Beet Necrotic Yellow Vein Virus and Its Relationship to Eight Sugar Beet Furo-like Viruses from the United States

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ABSTRACT

Wisler, G. C., Liu, H.-Y., and Duffus, J. E. Beet necrotic yellow vein virus and its relationship to eight sugar beet furo-like viruses from the United States. Plant Dis. 78:995-1001.

The degree of relatedness among five beet necrotic yellow vein virus (BNYVV) isolates and eight other rigid, rod-shaped viruses coined beet soilborne mosaic virus (BSBMV) isolated from sugar beet roots from the United States was evaluated by serology, electron microscopy, fungal transmission, the polymerase chain reaction (PCR), and host range. Polyclonal antisera to the C-terminal 60 amino acids of the BNYVV coat protein (CP), the 14- and 75-kDa nonstructural proteins, and seven monoclonal antibodies were specific to BNYVV in Western blots. Antisera to the BNYVV CP and its cloned CP reacted strongly with the 22-kDa CP of the BNYVV isolates, but weakly with the 24-kDa CP of the BSBMV. Antisera to the 42-kDa BNYVV nonstructural protein reacted with a 42-kDa protein of all BNYVV isolates and with a 44-kDa protein of all but one BSBMV isolate. No cross-reactivity was observed in reciprocal immunodiffusion tests between BNYVV and the BSBMV isolates using antisera to the CP of each virus. No products were observed for the BSBMV isolates analyzed in PCR using 10 BNYVV primer pairs. The eight BSBMV isolates investigated induced symptoms different from those of BNYVV in several hosts. Two BSBMV isolates tested were transmitted by *Polymyxa betae*. These eight BSBMV isolates appear to be furoviruses distinct from BNYVV.

Additional keywords: *Beta vulgaris,* rhizomania, soilborne virus, fungal vector

Beet necrotic yellow vein virus (BNYVV), which induces symptoms of rhizomania in sugar beet (*Beta vulgaris* L.), was first detected in the United States in 1983 (9). It is a member of the furovirus group, with a rigid rod-shaped particle morphology, and is transmitted by *Polymyxa betae* Keskin (19,24). Characteristics of rhizomania include bearding (proliferation of lateral roots) and a loss in both yield and sugar production (5,7). Control measures for BNYVV include the use of resistant sugar beet varieties and selective planting in soil found to be free of BNYVV based on ELISA.

Most isolates of BNYVV typically contain four single-stranded 5′-capped and 3′-polyadenylated plus-sense molecules (18). All four RNAs have been cloned and sequenced (2–4). RNA-1 encodes a single open reading frame (ORF) of a 237-kDa nonstructural protein with helicase and replicase motifs (16). RNA-2 encodes the coat protein ORF of about 22 kDa, in addition to a readthrough product with a theoretical molecular mass of 75 kDa and four additional nonstructural proteins of 42 kDa, 13 kDa, and 15 kDa (all involved in cell-to-cell movement) (19), and 14 kDa (function not fully determined). RNA-3 and RNA-4 encode a single ORF each of 25 kDa and 31 kDa, respectively. The 25-kDa protein is associated with leaf symptoms and root proliferation (11), and the 31-kDa protein is essential for fungal transmission (23).

Eight other virus isolates with rigid, rod-shaped particle morphologies similar to BNYVV have been isolated from sugar beet roots in the United States and will be referred to herein as beet soilborne mosaic virus (BSBMV) (14). Two were isolated from Texas (BSBMV-1 and -2), five from Nebraska, and one from Idaho. These isolates have been shown to cross-react with antisera to the BNYVV virion in ELISA and in Western blot analyses (29,30). The effect of these virus isolates on sugar beet is not fully known. In this study, serological analyses of both structural and nonstructural proteins, electron microscopy, fungal transmission, reverse transcriptase polymerase chain reaction (RT-PCR), and host range analyses were used to investigate the relationship among five BNYVV isolates and the relationship between the BNYVV isolates and the eight BSBMV isolates described above.

MATERIALS AND METHODS

Virus isolates. The BNYVV isolates addressed in this study originated from sugar beet fields in California (BNYVV-CA-1, BNYVV-CA-12, and BNYVV-CA-GH), Nebraska (BNYVV-NE-8-4), and Idaho (BNYVV-ID-47). The BNYVV-CA-GH was the original U.S. isolate found in California and has been maintained by continuous mechanical inoculation on *Beta macrocarpa* Guss. for several years in the greenhouse. Two BSBMV isolates originated from Texas (BSBMV-1 and -2), five from Nebraska (NE-8-1, NE-8-3, NE-8-5, NE-10, and NE-KW), and one from Idaho (ID-31051). Both BNYVV and BSBMV isolates were initially obtained by mechanical inoculation from infected roots and symptomatic leaf tissues collected from field samples onto leaves of *Chenopodium quinoa* Willd. and *B. macrocarpa* plants. Isolates derived from single local lesions were increased in *C. quinoa* and stored by lyophilization and in glycerol at −20 C.

Electron microscopy. Leaf extracts were prepared for examination with the electron microscope by chopping tissue with a razor blade into 0.01 M potassium phosphate buffer, pH 7.0, at a ratio of approximately 1 part tissue to 5 parts buffer. One drop of extract was placed on a 0.4% formvar-coated copper grid for 1 min, followed by 20 drops of buffer, 20 drops of water, and a final drop of 2% uranyl acetate containing bacitracin at 250 μg/ml. Purified preparations of virus isolates were examined by placing 10 μl (about 0.1 mg/ml) on a grid followed by rinsing and staining as described.

