenhouse soil. With small seeded plants such as clover or _Nicotiana_ sp., the seeds were sown in a nursery bed and the seedlings were transplanted into pots when plants were 2 cm in height. A minimum of 10 plants were inoculated for each species or cultivar and 5 plants were used as controls. The plants were kept for 15 - 20 days under greenhouse conditions with temperature range of 25^{o} - 35^{o}C.

The presence of the virus was confirmed by use of double antibody sandwich ELISA (DAS-ELISA).

Double antibody sandwich ELISA (DAS-ELISA).

The DAS-ELISA procedure described by Clark and Adams (1977) was followed. All ELISA solutions or plant sap were added to the ELISA plates at 200 ul/well and then sealed in plastic bags during incubation to prevent evaporation.

Purified IgG, diluted to a concentration of 1 ug/ml (with 0.05 M sodium carbonate buffer at pH 9.6, coating buffer), was added to a Immulon R 1, 96-well flat bottom polystyrene ELISA plates (Dynatech Laboratories, Inc, Chantilly, VA 22021). Plates were incubated at 37°C for 4 hr and then rinsed three times in phosphate-buffered saline (0.02 M phosphate buffer plus 0.15 M NaCl at pH 7.4 containing 0.05% Tween 20 (PBS-Tween)). Bean leaf extracts were prepared by grinding leaf tissue 1:10 (w/v) with an electric tissue grinder (Tissumizer, Tekmar R Model No. SDT 1810) in extraction buffer. Extracts were added to the plates in duplicate and plates incubated overnight at 4°C. The plates were again rinsed with PBS-Tween as above except several rinses were used as needed to completely remove all traces of plant material from the wells. Enzyme conjugated IgG was diluted 1:800 with PBS-Tween containing 2% (w/v) polyvinylpyrrolidone (PVP) and 0.2 % (w/v) egg albumin (extraction buffer), and added to the ELISA plates. After 4 hr incubation at 37°C, the plates were rinsed 3 times in PBS-Tween as above. The substrate p-nitrophenyl phosphate was diluted to 1 mg/ml in 0.97% (v/v) diethanolamine substrate buffer, pH 9.8, and added to the plate. The reaction was allowed to develop at room temperature for 15 to 35 min until negative controls (background) started to increase in absorbance. All plates were read in a Dynatech Microelisa R mini-reader MR 590 for absorbance at 405 nm. A sample was scored positive if the absorbance reading was twice that of the healthy controls.

Seed transmission of BMMV

To determine whether BMMV-Mich. is seed transmitted, 7-day-old bean plants of cultivars Orfeo-INIA, Black Turtle Soup and Domino were mechanically inoculated on the primary leaves with BMMV-Mich. Plants were grown to maturity in the greenhouse and seeds harvested. Periodic pesticide applications were used to control mites, white flies, thrips and powdery mildew.

The seeds from each cultivar were harvested and planted individually in 10 cm diameter sterile clay pots with greenhouse soil. Plants were observed for a period of 5 weeks after which symptoms were recorded and serology tests (DAS-ELISA) were conducted.

Detection of seed infection with BMMV in Michigan grown bean seeds.

Thirty lots of navy and black bean seeds were obtained from the Michigan Department of Agriculture and used to determine the presence of BMMV in seed.

One hundred seeds of each lot were planted into vermiculite contained in sterile aluminum trays and kept under greenhouse conditions for 5 weeks. Evaluation of seed transmission was made on the basis of symptomatology and electron microscopy (EM).

For electron microscopy observations, a bulk sample of leaves from the plants of each seed lot was used to prepared leaf dips which were negatively stained with 2% ammonium molybdate. Grids were observed in a Philips 201 transmission electron microscope at 60 KV.

To analyze the amount of seed transmission of BMMV, one seed lot was selected at random from those showing virus particles as determined from the EM study. One hundred seeds were planted in individual 10 cm diameter sterile clay pots containing greenhouse soil. Plants were kept under greenhouse conditions for 4 weeks, after which virus was assayed in individual plants by DAS-ELISA.

Distribution of BMMV and BYMV in Michigan dry bean fields

During the summer of 1988, a field survey was conducted in dry bean fields in the area of Munger, Saginaw County, and Tuscola county,Michigan. Plant samples were obtained from several bean cultivars located in separate fields. Samples were collected at random from plants showing mosaic symptoms, mottling, stunting.

In 1989 a second survey was performed in bean fields from 4 counties (Clinton, Saginaw, Tuscola and Gratiot). Samples were obtained at random and consisted of 5 - 20 leaves from individual plants at each site; leaves with and without symptoms were collected. A total of 30 samples were collected for the first year and 500 the second year.

Individual samples were analyzed by DAS-ELISA to detect the presence of BMMV and BYMV, using the methodology described previously. Table 1 summarizes the sites sampled in 1988 and 1989.

