Evidence of Cross-Protection Between Beet Soilborne Mosaic Virus and Beet Necrotic Yellow Vein Virus in Sugar Beet

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ABSTRACT

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A high degree of reciprocal cross-protection occurred in sugar beets between beet soilborne mosaic virus (BSBMV) and beet necrotic yellow vein virus (BNYVV) in greenhouse experiments. The incidence of cross-protection depended on the interval between inoculations with protecting and challenging virus; longer inoculation intervals enhanced the incidence of cross-protection. Cross-protection was most effective when the inoculation interval was between 5 and 10 days, a period during which virus accumulated to a maximum level in plants singly infected with BSBMV or BNYVV. Results obtained by enzyme-linked immunosorbent assay (ELISA) and Western blotting were consistent and indicated that cross-protection affected viral capsid protein accumulation. RNA of both protecting and challenging viruses was detected in doubly infected plants by using reverse transcription-polymerase chain reaction (RT-PCR), indicating that RNA of the challenge virus was present in protected plants, but capsid protein was undetected by serological tests. BSBMV is a closely related but serologically distinct virus that provides an excellent system for studies of cross-protection with BNYVV.

Rhizomania is a devastating disease of sugar beets worldwide (33). It was first identified in Italy in 1959 and has since been found in most of the sugar beet growing countries of Europe (21) and in Japan (38). In the United States, rhizomania was first identified in California and was partially responsible for a 40% reduction in acres planted to sugar beet between 1983 and 1995 (9). The disease also has been reported from Texas, Colorado, Idaho, Nebraska, Wyoming, and Minnesota (8,13,14,42).

Rhizomania is caused by beet necrotic yellow vein virus (BNYVV), which is vectored by the plasmodiophorous fungus, *Polymyxa betae* (11). Infected roots are stunted and constricted, develop a proliferation of rootlets, and have a greatly reduced sucrose content. If plants are infected in late season, root yields may be unaffected but sucrose content may still be reduced. Foliar symptoms are rare and variable and may include general chlorosis, wilting, and necrotic vein yellowing (16).

Development of effective control measures for rhizomania in sugar beet producing areas has been only partially successful. One recommended control has been soil fumigation with Telone II (1,3-dichloropropene), which is expensive and potentially hazardous to the environment (3,25). Recently, much work has been conducted on screening for resistance to *P. betae* and BNYVV, and cultivars with moderate tolerance have been released (2,23,30). Long crop rotations prevent excessive population buildup of the vector *P. betae*, but cystosori, the survival structure of *P. betae*, can survive in field soil and remain infective for many years (1,37). Therefore, control strategies offering economically acceptable levels of disease reduction are limited. The lack of effective control measures makes biological control an attractive method of controlling this disease.

Cross-protection, in which prior infection with one (protecting) plant virus prevents or interferes with superinfection by another strain or closely related virus (challenge), can be capitalized on for use in biological control (12,15,26,35). It has been used to investigate relationships among viruses and as a practical means of protecting plants against virus infection (6,28,44). Large-scale application of cross-protection is used to control tobacco mosaic virus (TMV) in tomato in Europe (10,31) and Japan (28), papaya ringspot disease in papaya in Taiwan (40), and citrus tristeza virus (CTV) in citrus in Brazil (27). The key to these practical applications is the availability of a mild strain or closely related virus that does not cause

severe damage and also provides a high degree of protection.

In Texas in 1988, a virus in sugar beet was identified as being morphologically similar to BNYVV but serologically distinct (24). The relationships between BNYVV and eight other rod-shaped viruses from Texas, Nebraska, and Idaho were serologically similar (42,43). The name beet soilborne mosaic virus (BSBMV) identifies these isolates and others with similar serological reaction. BSBMV is serologically different from BNYVV and causes foliar symptoms distinct from BNYVV in naturally infected sugar beets. Characterization of BSBMV showed its close resemblance to BNYVV (17). BSBMV shows a significant genetic homology with BNYVV. The sequence data for BSBMV RNA 2 (with regions showing more than 90% sequence homology to BNYVV RNA 2) has been submitted to a gene bank (accession no. AF 061869). BSBMV is a rod-shaped virus which is also transmitted by *P. betae*. Previous studies show that BSBMV causes mild damage to sugar beets compared to BNYVV (16,17).