Fungal transmission. Two separate experiments were performed to test for fungal transmission. The experimental design was similar in both tests, but *B. vulgaris* was the host plant in the first experiment, and *B. macrocarpa* was used in the second experiment. Holes were drilled in the bottom of 50-ml disposable centrifuge tubes. The tubes were plugged with two layers of cheesecloth, filled with builders' sand, and autoclaved. Approximately 10 seeds of the host were surface sterilized and planted in each tube. Two weeks after planting, when two to four leaves had fully expanded, the leaves were mechanically inoculated with either BNYVV, BSBMV-2, NE-10, or 0.1 M potassium phosphate buffer, pH 7.2. For each treatment above, three tubes were inoculated with a fresh root sample containing mature cystosori of *P. betae*. This root culture of *P. betae,* originally collected from a rhizomania-free sugar beet field, was routinely tested and determined to be free of BNYVV, BSBMV, and tobacco mosaic virus (TMV), a common contaminant in sugar beet roots. A second set of three tubes from each treatment was not inoculated with *P. betae*. Plants were placed in a growth

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Accepted for publication 19 July 1994.

room at 20 C constant temperature with a 16 hr day length at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$.

After 2–3 wk, root samples from each tube were examined with the light microscope. Seedlings inoculated with *P. betae* contained mature cystosori. Seedlings not inoculated with *P. betae* did not contain any visible cystosori. At this time, each tube was secured above another tube of 2-wk-old healthy seedlings grown in the same manner. As the top donor tubes were watered, they were allowed to drip into the bottom recipient tubes. The cheesecloth plugs in the tubes allowed zoospores to filter through to the recipient tubes, excluding pieces of plant material. After 2 mo, all roots were harvested, assayed for BNYVV and BSBMV by Western blot analysis, and used to mechanically inoculate leaves of *C. quinoa* and *B. macrocarpa*.

Serological analyses. The double antibody sandwich (DAS)-ELISA was done as described by Clark and Adams (8), with an immunoglobulin concentration of 1 μg/ml for coating the ELISA plates and a 1,000-fold dilution of the alkaline phosphatase conjugates. Plant extracts were obtained by macerating one part plant tissue in three parts of sample extraction buffer (0.1 M phosphate buffered saline, pH 7.4, with 2% polyvinylpyrrolidone and 0.2% ovalbumin). Root samples were derived from sugar beet (cv. USH11) seedlings grown in soil samples submitted from sugar beet fields. Healthy samples consisted of sugar beet seedlings grown in autoclaved soil. Samples were tested in pairs, and the average absorbance at 405 nm was recorded. Samples determined to be positive had average ELISA values three times the average healthy readings.

The Western blot procedure was conducted essentially as described by Towbin et al (27) using a Bio-Rad Mini-Protean II Electrophoresis Cell and Trans-Blot Electrophoretic Transfer Cell (Hercules, CA) according to manufacturer's instructions. In most instances, 12 and 15% sodium dodecyl sulfate (SDS) polyacrylamide gels were used. Plant tissues selected for assay were triturated in an extraction buffer (1:2, w/v) consisting of 75 mM Tris-HCl, pH 6.1, 9 M urea, 7.5% 2-mercaptoethanol, and 4.5% SDS (21). Samples were squeezed through dampened cheesecloth, heated at 95 C for 2 min, and centrifuged at 10,000 *g* for 2 min. Extracted samples were stored at −20 C. Each isolate was tested at least twice in both *B. macrocarpa* and *C. quinoa*.

In Western blots, polyclonal antisera and monoclonal ascites fluid were routinely diluted 1/1,000 and monoclonal tissue culture supernate at 1/10. Antisera to the C-terminal 60 amino acids of the BNYVV capsid protein and the 14-, 25-, 42-, and 75-kDa nonstructural proteins (16) were kindly supplied by K. Richards (Strasbourg, France). The BNYVV monoclonal antibodies (MAbs) 41 and 47 (10) were supplied by G. Grassi (Bologna, Italy), and MAbs 6, 7, 8, 9, and 10 (26) were supplied by L. Torrance (Dundee, England). Antiserum also was produced in this study to the BNYVV coat protein (CP), which was cloned (clone courtesy of K. Richards) and expressed in the pETh vector (15) with BL21DE3pLysS as the *Escherichia coli* expression host (Novagen, Madison, WI).

SDS-immunodiffusion tests were conducted as described by Purcifull and Batchelor (17). Immunodiffusion media consisted of 0.8% Noble agar, 0.5% SDS, 1% NaCl, and 0.05 M Trizma base, pH 8.0 (31). Antigens in leaf extracts of *C. quinoa* were prepared 1:1 (w/v) in 1.5% SDS. Purified virus preparations (about 0.1 mg/ml) were used 1:1 (v/v) in 3% SDS. All isolates were tested at least twice against antisera to both BNYVV, BSBMV-1, and BSBMV-2 using plant tissue as the antigen. In addition, selected isolates were tested as purified virus preparations.

Host plant inoculations. Test plants used for inoculations were held in the dark for 16–24 hr prior to inoculations. Initial virus inoculations were made by triturating sugar beet root tissues by mortar and pestle with 0.1 M potassium phosphate buffer, pH 7.2, containing 0.1% Na_2SO_3, with the addition of 600-mesh Carborundum. The slurry was

Table 1. Primers used in reverse transcription polymerase chain reaction (RT-PCR) analyses of beet necrotic yellow vein virus (BNYVV) and beet soilborne mosaic virus (BSBMV) isolates