Synergistic effect of BMMV and BYMV in several bean cultivars

Plants of bean cultivar Black Turtle Soup, individualy infected with either BYMV (C-20 isolate) or BMMV-Mich. were used as sources of inoculum. Inoculum was prepared by grinding leaves systemically infected with either viruses in 0.01 M phosphate buffer, pH 7.0 (1:10 w/v) in a sterile chilled mortar. Possible synergism was studied by inoculating plants with a mixture of both viruses compared with each individual virus. Such inoculum was prepared by grinding equal amounts of infected leaves obtained from plants infected with either viruses in a concentration 1:10 (w/v) in 0.01 M phosphate buffer, pH 7.0 in a sterile chilled mortar.

Sap containing the individual viruses or the mixture was rub-inoculated with a sterile foam rubber sponge onto the primary leaves of 7-day-old bean seedlings. The leaves were previously dusted with 300 mesh carborundum. Ten plants for each bean cultivar were inoculated with the different viruses or the mixture and five plants for each cultivar were inoculated only with buffer as controls.

All plants were kept under greenhouse conditions for 15 - 20 days with a temperature range of 25^{o} - $35^{o}C$.

Sixteen of the eighteen bean cultivars mentioned in

Table 1 : Field survey for BMMV and BYMV in Bean Fields in Central Michigan, 1988 and 1989.

Date of sampling	County	Location	Bean Type or cultivar	Symptoms	Number of samples
9/12/88	Saginaw	MSU Research Plot	Breeding lines	* mos,y	5
9/12/88	Tuscola	Nolette Rd./ M-138	Navy	y	1
9/12/88	Saginaw	MSU Research Plot	Montcalm	mos	1
9/22/88	"	"	Cranberry	mos	1
9/12/88	Tuscola	Nolette Rd.	Navy	mos	5
9/22/88	"	M-138/Vassar Rd.	Navy	mos,mot	10
9/12/88	"	Russell Rd.	Cranberry	mos,mot	5
9/12/88	Tuscola	M-138/Nolette Rd.	Aurora	e,mos	2
7/31/89	Clinton	French Rd./Dewitt Rd.	Navy	ns	10
7/31/89	"	"	Cranberry	vc	15
7/31/89	"	Dewitt Rd./ Livingston Rd.	Navy	y	20
7/31/89	"	Kinley Rd./Essix Center Rd.	Cranberry	ns	20
8/15/89	Saginaw	MSU Research Plot	Seafarer	mos	10
8/15/89	"	"	N84004	mos	5
8/15/89	"	"	I89006	mos,mot	10
8/15/89	"	"	Fleetwood	nd	10
8/15/89	"	"	N85074	mos,nd	5
8/15/89	"	"	Pinto UI 114	mm	5
8/15/89	"	"	Sierra	mos	5

Table 1 : cont'd

Date of sampling	County	Location	Bean Type or cultivar	Symptoms	Number of samples
8/15/89	Saginaw	MSU Research Plot	Domino	mos	5
8/15/89	"	"	N89302	mos	5
8/15/89	Tuscola	Knight Rd.	C-20	ns	20
8/15/89	"	Wolf Rd/Hack Rd.	C-20	sm	1
8/15/89	"	M-81/Knight Rd.	Seafarer	ns,mos	20
8/15/89	"	Portmouth Rd.	Seafarer	mos	20
8/15/89	"	Indiantown	Bunsi	ns,mos	10
8/15/89	"	Uncle Henry/ Knight Rd	C-20	mm	5
8/15/89	"	4650 James Rd.	Mayflower	mm,ns	20
8/15/89	"	5000 James Rd.	C-20	mos	20
8/15/89	Saginaw	MSU Research Plot	N85033	mot	5
9/5/89	Tuscola	M-46 East	Navy	ns	20
9/5/89	"	Garner Rd./M-46	N84032	mos	10
9/5/89	"	"	Bunsi	ns,mos	10
9/5/89	"	"	N80100	le,mo	5
9/5/89	"	Garner Rd./M-46	B87004	mm	10
9/5/89	"	"	Mayflower	mos	10
9/5/89	"	"	Sierra	ns	10
9/5/89	"	"	Aurora	y	10
9/5/89	"	"	Midland	mo	10
9/5/89	"	Block Rd./M-81	Mayflower	ms	20
9/5/89	Gratiot	Crapo Rd./ Lincoln Rd.	Bunsi	mos	20

Table 1 : cont'd

Date of sampling	County	Location	Bean Type or cultivar	Symptoms	Number of samples
9/5/89	Gratiot	Crapo Rd.	Black Magic	mos	10
9/5/89	"	"	K86002	ns	10
9/5/89	"	"	C-20	ns	10
9/5/89	"	"	894T23	ns	10
9/5/89	"	"	N 1 906	mos	10
9/5/89	"	"	N84032	y	10
9/5/89	"	"	Mayflower	ns	10
9/5/89	Tuscola	Nolet Rd./ Kinney Rd.	Seafarer	ns	20
9/5/89	"	Bradleyville Rd.	Seafarer	ns	20
9/5/89	Saginaw	MSU Research Plot	Domino	mos	10
9/5/89	"	"	P86291	mos	10
				TOTAL	538

* e = epinasty; le = leaf elongation; mm = mild mosaic; mos = mosaic; mot = mottling; nd = nutrient deficiency; ns = no symptoms; sm = severe mosaic; vc = vein chlorosis and y = yellowing

the host range study, were used in this experiment to inoculate them with the different viruses or the mixture. Plants were observed up to 20 days after inoculation. Symptoms considered as a susceptible reaction included any degree of mosaic, leaf curling, stunting, epinasty or necrosis. All test plants were assayed for the presence of both viruses by DAS-ELISA.

