Comparison of Serological Tests for the Detection of Two Soilborne Sugar Beet Viruses

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Rhizomania was first reported in the United States in 1984 in California (1). It has since been identified in other sugar beet–producing states including Texas, Colorado, Wyoming, Nebraska, Idaho, and Minnesota (7,11). The disease is caused by beet necrotic yellow vein virus (BNYVV) and is characterized by heavy lateral root proliferation, overall stunting, and constriction of the tap root (8). The soilborne virus is transmitted by *Polymyxa betae* Keskin (2), and infection by BNYVV reduces yield both in percent extractable sugar and tonnage.

Beet soilborne mosaic virus (BSBMV) was first reported in Texas in 1988 (6). BSBMV and BNYVV are closely related. BSBMV, like BNYVV, is a multiparticulate virus composed of rigid, rod-shaped particles and is transmitted by *P. betae* (4,10). The RNA species and coat protein sizes of both viruses are similar. Roots of sugar beets infected with BSBMV often appear healthy, though beets have been collected that exhibit typical symptoms of rhizomania but test positive only for BSBMV. BSBMV is found to systemically infect beets in the field more frequently than BNYVV, and foliar symptoms include broad yellow vein banding and mottling. To date, studies indicate that BSBMV causes some loss of yield, but not to the extent that BNYVV does. BSBMV has been identified in the same growing areas as BNYVV (7,11).

Since these viruses are similar and are found in the same growing areas, it is important to be able to differentiate them by serological testing. BSBMV and BNYVV are serologically distinct. However, depending on test conditions and the antiserum used, cross reaction may occur (4,10). There have been conflicting results from different labs that conduct BNYVV testing on field samples. This study was conducted to compare variation in results among different serological assays used to test field beet samples. A second part of the objective was to compare variation in test results when using antiserum developed to whole virus particles or denatured capsid of BNYVV and BSBMV.

Materials and Methods

In 1997, approximately 325 beets were collected from fields in Texas and Minnesota. In 1998, 235 beets were collected from fields in Minnesota, Colorado, Nebraska, and Texas. Results presented here are for beets collected in 1997. Beets that exhibited root or foliar symptoms indicating possible infection by BSBMV or BNYVV were selected for the study. Twelve replications, 20-30 beets each, were tested.

Antisera were developed in rabbits to BNYVV and BSBMV whole virus particles or denatured capsid. IgG was fractionated from the four antisera (BNYVV-whl, BNYVV-den, BSBMV-whl, and BSBMV-den; -whl indicates antiserum developed to whole virus particles, and –den indicates antiserum developed to denatured capsid).

Two ELISAs using these antisera were evaluated. The first, indirect DAS ELISA (which will be referred to as DAS ELISA in the remainder of this paper), plates were coated with IgG, samples were probed with a secondary biotin-labeled IgG, and the secondary antibody was detected with avidin-conjugated alkaline phosphatase (4). In the second type of ELISA, $F(ab')_2$ indirect ELISA, plates were coated with $F(ab')_2$ fragments generated from the four antisera, and samples were probed with the respective unfractionated antiserum. Protein-A conjugated alkaline phosphatase was used to detect the antiserum probe (3).

Samples were also tested by BNYVV ELISA reagents obtained from a commercial source (Bioreba Ag) and by Western blot analyses. Reagents obtained from Bioreba Ag were for a simple direct DAS ELISA. For Western blots, samples were extracted, denatured, and stored frozen until they were tested. Antiserum developed to denatured capsid of BNYVV or BSBMV were used to probe samples tested by Western blot (5,9).

Buffers used in all ELISAs were the same, and plates were incubated under the same conditions. Samples for ELISA were ground in extraction buffer at a ratio of 1:10 (w/v), and root samples prepared for Western analyses were extracted at a ratio of 1:3 (w/v).

To compare tests, Western analyses for BNYVV and BSBMV were chosen as standard tests. BNYVV and BSBMV ELISA results were compared to respective Western results on a beet-by-beet basis. If an ELISA result matched that of the same beet tested by Western blot, that was considered to be a match. The number of matches for beets tested by one ELISA from a replication were counted and converted to a percentage (number of matches divided by the number of beets tested). Data were analyzed to determine if results of any test matched those of Western blot analyses more closely than other test results.