Due to the many similarities with BNYVV, BSBMV is a natural candidate for cross-protection studies. Here we report on the efficacy, under greenhouse conditions, of BSBMV as a cross-protection agent against BNYVV.

MATERIALS AND METHODS

Viruses stock and maintenance. BSBMV and BNYVV were maintained in a susceptible local lesion host, *Chenopodium quinoa* (17). Root or leaf tissues from *Beta vulgaris* infected with the virus were ground in 0.01 M potassium phosphate buffer (PBS), pH 7.1 (1 g per 10 ml), and the extracts were rubbed on *C. quinoa* predusted with 600-mesh Carborundum. The plants were kept in the greenhouse at 24 to 30°C without supplemental light. Infection was checked visually and by a modified double antibody enzyme-linked immunosorbent assay (ELISA) as described by Heidel et al. (17).

Inoculation method for cross-protection. Young beet seedlings were mechanically inoculated with either BNYVV or BSBMV or both using a method adapted from Koenig and Stein (20). Seedlings (10- to 14-day-old, 15 to 24 per inoculation) were placed in a plastic 50-ml centrifuge tube with 5 ml of inoculum containing 0.12 g of 600-mesh Carborundum, and the

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seedlings were vortexed for 50 s on a Genie™ vortex mixer at a speed setting of 7-8. The inoculum consisted of 1:2.5 (leaf tissue:PBS, wt/vol) freshly expressed sap from infected *C. quinoa* leaves. After vortexing, plants were left for 5 min in the inoculum. Following inoculation, seedlings were washed in sterile distilled water and planted in commercial potting soil. Five to six seedlings were planted in each 3.7-liter pot.

Cross-protection experiment. Uniform 10- to 14-day-old sugar beet seedlings grown in sand were used to study cross-protection. Four sets of plants were used in each experiment. Plants in the first set were protected but not challenged, those in the second set were challenged but not protected, those in the third set were protected and challenged, and those in the fourth set were neither protected nor challenged but mock-inoculated with PBS and Carborundum. Plants were challenged with BNYVV at 0, 2, 5, 10, and 15 days following previous inoculation with BSBMV. All plants were challenged by the method just described. In each set, 25 to 30 plants were inoculated for each inoculation interval after protection with BSBMV (or BNYVV when previously inoculated with BSBMV in reciprocal cross-protection experiments). Plants were randomly arranged in five replicates and maintained in the greenhouse with natural light conditions. One plant from each of the five replicates (from each set of inoculations) was taken randomly at 10, 15, 20, and 30 days after the challenge inoculations. Samples were individually tested by ELISA and in some cases (details provided below) by Western blotting and reverse transcription-polymerase chain reaction (RT-PCR). The experiment was repeated once.

Sample processing. Harvested plants were washed with tap water and stored at

-20°C until processed. Leaf samples were prepared by extracting approximately 0.2 g of frozen leaf tissue in 1 ml of extraction buffer, 0.02 M sodium phosphate-buffered saline plus 1% bovine serum albumin, with a leaf presser (Ravened Specialties Co., Seneca, SC).

ELISA, data processing, and Western blotting. Virus was detected by an indirect double antibody sandwich (DAS)-ELISA method as previously described (17). Part of the leaf tissue from plants challenged after 5 and 10 days was used for ELISA and the remainder for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. A ratio between doubly and singly infected plants (D/S) was calculated, and virus detection frequency was determined (41). D/S ratio is the value obtained by calculating the “D” value (mean ELISA value for doubly infected plants minus mean ELISA value for healthy plants) divided by the “S” value (mean ELISA value for singly infected plants minus mean ELISA value for healthy plants). If the challenge virus is undetectable (complete cross-protection), the D/S ratio for the inhibited virus is considered to be zero. Virus detection frequency is the value obtained by dividing the number of infected samples by the total number of samples tested.