RESULTS

Identification of bean mild mosaic virus (BMMV) as a contaminant virus in bean yellow mosaic virus (BYMV) studies.

Mechanical transmission.

A virus was successfully transmitted by mechanical inoculation to plants of the Domino bean cultivar. At 10 days after inoculation inoculated plants showed mild mosaic symptoms, mottling, vein banding or mild curling of the leaves (Figure 1 and 2).

Electron microscopy

Grids were observed at 45,000 and 70,000 X magnification and a large number of spherical particles which measured approximately 28 nm in diameter were found in leaf dips from infected Domino plants (Figure 3).

The size distribution of 100 virus particles is shown on a histogram, and the modal size for the particles was 28.5 nm (Figure 4).

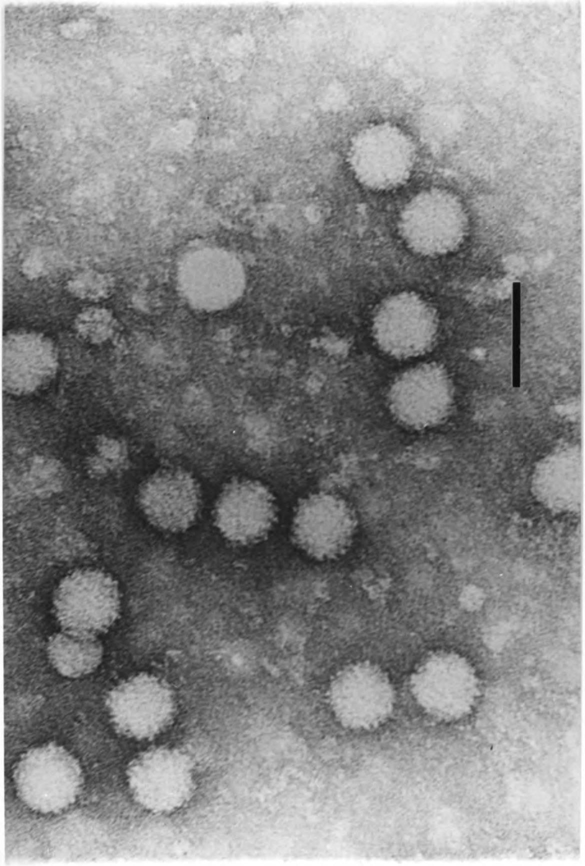
Serology

A positive reaction, consisting of a precipitin line, was only found between the BMMV-antiserum and the sap obtained from Domino infected plants (Figure 5). The clearest precipitin lines were observed at 1:4 dilution of

Figure 1 : Vein banding caused by BMMV-Mich. in bean cultivar Domino, 10 - 15 days after inoculation.

Figure 2 : Mottling caused by BMMV-Mich. in bean cultivar Domino, 10 - 15 days after inoculation.

Figure 3 : Transmission electron micrograph of bean mild mosaic virus-Mich. virus particles, negatively stained with 2% ammonium molybdate. Bar represents 50 nm.