Primer; direction of synthesis	Sequence[a]	Nucleotide position on RNA
1; 3′ to 5′[b]	5′-TTCACACCCAGTCAGTA-3′	6,704/RNA-1; 4,574/RNA-2; 1,735/RNA-3; 1,391/RNA-4[c]
2; 5′ to 3′	5′-TTTGTCTGATGCTATTG-3′	465/RNA-1
3; 5′ to 3′	5′-AGATAGTGCTATAAACGG-3′	5,649/RNA-1
4; 3′ to 5′	5′-CACTTTCCATATTGCGG-3′	1,029/RNA-1
5; 5′ to 3′	5′-CTGCGAATCTATCTATAA-3′	275/RNA-2
6; 5′ to 3′	5′-AACTTAAATGCAAGAAAC-3′	4,263/RNA-2
7; 3′ to 5′	5′-CGTACATTAGCAGATGC-3′	484/RNA-2
8; 5′ to 3′	5′-ACAGCCGGTTACATGGT-3′	1,501/RNA-3
11; 5′ to 3′	5′-GTTTCTGTTGAGATTCT-3′	111/RNA-4
12; 5′ to 3′	5′-GTTGGACGTGTACGTGT-3′	587/RNA-4
13; 3′ to 5′	5′-AACCTGACACCGACATA-3′	360/RNA-4
CP-1; 3′ to 5′	5′-ATGTCGAGTGAAGGT-3′	145/RNA-2
CP-2; 5′ to 3′	5′-CTATTGTCCGGGTGG-3′	711/RNA-2
42k-1; 3′ to 5′	5′-ATGGTCCAAGTACAG-3′	2,133/RNA-2
42k-2; 5′ to 3′	5′-CGCAAAAAGTATCTC-3′	3,276/RNA-2

[a] Sequences are derived from those reported for BNYVV by Bouzoubaa et al, 1985, 1986, and 1987 (2–4).

[b] Primers used as paris: 1 and 3, 1 and 6, 1 and 8, 1 and 11, 1 and 12, 2 and 4, 5 and 7, 11 and 13, CP-1 and CP-2, 42k-1 and 42k-2.

[c] The sequence for primer 1 corresponds to a conserved sequence at the 3′ end of each of RNAs -1, -2, -3, and -4.

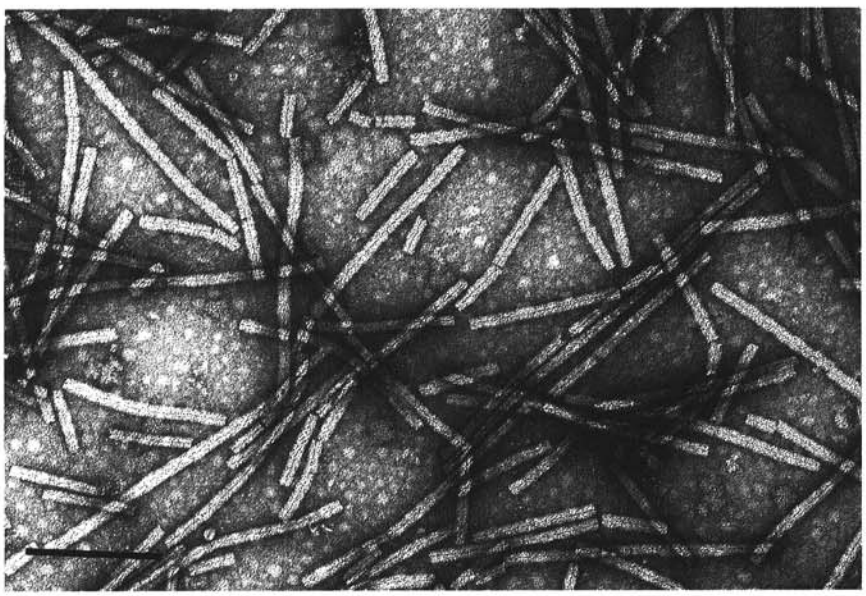

Fig. 1. Electron micrograph of a partially purified preparation of beet soilborne mosaic virus isolate 1 with 2% uranyl acetate. Bar represents 200 nm.

rubbed onto leaves, and plants were rinsed gently with water after inoculation. Test plants included *B. vulgaris, B. macrocarpa, C. quinoa, C. murale* L., *C. amaranticolor* Coste & Reyn., *C. capitatum* (L.) Aschers., *Spinacia oleracea* L., *Gomphrena globosa* L., *Nicotiana benthamiana* Domin., *N. glutinosa* L., and *N. tabacum* L. Three successive single local lesion transfers were made with each new isolate to be sure of purity and to escape possible contamination with TMV, commonly found in sugar beet roots. Host plant studies were repeated three times.

RT-PCR. Aliquots of purified virus preparations (about 0.1 mg/ml) were subjected to a phenol:chloroform extraction, followed by an ethanol precipitation. The extracted viral RNA was used as a template for production of cDNA using reverse transcriptase (Superscript, Gibco BRL, Grand Island, NY), followed by amplification by PCR as described by Robertson et al (20). Briefly, the RNA was resuspended in 10 μl of water treated with diethyl pyrocarbonate (DEPC) and was added to 50 pmol of the RNA-specific primers (primers 1, 4, 7, 13, CP-1, or 42k-1, Table 1) to a final volume of 12.5 μl. This was heated to 95 C for 5 min, then cooled to 40 C for 10 min. The annealed template was added to the first strand reaction mixture consisting of 200 units Superscript II RNase H^- (Gibco BRL) and 2.5 mM dNTPs, in a final volume of 25 μl for 1 hr at 37 C. The first strand buffer and dithiothreitol (DTT) were used according to manufacturers' instructions. cDNA samples were frozen or used directly in the PCR reaction. One isolate from each state was chosen for RT-PCR analysis (BNYVV-CA-1, -NE-8-4, and -ID-47), in addition to the original greenhouse isolate from California (BNYVV-CA-GH). The complete RT-PCR reactions were conducted a minimum of three times. Primers were prepared according to the published sequence of BNYVV (2–4,22) to represent the 5′ and 3′ regions of RNA-1, -2, -3, and -4 (courtesy C. Rush), and to include the CP gene and the 42-kDa protein-encoding gene of RNA-2 (19). For primer sequences and location on the BNYVV genome, see Table 1. PCR reactions were performed in a final volume of 100 μl with one unit of Taq polymerase (Promega, Madison, WI). The amplification profile included 94 C for 3 min, 45 C for 1 min, 72 C for 3 min (three cycles), 93 C for 1 min, 45 C for 1 min, 72 C for 3 min (35 cycles), and 72 C for 10 min (1 cycle). PCR products were analyzed on a 1.0% agarose gel and a 6% acrylamide gel, stained with 0.5 mg/ml ethidium bromide, and viewed with ultraviolet light.