Samples for SDS-PAGE were ground (1:10 wt/vol) in Tris-buffered saline, pH 7.2 (TBS, 0.05 M Tris-HCl, 0.2 M NaCl). The mixture was centrifuged at $14,000 \times g$ for 20 min to remove large particulates. The aqueous supernatant was mixed with sample buffer (0.125 M Tris-HCl, pH 6.8, 4% [wt/vol] glycerol, 5% [vol/vol] 2-mercaptoethanol, 0.004% bromophenol blue) in a 1:1 ratio, heated at 100°C for 5 min, and chilled in ice water. Samples were assayed by SDS-PAGE on vertical mini-slab gels with a 12% resolving gel and a

5% stacking gel for 45 min at 200 V (22). Prestained molecular standards (Bio-Rad Laboratories, Richmond, CA) were included on the gel for molecular weight determination.

After SDS-PAGE, proteins were transferred electrophoretically onto nitrocellulose membrane (0.45 microns; Bio-Rad) by the method of Towbin et al. (39) for 25 min at 18 V using a semidry transblotter as recommended by the manufacturer (Bio-Rad). Membranes were blocked overnight at 4°C with 5% nonfat dry milk in TBS, then incubated for 2 h in anti-viral antibodies (BNYVV antiserum 1.0 mg/ml, BSBMV antiserum 1.3 mg/ml) at a dilution of 1 µg/ml. The membranes were washed three times for 10 min in TBS, followed by an incubation for 2 h in a 1:1,000 (vol/vol) (0.8 mg/ml) solution of goat anti-rabbit antibody conjugated to alkaline-phosphatase (Sigma Chemical Co., St. Louis, MO). After washing with TBS, the presence of alkaline phosphatase was demonstrated by reacting the membranes with bromochloroindolyl phosphate/nitro blue tetrazolium substrate.

Preparation of total nucleic acid. Leaf tissue from plants challenged 5 and 10 days after protection with BSBMV was used for total nucleic acid extraction according to the method of Pappu et al. (29). One gram of tissue from each sample was ground in 4 ml of extraction buffer (200 mM Tris-HCl, 400 mM KCl, 200 mM sucrose, 35 mM MgCl₂, 25 mM EDTA), pH 9.0. Phenol/chloroform/isoamyl alcohol (25:24:1) was added, and the mixture was centrifuged at $14,000 \times g$ for 10 min at 4°C to obtain a crude nucleic acid preparation. This nucleic acid preparation was used as a template source for first-strand cDNA synthesis in reverse transcriptase reactions as previously described (32). Nucleic acid extract from leaves of healthy plants (mock-inoculated with PBS) was used as negative control, whereas nucleic acids extracted from leaves of sugar beet seedlings either protected or challenged with BSBMV or BNYVV, respectively, were used as positive controls.

Amplification of the specific sequence by reverse transcription-polymerase chain reaction. A virus-specific cDNA fragment was amplified from total nucleic acid derived from virus-infected tissues by RT-PCR as described by Rush et al. (32). A primer pair was commercially synthesized for BNYVV RNA 1 based on published nucleotide sequence data from European isolates (4). The downstream reverse primer, BNYVV 4 (5' CACTTT CCATATTGCGG 3'), corresponds to residues 1,029 to 1,046 of BNYVV RNA 1. The upstream primer, BNYVV 2 (5' TTT GTCTGATGCTATTG 3'), corresponds to residues 465 to 482 in BNYVV RNA 1. A second primer pair was commercially synthesized for BSBMV RNA 2 (accession no. AF 061869). The downstream reverse