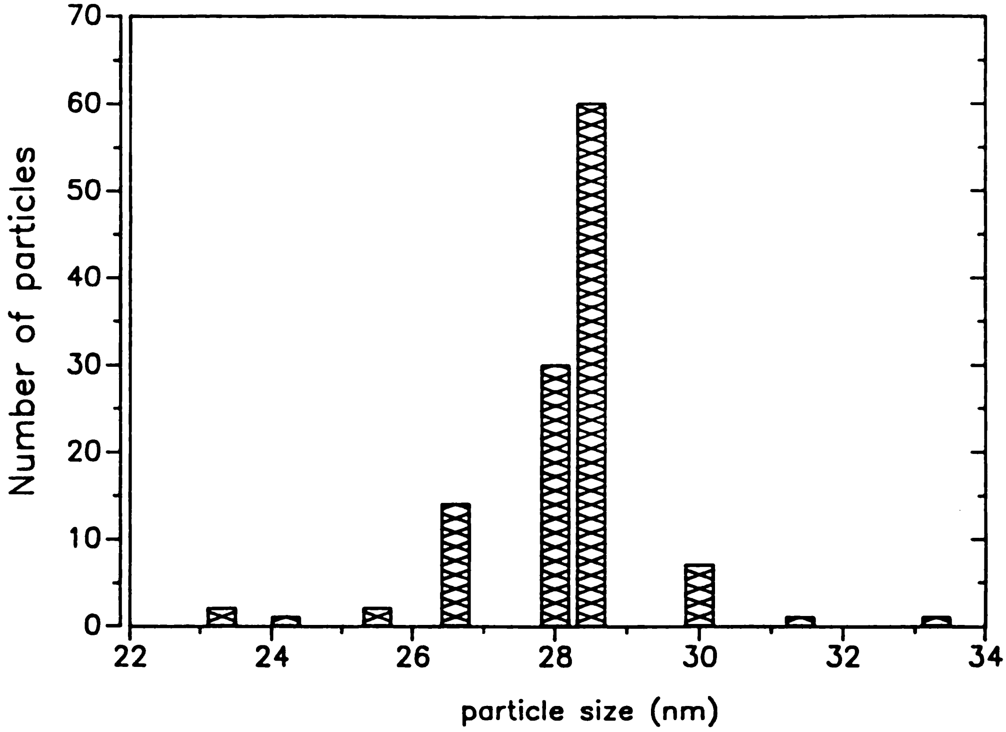


Figure 4. Distribution of particle size of BMMV in bean

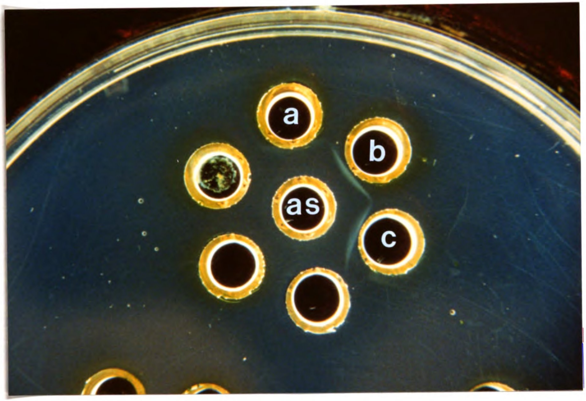


Figure 5 : Typical reactions of BMMV-Mich with homologous antiserum at a 1:4 dilution in agar double-diffusion tests.
Wells a : healthy Domino bean; b : positive control, bean inoculated with BMMV, c : Domino bean inoculated with BMMV-Mich.

the antiserum. No reactions were observed in tests with sap from healthy leaves. When sap containing BMMV-Mich. was placed in adjacent wells with sap from BMMV infected bean leaves, there was fusion of precipitating lines without spur formation, indicating that the two antigens were closely related if not identical.

Immunosorbent electron microscopy (ISEM).

The confirmation of BMMV as the causal agent of the symptoms observed on bean was by ISEM. A large number of particles were trapped by BMMV-antiserum. Decorated particles were easily and rapidly detected. Figure 6 shows a comparison between decorated and non-decorated particles of the virus. A distinctive dark halo is found in decorated particles, which corresponds to an additional amount of staining as a result of the virus-antiserum reaction.

Mechanical inoculation and host range.

The reactions of the different species to BMMV-Mich. are shown in Table 2. The results indicate that the virus has a very narrow host range. All bean cultivars were susceptible to the virus with a wide range of symptoms. Among other species, BMMV infected the three soybean cultivars (*Glycine max* (L.) Merr) and the Alaska pea cultivar (*Pisum sativum* L.). The symptoms in soybeans and beans included mosaic and curling of the leaves (Figures 7

Figure 6 : Immunosorbent electron microscopy of BMMV-Mich. virus particles negatively stained with 2% ammonium molybdate. A : decorated; B : non decorated particles. Bar represents 100 nm.

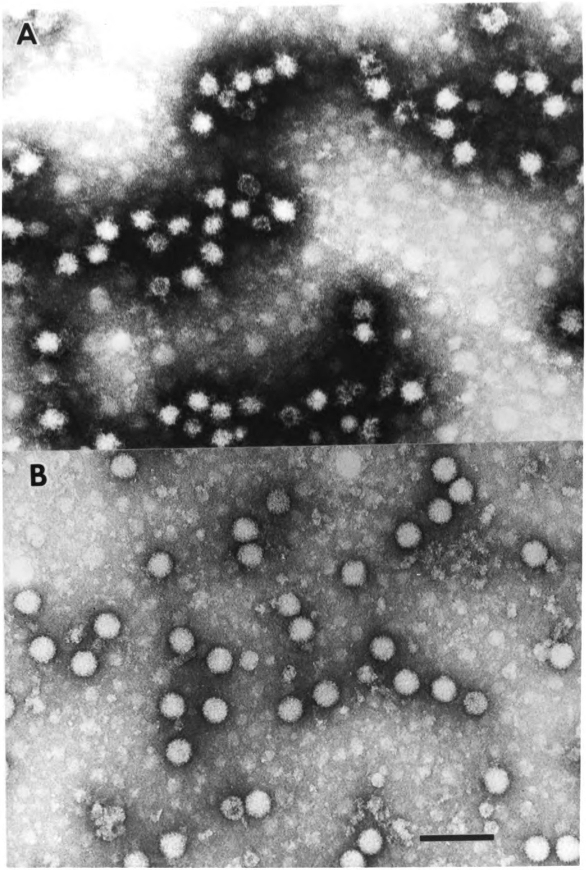

A
B

Table 2 : Symptoms in different species inoculated with BMMV-Mich.