Table 2. Absorbance values for beet necrotic yellow vein virus (BNYVV) and beet soilborne mosaic virus (BSBMV)-1 and -2 in double antibody sandwich (DAS)-ELISA

Isolates	Antisera		
	BNYVV	BSBMV-1	BSBMV-2
BNYVV-CA-1	0.815[a] (7.8×)[b]	0.206 (2.2×)	0.315 (2.0×)
BSBMV-1	0.209 (2.0×)	0.877 (9.1×)	0.997 (6.2×)
BSBMV-2	0.269 (2.6×)	0.719 (7.5×)	1.223 (7.6×)
Healthy	0.105	0.096	0.161

[a] A_{405} values reflect the average of two replications and are representative of the cross-reactivity seen between BNYVV and BSBMV isolates found outside of California. DAS-ELISAs were made at 1 μg/ml coating immunoglobulin and 1,000-fold dilution of alkaline phosphatase conjugate.
[b] Numbers in parentheses are the ratio of the average A_{405} values for each sample to its respective healthy control.

RESULTS

Electron microscopy. All of the isolates addressed in this study, including those identified as BNYVV and those which were serologically identical to BSBMV-1 and -2 contained rigid, rod-shaped virus particles with a central core, as shown in Figure 1. The virus particles were 20 nm wide and of varying lengths, seen in both leaf dips and purified preparations.

Fungal transmission. In both experiments, the roots from plants which were mechanically inoculated with BNYVV, BSBMV-2, or NE-10 gave positive reactions in Western blots for the respective viruses. For example, roots from BNYVV mechanically inoculated plants showed a positive reaction at

Table 3. Summary of Western blot analyses of sugar beet furoviruses using antisera to structural and nonstructural proteins of beet necrotic yellow-vein virus (BNYVV), and to beet soilborne mosaic virus (BSBMV)-1 and -2

Isolates	Antisera[a]							
	BNYVV coat protein	BSBMV -1, -2	BNYVV coat protein/C-terminus	BNYVV anti-P75	BNYVV anti-P42	BNYVV anti-P14	BNYVV anti-P25	BNYVV MAbs 41, 47 6–10
BNYVV isolates								
BNYVV-CA-GH	+,22k[b]	(+,22k)	+,22k	+,75k	+,42k	+,14k	–	+,22k
BNYVV-CA-1	+,22k	(+,22k)	+,22k	+,75k	+,42k	+,14k	+,25k	+,22k
BNYVV-CA-12	+,22k	(+,22k)	+,22k	+,75k	+,42k	+,14k	+,25k	+,22k
BNYVV-NE-8-4	+,22k	(+,22k)	+,22k	+,75k	+,42k	+,14k	+,25k	+,22k
BNYVV-ID-47	+,22k	(+,22k)	+,22k	+,75k	+42k	+,14k	–	+,22k
BSBMV isolates								
NE-8-1	(+,24k)[c]	+,24k	–	–	+,44k	–	–	–
NE-8-3	(+,24k)	+,24k	–	–	+,44k	–	–	–
NE-8-5	(+,24k)	+,24k	–	–	+,44k	–	–	–
NE-10	(+,24k)	+,24k	–	–	+,44k	–	–	–
NE-KW	(+,24k)	+,24k	–	–	–	–	–	–
ID-31051	(+,24k)	+,24k	–	–	+,44k	–	–	–
BSBMV-1	(+,24k)	+,24k	–	–	+,44k	–	–	–
BSBMV-2	(+,24k)	+,24k	–	–	+,44k	–	–	–
Noninoculated hosts								
Chenopodium quinoa	–[d]	–	–	–	–	–	–	–
Beta macrocarpa	–	–	–	–	–	–	–	–

[a] Antisera to nonstructural proteins courtesy of K. Richards; to monoclonal antibodies (MAbs) 41 and 47 courtesy of G. Grassi, and MAbs 6, 7, 8, 9, and 10 courtesy of L. Torrance.
[b] k = Kilodaltons; values reported are estimates of the protein molecular masses based on known protein standards.
[c] Parentheses indicate a weak heterologous reaction, unlike the strong homologous reaction.
[d] – = No detectable reaction.

about 22 kDa using BNYVV antiserum, whereas BSBMV-2 and NE-10 inoculated plants were positive for BSBMV-2 at about 24 kDa. Plants inoculated with buffer only were negative in Western blots for both BNYVV and BSBMV-2. Two to three weeks after inoculation with *P. betae,* roots were found to contain numerous cystosori, whereas no cystosori were observed in plants not inoculated with *P. betae.*

The bottom, recipient plants that received leachate from the *P. betae*- and virus-inoculated donor plants also became infected with the respective virus. Plants that received leachate from virus-infected donor plants without *P. betae* were not infected with either virus. Plants that received leachate from *P. betae*-treated or buffer-treated donor plants were likewise not infected with either virus. Infections were confirmed by both Western blot analyses and mechanical inoculations onto *C. quinoa* and *B. macrocarpa.*