Table 1. Effects of interval between beet soilborne mosaic virus (BSBMV, protecting) and beet necrotic yellow vein virus (BNYVV, challenge) inoculation on the incidence of cross-protection

Inoculation interval ^c	Virus tested	Virus detection frequency ^a (D/S ratio) ^b in leaves			
		10 days ^d	15 days	20 days	30 days
0	BSBMV	0.6 (0.9)	0.8 (1.0)	0.6 (1.0)	0.6 (1.0)
	BNYVV	0.8 (0.9)	0.8 (0.7)	0.6 (1.0)	1.0 (1.0)
2	BSBMV	1.0 (1.0)	0.8 (1.0)	0.6 (1.0)	0.8 (1.0)
	BNYVV	0.8 (0.7)	0.2 (0.3)	0 (0)	0.2 (0.4)
5	BSBMV	0.8 (1.0)	1.0 (1.0)	0.8 (1.0)	1.0 (1.0)
	BNYVV	0.2 (0.4)	0 (0)	0 (0)	0 (0)
10	BSBMV	0.8 (1.0)	0.8 (1.0)	0.8 (1.0)	0.8 (1.0)
	BNYVV	0 (0)	0.2 (0.1)	0 (0)	0 (0)
15	BSBMV	0.8 (1.0)	0.8 (1.0)	0.8 (0.8)	0.6 (1.0)
	BNYVV	0.2 (0.2)	0.2 (0.2)	0.4 (0.1)	0.2 (0.2)

^a Calculated by dividing the number of infected plants by the total plants tested.

^b Value obtained by calculating the “D” value (mean enzyme-linked immunosorbent assay [ELISA] value for doubly infected plants minus mean ELISA value for healthy plants) and dividing by the “S” value (mean ELISA value for singly infected plants minus mean ELISA value for healthy plants). For instance, if mean ELISA value for healthy samples is 0.04, for singly infected samples is 1.07, and for doubly infected samples is 0.972, then the D/S ratio will be as follows: D/S ratio = $(0.972 - 0.04)/(1.07 - 0.04) = 0.9$.

^c Interval in days between protecting (BSBMV) and challenge (BNYVV) inoculations.

^d Days after challenge inoculation. Samples were tested by double antibody sandwich (DAS)-ELISA.

primer, BSBMV Cp5-1 (5' CACAGCTCT CACATTGC 3'), corresponds to residues 3,030 to 3,046 of BSBMV RNA 2. The upstream primer, BSBMV Cp5-15 (5' CAGCATAAGCAGAATGC 3'), corresponds to residues 2,044 to 2,060 of RNA 2.

The RT-PCR mixture reaction (50 µl) contained 4 µl of 10 pmol of each primer, 10 mM dithiothreitol, 10 mM of each dNTP, 3.0 units of *Vent* DNA polymerase (New England Bio Labs, Beverly, MA), 5 µl of the 10× buffer supplied with the enzyme, and 37 µl of water. Nucleic acid extracts, previously described, were used as templates for first-strand cDNA synthesis in reverse transcriptase reactions. Heat denatured nucleic acid was added to the reaction mixture, which was incubated at 42°C for 1 h to allow first-strand cDNA synthesis. The PCR mixture was subjected to 35 cycles of 1 min at 94°C, 1 min at 41°C, and 2 min at 72°C. During the first and last cycle, the extension step was held at 72°C for 10 min, followed by holding the reaction mixture at 4°C until it was removed from the thermocycler. PCR products were resolved by 1% agarose gel electrophoresis and visualized by ethidium bromide staining. These tests were performed three times.