SPECIE	COMMON NAME	SYMPTOMS
Chenopodium amaranticolor Coste & Reyn.		- *
Chenopodium quinoa Willd.		-
Glycine max L. Merr	soybean	
Corsoy		mos, mot
Hobbit		mos, mot
Hodgson 78		mos, mot
Gomphrena globosa L.		-
Lens culinaris Medik.	lentil	
Araucana INIA		-
Tekoa		-
Nicotiana glutinosa L.		-
Nicotiana rustica L.		-
Nicotiana tabacum L.	tobacco	-
Phaseolus lunatus L.	lima bean	
Henderson Bush		-
Pisum sativum L.	pea	
Alaska		latent
Burpee Sugar Snap		-
Early Perfection		-
Trifolium incarnatum L.	crimson clover	-
Trifolium pratense L.	red clover	-
Vicia faba L.	faba bean	
Minor		-
Vigna unguiculata L.	cowpea	
Blackeye		-

Table 2 : cont'd.

SPECIE	COMMON NAME	SYMPTOMS
Phaseolus vulgaris L.	common bean	
Amanda		mm
Black Turtle Soup		mod mos,vb
Blanco INIA		mod mos,curl
Blue Lake Stringless		mm
common cranberry bean		mod mos
Domino		mod mos,curl,vb
Great Northern 31		mod mos,vb
Great Northern 123		mm
Isabella		mm
Michelite 62		mod mos,vb
Montcalm		mm
Olathe		mod mos,ln
Orfeo INIA		mod mos,mot,curl
Pinto UI 114		mod mos
Redkloud		mm
Stringless Green Refugee		mm,curl
Top Crop		mm
Tortola INIA		mod mos

* curl = curling of leaves, latent = no symptoms, however virus was detected by DAS-ELISA, ln = local necrosis, mm = mild mosaic, mod mos = moderate mosaic, mos = mosaic, mot = mottling, vb = vein banding, - = no symptoms, and absence of the virus confirmed by DAS-ELISA.

Figure 7 : Mild mosaic symptoms caused by BMMV-Mich. in Corsoy soybean, 10 - 15 days after inoculation.

and 8). Ten to fourteen days after inoculation, the first trifoliolate of bean plants usually showed a mild vein yellowing or vein banding that later developed into mild to moderate chlorotic mosaic. The symptoms were usually slightly more severe in the second trifoliolate. As the plant matured, symptom severity was reduced in some cultivars and only a very mild mosaic was visible, as in the case of Isabella, Montcalm and Blue Lake Stringless cultivars. In other cultivars (Domino, Black Turtle Soup and Orfeo INIA) mosaic symptoms remained moderate to severe.

Virus presence was confirmed by DAS-ELISA in tissue taken from all bean cultivars plus soybeans and Alaska pea. No symptoms were observed in Alaska pea even though the virus was present in the tissue; this was considered a latent infection.

Seed transmission of BMMV

Healthy looking pods and seeds were collected from inoculated bean plants and seed transmission of BMMV-Mich was demonstrated in all three cultivars.

After 5 weeks very mild mosaic or no symptoms were observed in plants of the different cultivars, but the virus was detected by DAS-ELISA in all of the cultivars. Table 3 summarizes the percentage of seed transmission found in the different cultivars which ranged from 3.3% in Black Turtle Soup to 5.0% in Orfeo INIA.

Figure 8 : Moderate mosaic and curling of the leaves caused by BMMV-Mich. in Domino bean, 10 - 15 days after inoculation.

Table 3 : Seed transmission of BMMV-Mich. in three bean cultivars

Cultivar	Number of seeds planted	Number of seeds germinated	Number of plants infected *	% transmission**	DAS-ELISA absorbance values Min.	Max.	Mean ***
Black T. Soup	100	90	3	3.30	.13	1.39	.90
Domino	140	129	5	3.87	.14	.27	.20
Orfeo INIA	70	60	3	5.00	.12	1.50	1.01

* virus detected by DAS-ELISA

** percentage (%) of seed transmission was calculated from the number of germinated seeds

*** absorbance values read at A_{405}nm after 15 min incubation of substrate. Absorbance values for non-infected control, min = .02; max = .05; mean = .04
Absorbance value for infected tissue, min = 1.44; max = 1.53; mean = 1.49
Samples were considered positive when absorbance value was higher than twice mean of non-infected control

Detection of seed infection with BMMV in Michigan grown bean seeds

Seed infection and seed transmission of BMMV were found in 11 of the 30 bean seed lots tested.