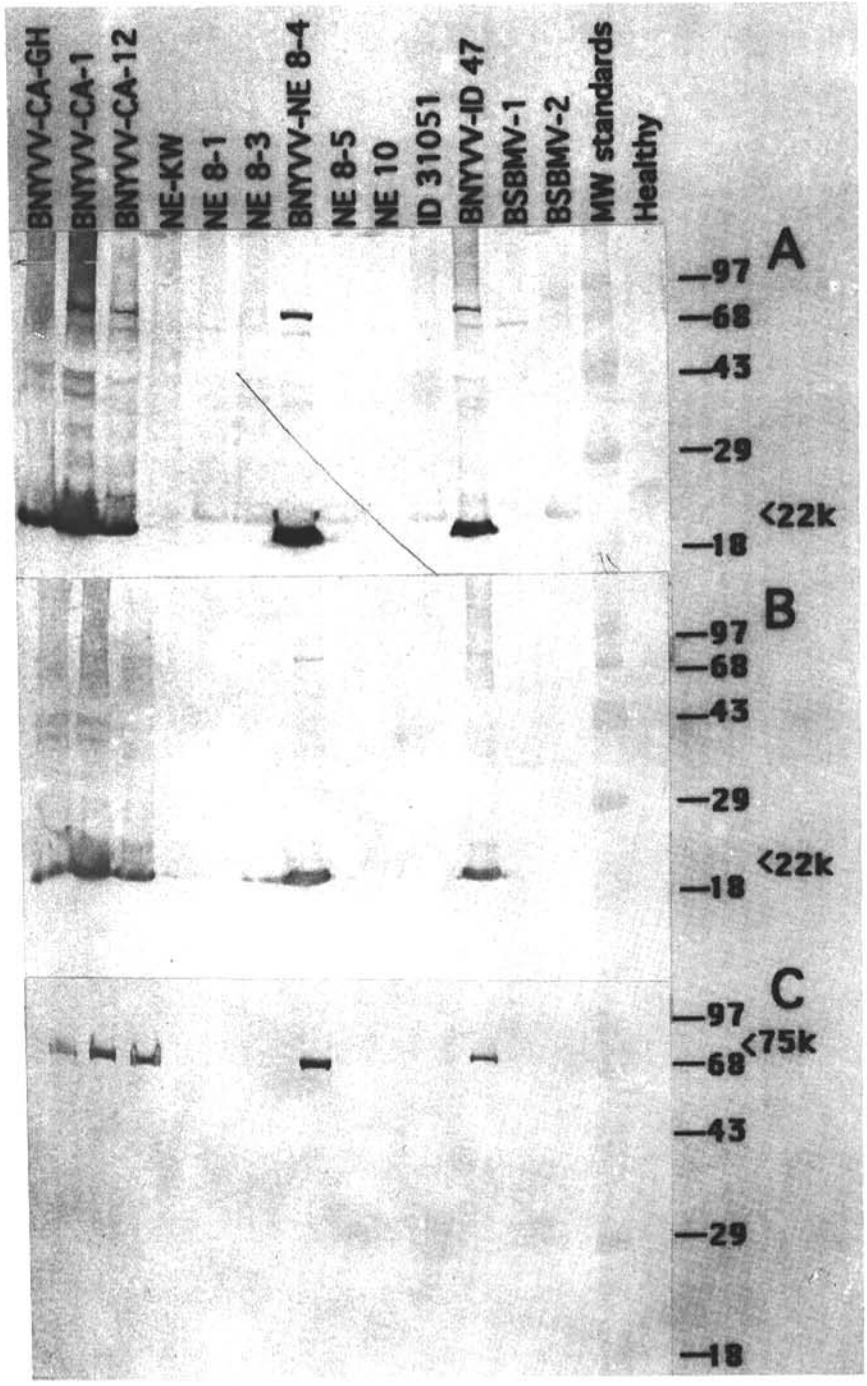

Fig. 2. Western blot analyses of five beet necrotic yellow vein virus (BNYVV) isolates and eight beet soilborne mosaic virus (BSBMV) related furovirus isolates (NE-KW, NE 8-1, NE 8-3, NE 8-5, NE-10, ID 31051, BSBMV-1, and -2). All isolates were tested against **(A)** polyclonal antiserum to the purified BNYVV virion, **(B)** polyclonal antiserum to the C-terminal 60 amino acids of the CP of BNYVV which was cloned and expressed in *Escherichia coli,* **(C)** polyclonal antiserum to the BNYVV 75-kDa nonstructural protein. Healthy lane refers to noninoculated *C. quinoa* tissue. Molecular mass standards (MW standards), in kilodaltons (kDa) are: phosphorylase b (97), bovine serum albumin (68), ovalbumin (43), carbonic anhydrase (29), and *b*-lactoglobulin (18). Arrows indicate approximate molecular mass of respective protein.

Serological analyses. Weak cross-reactivity was seen in ELISA between BNYVV, and BSBMV-1 and -2 (Table 2). Homologous reactions were greater than three times the healthy mean A_{405} reading, whereas heterologous readings often ranged from approximately two to three times the healthy mean.

Results from Western blot analyses are summarized in Table 3. Antisera to the whole CP of BNYVV and to the cloned CP of BNYVV reacted strongly at about 22 kDa with the five BNYVV isolates tested (Fig. 2A). These same antisera reacted weakly, however, with all eight of the BSBMV isolates at about 24 kDa. Reciprocal tests in Western blots were made using antisera to the CP of the BSBMV isolates -1 and -2 from Texas. Both antisera reacted strongly with all eight of the BSBMV isolates at about 24 kDa, but reacted weakly with the five BNYVV isolates at about 22 kDa. Antiserum to the C-terminal 60 amino acids of the BNYVV capsid protein was specific to BNYVV and reacted exclusively with the five BNYVV isolates at about 22 kDa (Fig. 2B).