RESULTS

Cross-protection between BSBMV and BNYVV. The characteristic symptoms of BNYVV were not observed within 30 days in plants that were protected and challenged. Expected symptoms developed in plants that were challenged but not protected. Based on ELISA, cross-protection occurred when plants were protected by BSBMV and challenged with BNYVV (Table 1). With a smaller interval between protecting and challenging virus, a greater number of plants had detectable amounts of challenge virus. But as the inoculation interval increased, the incidence of cross-protection increased to almost complete cross-protection when the interval was 5 and 10 days (Table 1), a period during which virus accumulated to maximum level in plants singly infected with BSBMV (data not shown). No cross-reactivity of antisera by these viruses was found. Results between repeated tests were similar.

Cross-protection between BSBMV and BNYVV also was demonstrated in experiments in which plants were tested by Western blotting assay. The results were consistent with the corresponding ELISA results (Table 2). A protein band with migration of approximately 22.5 kDa was observed with samples positive for BNYVV (Fig. 1A). BSBMV capsid protein migrated slightly more slowly than BNYVV in SDS-PAGE analyses (Fig. 1B). In many sample preparations, an additional protein band of approximately 20 kDa was also detected. This was possibly a degra-

dation product of the capsid protein of BSBMV, as has been reported for other viruses (5,34). In Western blots and ELISA, samples found to be positive for BNYVV proteins reacted only to antiserum made to BNYVV, and not to antiserum to BSBMV, and vice versa. No mock-inoculated controls reacted with either antiserum.

Reciprocal cross-protection between BSBMV and BNYVV. Table 3 shows the results for time course studies of reciprocal

cross-protection experiments with BNYVV (protecting) and BSBMV (challenge) when plants were tested by ELISA. Cross-protection was evident in the protected plants within 15 days after the challenge inoculation when the inoculation interval was 5 days. Some of the protected plants showed signs of cross-protection 10 days to 30 days after the challenge virus inoculation when the inoculation interval was 2 days but the effectiveness of cross-protection was not consistent. For example, 15 days

Table 2. Detection of beet soilborne mosaic virus (BSBMV, protecting) and beet necrotic yellow vein virus (BNYVV, challenge) by Western blotting in cross-protection experiments

Inoculation interval ^a	Virus tested	Virus detection in leaves			
		10 days ^b	15 days	20 days	30 days
5	BSBMV	3/4 ^c	4/4	3/4	3/4
	BNYVV	0/4	0/4	1/4	1/4
10	BSBMV	4/4	4/4	4/4	3/4
	BNYVV	0/4	0/4	0/4	0/4

^a Interval in days between protecting (BSBMV) and challenge (BNYVV) inoculations.

^b Days after challenge inoculation.

^c Number infected/number tested.

Fig. 1. Detection of (A) beet necrotic yellow vein virus (BNYVV) and (B) beet soilborne mosaic virus (BSBMV) by Western blotting after probing with antibodies to BNYVV and BSBMV (1.0 and 1.3 mg/ml, respectively, at 1:1,000 dilution) and anti-rabbit goat antibody alkaline-phosphatase conjugate (0.8 mg/ml at 1:1,000 dilution). Lane 1, molecular mass standards; lane 2, extracts from healthy sugar beet; lane 3, known positive control, (A) BNYVV-infected sugar beet and (B) BSBMV-infected sugar beet; lanes 4 and 5, sugar beet vortexed with BSBMV; lanes 6 and 7, sugar beet vortexed with BNYVV; and lanes 8 and 9, sugar beet protected with BSBMV and challenged after 10 days with BNYVV. Figures on left margin denoted sizes of molecular markers (kDa).

after the challenge inoculation, the challenge virus was detected in 80% of protected plants, but 30 days after the challenge inoculation, no protected plants were detected with challenge virus. In these experiments, the cross-protection persisted for 30 days when the inoculation interval was 10 days.