Only mild mosaic or yellowing were observed in some plants of specific seed lots after 5 weeks under greenhouse conditions. These symptoms were not always correlated with the presence of virus particles as demonstrated by electron microscopy. The number of virus particles found in leaf samples from different seed lots varied from only a few to many (Table 4). Table 4 shows that seed transmission was found in 9 of 20 navy bean seed lots and 2 of 10 black bean seed lots.

A 2% incidence of seed transmission was found in navy bean seed lot 921011, a seed lot chosen at random from those showing virus particles in leaf dips. The presence of the virus in these plants was confirmed by a positive DAS-ELISA test, even though plants showed no symptoms under greenhouse conditions even after 5 weeks.

Distribution of BMMV and BYMV in Michigan dry bean fields

Bean yellow mosaic virus was detected in all 30 samples collected in 1988, but BMMV was not detected in the same samples. The following season, 1989, both viruses were found in field grown bean plants but the incidence was very low. From 508 samples collected during 1989, BYMV was

Table 4 : Transmission of BMMV in lots of Michigan grown bean seed

Seed lot	Seed type / cultivar	BMMV particles*	Symptoms**
921011	navy	few	-
921360	black/Midnight Black	-	-
921361	navy/Seafarer	few	-
921362	navy/Fleetwood	-	-
921363	navy	-	-
921368	navy	-	-
921369	navy/Bunsi	-	-
921375	black/Black T Soup	-	-
921378	black/T-39	-	-
921380	navy	-	-
921388	black/Midnight Black	many	-
921389	black/Midnight Black	few	-
921393	navy/B-155	-	-
921395	navy/C-20	-	-
921557	navy	-	-
921610	navy	few	-
921718	navy/Aurora	few	mm
922142	navy/Seafarer	few	-
922144	navy/C-20	-	-
922176	navy	few	mm
922185	navy/Seafarer	-	-
922186	navy	-	-
922210	black/Black T Soup	-	mm
922313	navy	-	-
922314	navy/Seafarer	few	mm
922417	navy	few	-
922433	black	-	-
922434	navy	few	mm
922446	navy	-	mm
922459	navy	-	-

* virus particles observed in leaf dips, few = less than 10 particles/field; many = more than 10 particles/field.

** mm = mild mosaic, - = no symptoms.

found in seven while BMMV was only present in five samples.

Table 5 sumarizes the distribution of viruses among the counties and indicates that both viruses were never present together in the same plant, but they were found in the same field as in the case of a navy bean lot in Clinton county, (Dewitt Rd./Livingston Rd.) (Table 1).

Also tested for the presence of BMMV and BYMV were twenty samples of weeds collected in 1988 and 1989, in and around virus infected bean fields. Included among the samples were plants identified as: buckhorn Plantain (Plantago lanceolata), common milkweed (Asclepias syriaca), lambsquarter (Chenopodium album), Pennsylvania smartweed (Polygonum pensylvanicum), prostrate pigweed (Amaranthus blitoides). All samples tested negative for the presence of virus.

Synergistic effect of BMMV and BYMV on several bean cultivars

After 7 days, plants inoculated with either BMMV-Mich. or BYMV (C-20 isolate), or both exhibited different degrees of mosaic, epinasty, mottling, leaf necrosis and/or stunting (Figures 9, 10 and 11).

Analysis by DAS-ELISA showed that cultivars Amanda, Blanco INIA, Great Northern 31 and Tortola INIA were immune to BYMV when infected alone but not to BMMV (Table 6).

Some synergistic effects were observed in plants of Amanda and Great Northern 31 cultivars inoculated with both

Table 5 : Distribution of BMMV and BYMV in field grown bean plants in three counties in central Michigan, 1988 and 1989.

Year	County	Bean type or cultivar	Number of samples containing		DAS-ELISA absorbance values **					
					BMMV			BYMV		
			BMMV	BYMV	min.	max.	mean	min.	max.	mean
1988	Saginaw	Montcalm	-	1 *				.23	.28	.26
	Saginaw	breeding lines	-	5				.21	.70	.40
	Saginaw	cranberry	-	1				.38	.39	.39
	Tuscola	navy	-	15				.21	1.09	.78
	Tuscola	cranberry	-	5				.24	1.48	.77
	Tuscola	Aurora	-	2				.19	.27	.23
1989	Clinton	navy	1	1	.14	.15	.15	.14	.15	.15
	Saginaw	N85074	-	1				.25	.29	.27
	Saginaw	Sierra	-	1				.17	.24	.20
	Saginaw	navy	3	-	.21	.39	.30			
	Tuscola	C-20	-	1				.15	.26	.20
	Tuscola	navy	1	-	.25	.29	.27			
	Tuscola	Mayflower	-	3				.16	.51	.32

* presence of the virus determined by DAS-ELISA

** absorbance values read at A_{405}nm after 15 or 60 min incubation of substrate for BMMV and BYMV, respectively
absorbance values for non-infected control: 1988: min = .04, max = .06, mean = .05;
1989 min = .07, max = .09, mean = .08
absorbance values for infected tissue: 1988: BYMV, min = .50, max = 1.02, mean = .76;
1989: BMMV, min = 1.44, max = 1.46, mean = 1.45; BYMV, min = 1.06, max = 1.10, mean = 1.08
Samples were considered positive when absorbance values were higher than twice mean of non-infected control

Figure 9 : Moderate mosaic and mottling in Black Turtle Soup bean caused by BMMV-Mich., 10 - 15 days after inoculation.