Antisera to the nonstructural 75-kDa and 14-kDa proteins of BNYVV likewise reacted in Western blots only with the five BNYVV isolates, at 75 kDa (Fig. 2C) and 14 kDa, respectively. Antisera to the 25-kDa BNYVV nonstructural protein reacted with two BNYVV isolates from California (BNYVV-CA-1 and -12), and with one from Nebraska (BNYVV-NE-8-4), but not with an isolate of BNYVV from Idaho (BNYVV-ID-47) or with an isolate from California that had been maintained in a greenhouse by mechanical inoculation for several years (BNYVV-CA-GH). All seven BNYVV MAbs reacted specifically to the BNYVV isolates but not to any of the BSBMV isolates. Only the antiserum to the BNYVV 42-kDa nonstructural protein reacted strongly with all BNYVV isolates and with all but one BSBMV isolate (NE-KW). In this Western blot, the BNYVV isolates reacted with a molecular mass of about 42 kDa, whereas the seven BSBMV isolates reacted with a molecular mass of about 44 kDa.

No cross-reactivity was observed in reciprocal SDS-immunodiffusion tests between any of the five BNYVV isolates and the eight BSBMV isolates. Reactions of identity were observed among all BNYVV isolates when they were tested against BNYVV antiserum (Fig. 3). Likewise, reactions of identity were observed among all eight BSBMV isolates when they were tested against antisera to either BSBMV-1 or BSBMV-2. Reactions

using purified virus preparations were stronger than those using plant extracts as the antigens (data not shown), but the same specificity was seen in each case.

Host range studies. All BNYVV isolates induced similar symptoms on the indicator plants tested. These reactions included characteristic chlorotic to bright yellow local lesions, which spread into the veins in *C. quinoa.* All five BNYVV isolates also produced yellow local lesions on *B. macrocarpa* and sugar beet, with systemic vein clearing and distortion on *B. macrocarpa.*

The BSBMV isolates from sugar beet induced reactions on the indicator plants distinct from those of BNYVV and varied according to isolate (Table 4). For example, symptoms on *C. quinoa* ranged from diffuse, chlorotic local lesions with BSBMV isolates -1 and -2, to necrotic local lesions for isolates NE-10 and NE-KW. Symptoms on *B. macrocarpa* ranged from necrotic local lesions for NE-8-1 to a systemic necrosis of the midrib veins for ID-31051 and NE-8-3. No infection was detected when either BNYVV or BSBMV isolates were mechanically inoculated onto plants of *N. benthamiana, N. glutinosa,* or *N. tabacum.*

RT-PCR analyses. Products observed in repeated RT-PCR analyses among the four BNYVV isolates tested were identical for RNA-1 (primer pairs 1 and 3, and 2 and 4), RNA-2 (primers 1 and 6, 5 and 7, CP, and 42-kDa protein), and RNA-3 (primers 1 and 8) (Fig. 4). The products for RNA-4 of the BNYVV isolates (primers 1 and 12, and 1 and 11) were not identical. Primers 1 and 12, which amplify the 3′ region of RNA-4, showed two or three major products depending on the BNYVV isolate, indicating possible mispriming. Primers 1 and 11, which amplify most of the full length of RNA-4, produced an approximately 750-bp product for the greenhouse isolate of BNYVV (CA-GH), and an approximately 1,100-bp product for the BNYVV isolates CA-1, NE-8-4, and ID-47. The PCR products obtained for RNA-1, -2, and -3 were similar to the expected sizes based on the published sequences (2–4) in each case. Primer pair 1 and 8, which corresponds to the 3′ end of RNA-3, gave a very faint reaction for BNYVV-CA-GH. This corresponds to Western blot data where BNYVV-CA-GH did not react with antisera to the 25-kDa protein encoded by RNA-3. In addition to the expected products observed for primers representing the CP (about 550 bp) and the 42-kDa encoding genes (about 1,100 bp), each primer pair produced a smaller sized product of about 240 and 260 bp, respectively, indicating possible mispriming in those regions (Fig. 4).

No distinct products were detected using any of the BNYVV primer pairs for BSBMV-1 or -2, or for a related BSBMV isolate from Nebraska, NE-10. In some cases, a smear or faint multiple bands were observed, indicating a possible lack of specificity.

DISCUSSION

Based on reciprocal cross-reactivity in Western blots using whole virus CP antisera, BNYVV is related to but distinct from the BSBMV isolates addressed in this study. Antiserum to the cloned CP of BNYVV expressed in and purified from *E. coli* showed the same cross-reactivity as the antiserum to the purified BNYVV virion, confirming the results obtained with whole-virus antiserum.