RT-PCR. When total nucleic acid extracted from leaf tissue from doubly infected plants was used as template material for RT-PCR, cDNA fragments representing the BNYVV and BSBMV sequences were amplified (Fig. 2). The resulting RT-PCR products were of expected size (~1,000 bp for BSBMV, ~500 bp for BNYVV) and

comigrated in agarose gels with products derived from the positive controls (Fig. 2, lanes 4 and 7, respectively). No PCR products were detected from total nucleic acid extracted from healthy controls (Fig. 2, lanes 2 and 3). Only those samples (~64 samples) that showed the phenomenon of cross-protection in Western blot assays were analyzed by RT-PCR. Results from all repeated tests were identical.

DISCUSSION

These results are the first report of cross-protection of BNYVV by BSBMV. ELISA and Western blotting provided quantitative evidence that BSBMV was

able to confer cross-protection against BNYVV. The antisera used in these studies were specific to their homologous viruses, which allowed accurate discrimination between BNYVV and BSBMV in doubly inoculated plants. Reciprocal cross-protection between these two viruses was also demonstrated by ELISA and Western blotting (data not shown). However, use of the specific primers for BNYVV and BSBMV in RT-PCR reactions indicated that viral RNA of the challenge virus survived in protected plants.

In our study, the effectiveness of cross-protection was greatly affected by the inoculation interval. Effective cross-protection occurred when the inoculation interval was 5 days or more. This suggests that a minimum time interval between protecting and challenging inoculations is required. Beyond this minimal interval, the degree of cross-protection increases. Our results are similar to those of Wen et al., who reported cross-protection among strains of barley yellow dwarf virus (41), although they extended their postinoculation interval beyond 15 days. We were unable to challenge inoculate after 15 days because the vortex inoculation method was ineffective with plants older than 15 days.

Cross-protection is generally studied by mechanical inoculation with the protecting and challenging viruses in the plant. Occasionally, challenge viruses have been inoculated by using natural vectors such as aphids (6,19,41). Mechanical inoculation of sugar beets by hand with BNYVV or BSBMV is time-consuming and yields unsatisfactory results. Also, since BNYVV and BSBMV are naturally vectored by *P. betae*, a soilborne obligate parasite, it would have been difficult to use *P. betae* for cross-protection studies. Furthermore, systemic movement of these viruses from naturally infected roots is unreliable. The vortexing method of inoculation resulted in a high incidence of systemically infected young plants that were protected from symptoms of the challenge virus. Although this technique is not applicable to virus interactions in nature, it provided a means of investigating cross-protection between these two closely related viruses.

This is the first report in which RT-PCR was used to identify both protecting and challenge viruses in cross-protection studies. We designed primers that amplified specific PCR products from BNYVV and BSBMV RNA in doubly infected plants. However, this method was not useful for determining whether challenge virus RNA was inhibited or decreased. Although we were unable to detect challenge virus capsid in doubly infected plants by ELISA or Western blotting, RNA of the challenge virus was still present in the doubly inoculated plants. This suggests that the challenge virus was unable to replicate in the plants. This would explain reports in which cross-protection eventually breaks down

Table 3. Effects of interval between beet necrotic yellow vein virus (BNYVV, protecting) and beet soilborne mosaic virus (BSBMV, challenge) inoculation on the incidence of cross-protection

Inoculation interval ^c	Virus tested	Virus detection frequency ^a (D/S ratio) ^b in leaves			
		10 days ^d	15 days	20 days	30 days
0	BNYVV	0.8 (0.6)	0.6 (0.8)	0.8 (0.6)	0.8 (1.0)
	BSBMV	0.8 (0.6)	0.6 (0.8)	0.8 (1.0)	0.8 (0.9)
2	BNYVV	0.8 (1.0)	1.0 (1.0)	1.0 (1.0)	0.8 (0.9)
	BSBMV	0.2 (0.2)	0.8 (0.8)	0.8 (0.6)	0 (0)
5	BNYVV	0.6 (1.0)	1.0 (1.0)	0.8 (1.0)	1.0 (1.0)
	BSBMV	0.6 (0.9)	0.2 (0.1)	0 (0)	0.2 (0.1)
10	BNYVV	0.8 (1.0)	1.0 (1.0)	1.0 (1.0)	1.0 (1.0)
	BSBMV	0 (0)	0.2 (0.1)	0 (0)	0 (0)
15	BNYVV	0.8 (1.0)	1.0 (1.0)	1.0 (1.0)	0.8 (1.0)
	BSBMV	0.2 (0.4)	0.4 (0.3)	0 (0)	0.4 (0.2)

^a Value obtained by dividing the number of infected plants by the total plants tested.