Figure 10 : Local necrosis in Orfeo INIA bean inoculated with BMMV-Mich. and BYMV (C-20 isolate), 10 - 15 days after inoculation.

Figure 11 : Epinasty caused by BYMV (C-20 isolate) in Domino bean, 7 - 10 days after inoculation.

Table 6 : Symptomatology and detection by DAS-ELISA of BMMV and BYMV in plants of different bean cultivars inoculated either with one or the other or both viruses.

Cultivar	Plants inoculated with			
	BMMV	BYMV	BMMV + BYMV	
	* **			
Amanda	+ ns	- ns	+	+ mot,mos
Black T. Soup	+ vb,mos	+ se,s,mos	+	+ se,s,mos
Blanco INIA	+ mot,vb	- ns	+	- mm
Blue L. Stringless	+ ns	+ e,mm,s	+	+ s,mm
common cranberry bean	+ mm	+ mos	+	+ mos
Domino	+ vb,mos	+ se,mos,s	+	+ s,se
Gr. Northern 31	+ mm	- ns	+	+ mot,mos
Gr. Northern 123	+ mm	+ ns	+	+ mm
Isabella	+ ns	+ ns	+	+ mm
Michelite 62	+ mos,vb	+ s,mos,mot	+	+ s,e,mos
Montcalm	+ mos	+ mos	+	+ mos
Orfeo INIA	+ mos	+ sm,e	+	+ sm,s,ln
Pinto UI 114	+ vb,mos	+ mot	+	+ mos,mot,e
S. G. Refugee	+ mm	+ e,s,mos	+	+ e,mos,ln
Top Crop	+ mm,vb	+ mm	+	+ s,e,ln
Tortola INIA	+ mm	- ns	+	+ mos

* + = presence of virus detected by DAS-ELISA
- = virus not detected by DAS-ELISA
Absorbance values for DAS-ELISA for both viruses ranged from .60 to 1.44; non-infected tissue ranged from .03 to .09;infected tissue ranged from .98 to 1.45.

** e = epinasty; ln = local necosis; m = mos; mm = mild mosaic; mot = mottling; ns = no symptoms; s = stunting; se = severe epinasty; sm = severe mosaic; vb = vein banding.

viruses. Mottling and mosaic symptoms were observed in doubly infected plants while singly infected plants showed no symptoms. Both viruses were recovered by DAS-ELISA from doubly infected plants while only BMMV was recovered from single infections. Cultivar Amanda showed no symptoms, however.

Expression of symptoms was more severe in doubly infected plants compared with single infection for some cultivars (Orfeo INIA, Stringless Green Refugee and Top Crop) (Table 6).

No symptoms were observed on Isabella cultivar up to 15 days after single inoculations, however both viruses were detected on leaves by DAS-ELISA.

DISCUSSION

BMMV was confirmed as the causal agent of the symptoms observed in non-inoculated control plants and resistant bean cultivars by the use of ISEM, gel double diffusion and DAS-ELISA tests. These techniques have been successfully used in the identification of several different viruses (Milne and Luisoni, 1975; Milne and Lesemann, 1978; Roberts and Harrison, 1979; Lesemann, 1982; Haufler and Fulbright, 1983).

Further studies revealed the presence of BMMV in some of the BYMV isolates. This was determined by the use of leaf dips from plants infected with several isolates of BYMV. The different morphology of the virus particles allowed an easy identification of both viruses. The BYMV is a flexous rod that measures approximately 750 nm long and 15 nm wide. This virus belongs to the potyvirus group which includes a number of different viruses (Bos, 1970). BYMV is considered one of the most important viruses in bean production and has a worldwide distribution (Morales, 1986, Schwartz and Galvez, 1980).

Bean mild mosaic virus was readily transmitted by mechanical inoculation to bean plants, transmission to 100% of inoculated plants was obtained in most inoculations. Similar results were observed by Jayasinghe (1982) in the transmission of BMMV-CIAT.

The virus particle size of BMMV-Mich. was found to be around 28 nm in diameter, which corresponds well to the size mentioned by Waterworth et al (1977); BMMV-CIAT particles were slightly larger having an average size of 32.2 nm in diameter (Jayasinghe, 1982).

No relationship was found between BMMV-Mich. and other antisera tested, which supports the results of authors who were unable to place BMMV into any of the five serogroups of the comoviruses (Waterworth et al, 1977; Jayasinghe, 1982). Waterworth (1981) also mentioned that BMMV is not serologically related to ten other spherical viruses that are usually associated with legumes. Moreover, Morales and Gamez (1989) indicated that even though BMMV has similar morphology and physicochemical properties to other viruses, it has not yet been included into any of the recognized plant virus group.