To determine if any test (including Western blot) was consistently detecting the highest number of positive samples, tests were ranked by replication in terms of which test detected the highest percentage of positive samples. A ranking of 1 was assigned to the test or tests that detected the highest percentage of positive samples, and rankings of 2, 3, etc., were assigned to tests that detected lower percentages of positive samples, respectively, within a replication. Tests detecting the same percentage of positive samples within a replication were assigned equal rankings.

Results and Discussion

The percentage of positive samples, by test and replication, are indicated in Table 1. Most replications included beets positive for BNYVV and/or BSBMV.

Percentage of matching results for BSBMV tests and the range of percent matches for each test are in Table 2. Results of beets tested by DAS ELISA using BSBMV-whl and BSBMV-den antisera matched those of Western blot analyses more closely than results from $F(ab')_2$ ELISA using BSBMV-den antiserum. The range of percent matches was from 15-100 for all tests. In three $F(ab')_2$ BSBMV-den ELISA replications, fewer than 25% of the results matched those of beets tested by Western blot. In these replications, Western blot analyses and the other ELISAs usually indicated that most of the beets were negative for BSBMV; this test indicated that most of the beets were positive for BSBMV.

Among BNYVV tests (Table 3), results of DAS ELISA using antiserum developed to BNYVV denatured capsid matched those of Western blot analyses significantly less frequently than results of all other ELISAs. No differences in percentage of matching results were indicated among the other four tests.

Future analyses of the data will take into consideration how results of ELISA tests varied from those of Western blot analyses. In other words, ELISA results not matching Western results will be scored in terms of whether the ELISA result was positive and the Western result was negative, or vice versa.

Rankings of test results for BSBMV and BNYVV are in Tables 4 and 5. Among BSBMV tests, DAS ELISA using antiserum developed to whole virus particles detected the highest

Table 1. Percentage of beet root samples positive for BNYVV or BSBMV. Approximately 20-30 beets per replication were tested.

	DAS		Comm.[2]	Western		DAS		F(ab')$_2$			
Test	T-den[1]	B-den	BNYVV	T-den	B-den	B-whl	T-whl	B-whl	B-den	T-whl	T-den
Replication											
EBS	11	64	86	7	86	79	0	79	71	39	93
WBS	14	34	97[3]	24	83	100	14	93[3]	86	21	69
TEA	33	57	73	57	80	70	30	63[3]	60	33	40
F 9-26	95	81	71	95	48	86	90	86[3]	62	95	100
BMN	17	97	100[4]	33	83	90	27	97[4]	100	27	7
F10-10	81	95	95	76	86	95	81	33[3]	67[3]	52	67
FHP	10	63	100	20	100	97	10	100[4]	73	50	33
N 9-26	55	75	90	30	45	90	45	80[4]	75	55	50
EBS 3	7	83	79	3	53	90	20	83	62	59	79
DFR	57	93	100[3,4]	17	83	100	27	87[3,4]	100	70	100
EAC	37	20	43[3,4]	10	0	30	7	0[3,4]	0	0	0
SBS	58	25	100[4]	24	90	100	23	89[3,4]	46	32	71

[1]T indicates BSBMV (TX7); B indicates BNYVV; –den indicates antiserum developed to denatured capsid; –whl indicates antiserum developed to whole virus particles.
[2]ELISA reagents obtained from a commercial source.
[3]BSBMV control tested positive.
[4]BSBMV-like isolate (RC) tested positive.

Table 2. Percentage of BSBMV ELISA results matching those of Western blot analyses.

ELISA	Antiserum	% Match	Range[2]
DAS	-whl[1]	83.8 a	67-95
DAS	-den	75.4 ab	47-97
F(ab')$_2$	-whl	70.1 b	41-100
F(ab')$_2$	-den	56.0 c	15-95

[1]-whl indicates antiserum developed to whole virus particles; -den indicates antiserum developed to denatured capsid.
[2]Range indicates the highest and lowest percent match values.

Table 3. Percentage of BNYVV ELISA results matching those of Western blot analyses.

ELISA	Antiserum	% Match	Range[3]
Comm.[1]	-	79.0 a	55-100
DAS	-whl[2]	76.3 a	52-97
F(ab')$_2$	-whl	74.3 a	35-100
F(ab')$_2$	-den	73.3 a	50-100
DAS	-den	58.1 b	21-81

[1]Commercially available BNYVV ELISA reagents.
[2]-whl indicates antiserum developed to whole virus particles; -den indicates antiserum developed to denatured capsid.
[3]Range indicates the highest and lowest percent match values.

percentage of positive samples least often, and F(ab')$_2$ ELISA using antiserum developed to denatured capsid detected the highest percentage of positive samples most frequently. Among BNYVV tests, ELISA using commercial reagents and DAS ELISA using antiserum developed to whole virus particles detected the highest number of positive samples more frequently than other tests. No differences were indicated in rankings among Western analyses and F(ab')$_2$ and DAS ELISAs using antiserum developed to denatured capsid.