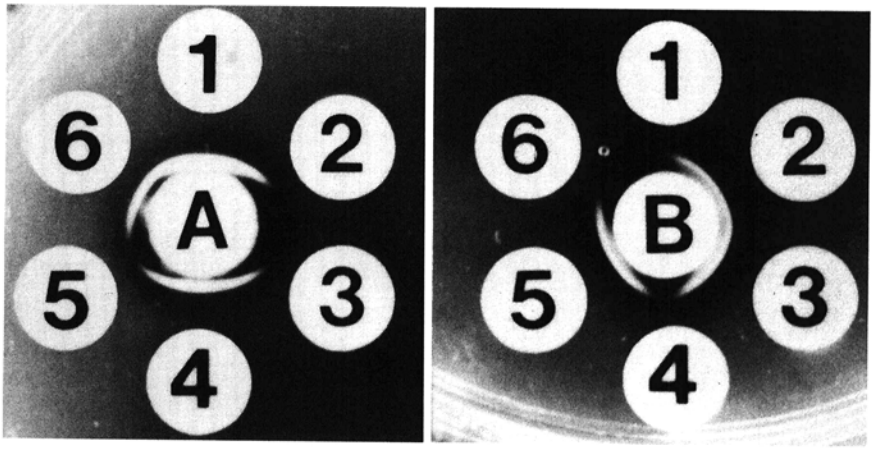

Fig. 3. Sodium dodecyl sulfate (SDS)-immunodiffusion tests with BNYVV and BSBMV-2. Reactions shown use purified virus extracts (about 0.1 mg/ml) as the antigen. Samples are (1) beet necrotic yellow vein virus (BNYVV)-CA-1, (2) beet soilborne mosaic virus (BSBMV)-1, (3) BSBMV-2, (4) BNYVV-NE-8-4, (5) NE-10 (serologically identical to BSBMV isolates), and (6) BNYVV-ID-47, prepared 1:1 with 3% SDS. Antisera are **(A)** polyclonal antisera made to the cloned capsid protein of BNYVV and **(B)** polyclonal antisera made to the purified BSBMV-2 virion.

Table 4. Selected host range and symptoms of eight beet soilborne mosaic furovirus (BSBMV) isolates

	BSBMV isolates[a]							
Host plants	**NE 8-1**	**NE 8-3**	**NE 8-5**	**NE-10**	**NE-KW**	**ID-31051**	**BSBMV-1**	**BSBMV-2**
Beta vulgaris	chl[b] ringspot	chl LL	chl LL	nec LL	chl ringspot	chl ringspot	sys mos	sys mos
B. macrocarpa	nec LL	nec LL & sys nec	chl LL & sys mos	chl LL & sys mos	nec LL	nec LL & sys nec	nec LL & sys mos	nec LL & sys mos
Chenopodium quinoa	chl LL	chl LL	chl LL	nec LL	nec LL	chl LL	chl LL	chl LL
C. murale	nec LL & sys mos	nec LL	chl LL	nec LL	nec ringspot	chl LL	chl LL	nr
C. amaranticolor	chl LL	chl LL	chl LL	nec LL	nec LL	chl LL	chl LL	nr
C. capitatum	nec LL	nec LL & sys mos	chl LL	nec LL sys nec	nec LL	nec LL	chl LL	nr
Spinacia oleracea	nr	sys mottle	chl LL	chl LL	sys mottle	nr	nr	nr
Gomphrena globosa	nr	nec LL	nr	nr	nr	nr	nr	nr

[a]NE = Nebraska, ID = Idaho, BSBMV = beet soilborne mosaic virus isolates from Texas.
[b]chl = Chlorotic, LL = local lesion, nec = necrotic, sys mos = systemic mosaic, nr = no reaction.