^b Value obtained by calculating the "D" value (mean enzyme-linked immunosorbent assay [ELISA] value for doubly infected plants minus mean ELISA value for healthy plants) and dividing by the "S" value (mean ELISA value for singly infected plants minus mean ELISA value for healthy plants).

^c Interval in days between protecting (BNYVV) and challenge (BSBMV) inoculations.

^d Days after challenge inoculation. Samples were tested by double antibody sandwich (DAS)-ELISA.

Fig. 2. Agarose gel electrophoresis of polymerase chain reaction products representing beet necrotic yellow vein virus (BNYVV) and beet soilborne mosaic virus (BSBMV). Lanes 1 and 10, 1-kb DNA ladder (Gibco BRL, Gaithersburg, MD); lanes 2 and 3, negative control from healthy sugar beet leaves inoculated with phosphate buffered saline; lanes 4 and 5, positive controls, representing BSBMV-inoculated sugar beet leaves; lanes 6 and 7, positive controls, representing BNYVV-inoculated sugar beet leaves; and lanes 8 and 9, sugar beet leaves challenged with BNYVV 10 days after protection with BSBMV and harvested for extraction at 20 days postinoculation interval. Lanes 2, 4, 6, and 8 represent samples using BSBMV primers BSBMV cp-1 and cp-15. Lanes 3, 5, 7, and 9 represent samples using BNYVV primers BNYVV 2 and 4. This shows that RNA of the challenge virus survives in the protected plants.

and the host succumbs to the challenge virus.

Huss et al. reported the breakdown of cross-protection when *C. quinoa* was challenged with grapevine fan leaf virus-F13 (GLFC-F13), a nepovirus (18). In their experiments, during the first 15 to 20 days after GLFC-F13 (challenge) inoculation, the quantity of this isolate remained very low. After that time, F-13 increased in such a way that the amounts in reference plants and in test plants remained the same. Dodds et al. also reported the breakdown of cross-protection with cucumber mosaic virus (CMV-P), a cucumovirus (7). Cross-protection was overcome as indicated by the detection of dsRNA and virions of CMV-P in the challenge-inoculated leaves of tomato when the challenge inoculum was RNA. Sherwood and Fulton reported that local lesions caused by necrotizing strains of tobacco mosaic, a tobamovirus (TMV), were formed in light green islands in *Nicotiana sylvestris* leaves previously infected with TMV when inoculated with RNA but not with virus (36). These reports and our detection of RNA of challenge virus in doubly inoculated plants support the theory that some mechanism(s) for prevention of superinfection involves capsid protein of the challenge virus in cross-protection. Whether or not cross-protection between BNYVV and BSBMV occurs in this manner is speculative.

BNYVV poses a significant threat to sugar beet production, and the presence of BNYVV nationwide increases the risk of disease problems in this important crop. This study provides impetus to investigate the ecological and epidemiological implications of cross-protection as they relate to rhizomania disease of sugar beets. These results also raise additional questions concerning the taxonomic relationship between BNYVV and BSBMV, since cross-protection usually occurs between virus strains and not between closely related viruses. Although BNYVV and BSBMV are separate viruses, regions with greater than 90% nucleotide sequence homology exist on RNA2 of these two viruses. These regions may be implicated in the cross-protection observed in this study, which would add to our understanding of mechanisms involved in cross-protection between multipartite soilborne viruses.

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