Bean mild mosaic virus host range was found to be very narrow among different species. However, all bean cultivars inoculated were found to be susceptible to the virus even though symptoms were difficult to see in some plants. No local lesion host or resistant cultivar was found. These results agree with those of Jayasinghe (1982).

In contrast to the results found by Waterworth et al (1977), BMMV-Mich. infected Alaska pea (*Pisum sativum* L.), causing a latent infection detected only by DAS-ELISA.

BMMV-Mich., did not infect *Chenopodium quinoa* Willd. and *Gomphrena globosa* L. Experiments conducted by Jayasinghe (1982) at CIAT (Colombia) demonstrated that BMMV-CIAT had a host range similar to the one found in this study, even though he did not inoculate pea. The differences in host ranges between the present study and that of Waterworth *et al* (1977) could be explained by different environmental conditions according to Jaysinghe (1982).

Seed transmission of BMMV-Mich. was higher than that reported by Jayasinghe (1982) for BMMV-CIAT. BMMV-Mich. was seed transmitted in a range of 3 - 5% in different bean cultivars. This percentage could be considered low by other authors who mentioned 10% seed transmission for other beetle transmitted viruses (Shepherd, 1964).

Seed infection was found in bean seed lots grown in Michigan and 2% of seed transmission was achieved in one of these seed lots. This is the first report of seed infection by BMMV under natural conditions in Michigan and United States. The virus has been reported infecting beans in Central and South America (Waterworth *et al*, 1977; Schwartz and Galvez, 1980; Morales, 1986) and as a contaminant virus in greenhouse studies in Corvallis, Oregon (Hampton and Hancock, 1981).

The fact that BMMV was found in field grown seed lots suggests that the virus is well distributed among the bean producing areas of Michigan. The percentage of seed transmission from field infected seed was lower than that obtained under experimental conditions, but even a low incidence of transmission could allow establishment of primary inoculum sources in the field. Moreover, the Mexican bean beetle, an efficient vector, is commonly found in many Michigan bean fields. In addition there also exists the possibility that BMMV could survive in infected crop debris from one season to the next (Hampton and Hancock, 1981), which could constitute another source of inoculum.

Economic losses due to BMMV have not been determined; however the presence of the virus in the seed could represent a potential problem under Michigan field conditions. In addition, the inability for easy detection of seed transmitted virus infection due to the very mild mosaic or absence of symptoms in some cultivars, could be a problem in attempting to eliminate the primary inoculum. Of concern is, the synergistic interactions between BMMV and other viruses which could represent a cause for economic losses in bean fields (Waterworth et al, 1977; Morales, 1986)

Seed transmission of BMMV could constitute a real problem in efforts to breed for disease resistance at Michigan State University. This is specially true because the symptoms in bean plants could be confused with those associated with BYMV or BCMV. The fact that the limited screening of bean cultivars for reaction to BMMV showed no resistance could also further complicate breeding efforts if BMMV is a contaminant of BYMV or BCMV inoculum sources. (Dr. James Kelly, personal communication).

Results obtained in this study confirmed the presence of BMMV in bean fields in Michigan. The virus was found in bean samples collected from three different counties in 1989. BMMV was not present in any sample collected in 1988 possibly because of the small number of sites sampled. The results indicate that BMMV is most likely to be found in navy bean fields. However, in light of the seed transmission studies and infectivity of the virus in other cultivars, the virus could easily be found in any other cultivars, so further studies are necessary to adequately address this question.

Bean yellow mosaic virus was present in all samples in 1988 but only in few of 1989. This situation could be due to a sampling effect or to the dry conditions in 1988 which may have favored greater numbers of aphid vectors and therefore greater disease spread. Climatic conditions were more humid and temperate in 1989.

The fact that BMMV and BYMV were never found together in the same plant in the field in this study, does not rule out the possibility that both viruses could affect bean plants under field conditions. The evidence that BMMV was present in some BYMV isolates is possible support for this.

Bean mild mosaic virus has been mentioned before as causing severe mosaic symptoms in mixed infections (Waterworth et al, 1977). This was confirmed in this study when several bean cultivars showed more severe symptoms when BYMV (C-20 isolate) and BMMV were inoculated together. Examples of such synergistic effects were cultivars Amanda and Great Northern 31 cultivars inoculated with both viruses. Bean yellow mosaic virus was able to infect these bean cultivars in the presence of BMMV but not when BYMV (C-20 isolate) was inoculated alone to the same cultivars. Other more severe reactions were also observed in some cultivars when both viruses were together in comparison with single infection, as observed in Orfeo INIA and Pinto UI 114.

The real economic importance of BMMV for beans under Michigan conditions could be an interesting topic to study in the next few years, considering the seed transmission, synergistic effects and vector-virus relationship of this new pathogen.

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