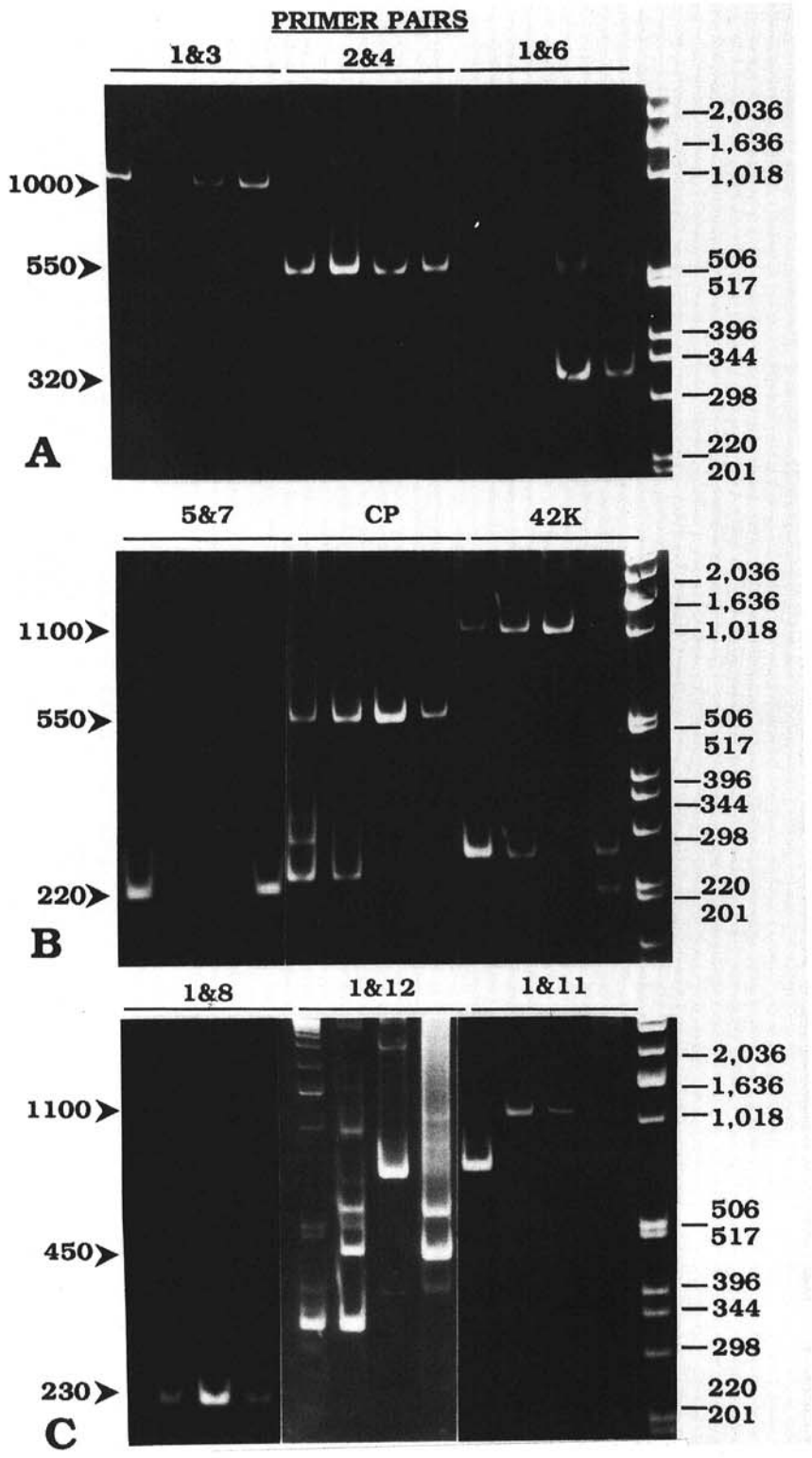

Fig. 4. Results from reverse transcriptase polymerase chain reaction (RT-PCR) using primers specific to beet necrotic yellow vein virus (BNYVV). Primer pairs are indicated above each of four BNYVV isolates, two from California (CA) and one each from Nebraska (NE) and Idaho (ID). Each group of four reactions is indicated by the primer pair tested. Each isolate is shown in the same order for each primer pair tested as: BNYVV-CA-GH, BNYVV-CA-1, BNYVV-NE-8-4, and BNYVV-ID-47. Primer pairs 1 and 3, and 2 and 4 represent regions of RNA-1. Primer pairs 1 and 6, 5 and 7, capsid protein (CP), and 42K represent regions of RNA-2. Primer pairs 1 and 8 represent a region of RNA-4, and pairs 1 and 12, and 1 and 11 represent RNA-4. Primers 1 and 11 represent most of the full length of RNA-4 (941 bp). Standards are the 1KB DNA ladder (Gibco/BRL, Grand Island, NY). Arrows and respective values, in bp, indicate the approximate sizes of the major products obtained for each primer pair tested.

Antiserum to the C-terminus of the BNYVV coat protein, however, was specific to BNYVV and did not cross-react with the BSBMV isolates. Ward and Shukla reported similar results for potyviruses (28), where antisera to the N- or C-termini of the coat protein are highly specific, whereas antisera to the core are cross-reactive. The 42-kDa antiserum was also cross-reactive among all but one BSBMV isolate (NE-KW) used in this study. This result is not surprising, because the 42-kDa protein is conserved among several plant virus groups, including potex-, carla-, furo-, and hordeiviruses (1) and has been implicated in cell-to-cell transport. All seven BNYVV MAbs used in this study were specific to the five BNYVV isolates tested from the United States. Four of the MAbs supplied by L. Torrance (MAb 6, 7, 8, and 9) also reacted with all 19 European BNYVV isolates tested (26). The cross-reactivity between BNYVV and BSBMV isolates seen in Western blots and ELISA using antisera to the respective coat proteins was not seen in the immunodiffusion tests, probably due to the lower sensitivity of this test.

The similarity among BNYVV isolates in serological tests was likewise noted for products obtained for the RNA-1, -2, and -3 in RT-PCR. The variation in the PCR products seen in this study for RNA-4, however, is not surprising, given that it has been reported to be more diverse in size among previously tested isolates (6,12,25). Variation in the presence or level of expression of RNA-3 was seen in Western blot analyses of its 25-kDa protein, the product of which was not detected in the BNYVV isolates CA-GH and ID-47. A study by Lemaire et al (13) indicated that, although RNA-3 and -4 may not be detected, they can reappear after isolates of BNYVV are successfully transmitted to sugar beets via *P. betae*. Thus, these RNAs may exist at extremely low levels after repeated mechanical transmission. A relationship between the BNYVV isolates and BSBMV-1, -2, and NE-10 was not observed in the RT-PCR analyses.

The eight BSBMV isolates addressed in this study are not known to be responsible for the rhizomania disease symptomatology, and in host range studies they induced symptoms distinct from those observed for BNYVV. However, the effect of these virus isolates on sugar beet production is not fully known. These isolates have been grouped together in this study because of their reactions of identity with BSBMV-1 and -2 in immunodiffusion tests and their identical molecular masses (about 24 kDa) in Western blots. Transmission experiments using isolates BSBMV-2 and NE-10 indicate both of these to be transmitted by *P. betae*. The cross-reactivity seen in Western blots between the eight BSBMV isolates and the BNYVV CP

and 42-kDa protein antisera, the molecular mass of the CP (24 kDa), the rigid rod-shaped particle morphology, and transmission by *P. betae* indicate that these isolates belong to the furovirus group, but are distinct from BNYVV. Whereas the five BNYVV isolates caused similar symptoms on respective indicator plants, the eight BSBMV isolates produced a variety of different symptoms on the host range studied. These eight BSBMV isolates thus appear to be biologically more diverse than the BNYVV isolates studied.

It is possible, in sensitive serological tests such as ELISA or Western blots, using antisera to the whole virion of BNYVV, that false positive results could occur due to the presence of one or more of the related furoviruses of sugar beet. Since BNYVV is an important pathogen of sugar beet, and subject to quarantine restrictions, these relationships could cause unnecessary regulatory problems for the sugar beet industry. Although Western blot analysis is not well suited to large-scale diagnostic tests, with the proper controls, it can distinguish between BNYVV and the other rigid, rod-shaped isolates addressed in this study. To date, no isolate similar to the BSBMV-1 or -2 has been detected from California. Future studies will focus on the genomic characterization of the eight furoviruses addressed in this study and will attempt to determine their effect on, and importance to, the sugar beet industry.

ACKNOWLEDGMENTS

We thank Ray Perry for assistance with isolation and maintenance of virus cultures, and Alice L. Pilgeram for assistance with fungal transmission experiments.

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