Table 4. Rankings of test results based on percentage of beets testing positive for BSBMV within a field[1].

Assay	Antiserum	Rank
$F(ab')_2$	-den[2]	1.9 a
$F(ab')_2$	-whl	2.4 b
DAS	-den	2.6 b
Western	-den	2.9 c
DAS	-whl	3.4 d

[1]A ranking of 1 was assigned to the test within a replication that detected the highest percentage of positive samples. Rankings increased numerically as the percentage of positive samples indicated by a test decreased.

[2]-den indicates antiserum developed to denatured capsid; -whl indicates antiserum developed to whole virus particles.

Table 5. Rankings of test results based on percentage of beets testing positive for BNYVV within a field[1].

Assay	Antiserum	Rank
Comm.[2]	-	1.5 a
DAS	-whl[3]	1.6 a
$F(ab')_2$	-whl	2.6 b
Western	-den	3.2 c
$F(ab')_2$	-den	3.3 c
DAS	-den	3.3 c

[1]A ranking of 1 was assigned to the test within a replication that detected the highest percentage of positive samples. Rankings increased numerically as the percentage of positive samples indicated by a test decreased.

[2]Commercially available BNYVV ELISA reagents.

[3]-whl indicates antiserum developed to whole virus particles; -den indicates antiserum developed to denatured capsid.

The weakness in ranking data in this way is that information on how much the values of percentage of positive samples detected varied among tests within a replication was not indicated. Rankings of 1 and 2 might mean that one test detected 90% positive samples and another test detected 60% positive samples. Or rankings of 1 and 2 might mean that one test detected 35% positive samples and another test detected 34% positive samples. A way to avoid this would be to group percent positive values into class rankings and assign numerical rankings to different classes instead of to individual scores.

Cross-reaction has been reported previously between BNYVV and BSBMV, depending on the test, test conditions, and antiserum used (4,10). BNYVV and BSBMV controls were included in all tests used in this study, and, for most tests, controls reacted as expected. However, in seven $F(ab')_2$ ELISAs using BNYVV-whl antiserum, one $F(ab')_2$ ELISA using BNYVV-den antiserum, and three commercial BNYVV tests, the BSBMV control tested positive. In six $F(ab')_2$ ELISAs using BNYVV-whl antiserum and four commercial BNYVV tests, an isolate referred to as RC reacted positively. RC typically reacts positively for BSBMV, but with a much weaker reaction than a standard BSBMV positive control. Tests which indicated cross reactions mentioned above are noted in Table 1. For the most part, cross reaction was not observed in control samples.

Given the potential for cross reaction between BNYVV and BSBMV, it is important to include a BSBMV positive control in BNYVV tests and vice versa, particularly when evaluating new assays. When testing samples collected in 1998, reagents for BNYVV ELISA were obtained from a commercial supplier different from the one used in 1997 tests. At the manufacturer's recommended 1:100 dilution of BNYVV IgG and alkaline phosphatase-conjugated IgG, there was a strong positive reaction by the BSBMV control. When reagents were diluted to 1:750, results were similar to those obtained using Bioreba Ag reagents.

Besides cross reaction, a possible reason for variation in test results observed could be differences that can occur among ELISAs in terms of sensitivity and specificity. It has been

reported that indirect ELISAs, such as $F(ab')_2$ tests, can be more sensitive and less specific than direct DAS ELISAs. $F(ab')_2$ tests can detect a broader range of serologically related viruses (3).

Even though variation in results occurred, most ELISA results were within 70-80% agreement of Western results. For speed and ease of handling large numbers of samples, ELISA is a suitable test. However, samples should be tested in more than one way if results are in question.

Use of assays which incorporate molecular probes specific for BNYVV and BSBMV would provide further verification of serological results. Northern hybridization and RT-PCR would be appropriate tests. However, RT-PCR would be more sensitive and better suited to detect BNYVV or BSBMV in field beet samples in which the titer might be low and difficult to detect by Northern hybridization.

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