Presence and distribution of BNYVVand BSBMV in the Glyndon Rhizomania research site.

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Sugarbeet Rhizomania is a serious disease in many growing regions of the world that effects root growth and morphology and beet yield (Duffus and Ruppel, 1993). Disease symptoms are caused by beet necrotic yellow vein virus (BNYVV; see http://image.fs.uidaho.edu/vide/descr086.htm for a description) infection primarily in root tissues although virus movement to upper leaves of the plant may cause classic vein necrosis or fluorescent yellowing of the leaves. The virus is transmitted to beet roots by the Plasmodiophoromycete fungus *Polymyxa betae* which uses a motile zoospore to seek out root surfaces. The fungal vector *P. betae* transmits other viruses of sugarbeet having a morphology similar to that of BNYVV, namely beet soilborne mosaic virus (BSBMV), beet soilborne virus (BSBV) and beet virus Q (BVQ). Whereas BSBMV and BSBV are known to exist in the U.S., BVQ has only been reported to occur in Europe.

Rhizomania disease of sugarbeet was first detected in growing regions of Minnesota and North Dakota in the mid 1990's prompting concern about the future impact of this disease on the industry. With the discovery of BSBMV (Heidel et al., 1997), it became clear that at least two viruses might have to be addressed in strategies to control Rhizomania and similar diseases. Although BSBMV symptoms generally are distinguishable from those produced by BNYVV, confusion in diagnosis may occur. Using of antisera specific for each virus, confirmation of which virus is associated with the diseased symptoms observed can be made.

The Glyndon MN research site for Rhizomania studies consists of 80 acres previously confirmed for the presence of BNYVV. Determination of the spatial distribution of BNYVV and related viruses across the research site would produce data that could be used by other investigators as they evaluate the performance of varieties and/or treatments tested at the location. This study reports the second year testing of sugarbeet acres planted at the site in 2004 using enzyme-linked immunosorbant assay (ELISA).

Experimental Procedures

Samples to be tested were harvested during the week of July 12, 2004 when sugarbeets at the site were at the 6 to 8 leaf stage. Since the research site was planted predominantly to 6-row plots, the first row (from left to right) planted with a BNYVV-susceptible variety was used for sampling. Progressing from south to north across the study, alternating plots were sampled from, resulting in 22 plots sampled in this direction. Longitudinal sampling was performed by generating 49 grid points in an east-to-west direction, with

30 feet separating each grid point. Combined, this generated 1078 sampling grid points covering approximately 85% of the total acreage planted to sugarbeet at the site in 2004.

Each sample consisted of 3 beet plants pulled at random from the susceptible check row. Where a grid point was located in an alley way, 3 beets were harvested from the susceptible check row closest to that point. Also, where sampling grids coincided with strip trial sugarbeets at the west end of the site, beets closest to the grid points were harvested for testing. Harvested beets were placed into labeled paper bags and stored at 4°C less than one week during processing.

Sampled sugarbeets were washed free of soil, blotted dry and lateral roots, adjacent root epidermis, and adjacent root cortex were excised and weighed to yield 2 g of root material. Sample tissue was transferred to plastic grinding bags (AgDia,) and pulverized in the presence of 4 ml phosphate-buffered saline (PBS pH 7.4) containing 0.05% TWEEN 20, 2% polyvinylpyrrolidone, and 0.2% chicken albumin (Sample Buffer). Two 1.5-ml volume microfuge tubes were filled with the pulverized slurry and centrifuged at 16,000 x g for 5 min. Supernatants were transferred to two labeled microfuge tubes for storage at –80°C prior to testing. For testing of soil, seed of variety ACH9369 were distributed on the surface of Sunshine Mix #1 in 4" pots and overlayed with ~0.5" of test soil sample. Seedlings were sprouted in a greenhouse maintained at 24°C under a 16 hr daylength and harvested at 14 days post-germination. Extracts were prepared for analysis as above.

Negative control extract was prepared by grinding greenhouse-raised sugarbeet (ACH 9369) grown in Sunshine Mix #1. Positive control extract was prepared by grinding leaves exhibiting viral lesions of BNYVV or BSBMV after mechanical inoculation in the greenhouse. ELISA-grade plates (96-well) were incubated with rabbit antiserum (kindly provided by Dr. Hsing-Yeh Liu, USDA-ARS, Salinas, CA) reactive with either BNYVV or BSBMV in the presence of sodium carbonate buffer (pH 9.6) for 16-18 hr at 4°C. Wells were rinsed several times with PBS containing 0.05% TWEEN 20 (PBST), after which 75 ul of thawed sample and control extracts were transferred to the wells. All samples were tested in duplicate and the outside rows of the plate were not used. Plates were then incubated for 4 hr at room temp. followed by rinsing of the wells several times with PBST.

Anti-virus antiserum conjugated to alkaline phosphatase was diluted in Sample Buffer and 100 ul was added to each well. The plate was incubated at 37°C for 1 hr and the wells subsequently rinsed several times with PBST. Finally, a solution containing 1 mg/ml paranitrophenyl phosphate (pNPP) in diethanolamine buffer (pH 9.6) was transferred at a rate of 200 ul per well. Plates were incubated at room temperature and absorbance in the wells recorded at a wavelength of 405 nm using a microplate reader (Tecan GEnios Reader). Plates were recorded at 30' and 70' after substrate addition. Data were transferred to spreadsheet software (Microsoft Excel) for analysis.

Results and Discussion

Of the 1078 root samples tested for the presence of BNYVV, 3.1% were confirmed positive (Fig. 1). Samples are considered to be positive when their absorbance values exceed 3X the value obtained for the negative control sample. For soil samples, 2.9% of the samples tested positive for BNYVV (Fig. 1), indicating that the soil test is comparable to root testing for detecting the virus in a test site. The greatest number of BNYVV-positive samples was found near the north central edge of the research site (Fig. 1). This would be closest to ditch water flow, commonly associated with the distribution of Rhizomania. All 1078 samples tested with antiserum to BSBMV were negative for this virus even though positive controls on the plate were clearly positive (not shown).

The data presented represent the testing of the second 20 of an 80-acre rotation scheduled to be planted to sugarbeet for future studies. Apparent concentration of BNYVV near the north end of this 20 acre parcel should be taken into account when the performance of treatments in the 2004 replicated plots are evaluated. It should be noted that competition between BNYVV and BSBMV for infection establishment previously has been documented (Piccinni et al., 2000). Therefore, it is possible that BSBMV does in fact exist in this 20 acre parcel, but was masked by earlier infection with BNYVV. This could be tested by evaluating Rhizomania-resistant sugarbeet varieties that are known to be susceptible to BSBMV (bred for resistance to BNYVV) in this location. Nevertheless, concurrence of the data between tested roots and tested soil in 2004, along with data from 2003, suggests that BSBMV is very low to absent in this location.

References

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Figure 1

	V	U	T	S	R	Q	P	O	N	M	L	K	J	I	H	G	F	E	D	C	B	A
1	1.8	0.9	1.6			1.8														2.1		
2									1.5													
3																						
4																						
5																						
6																						
7					1.6																	
8	1.8																					
9																						
10	1.8							1.6												1.1		
11																0.9						
12																0.9						
13																0.9						
14								0.9	2.3													
15								0.9														
16																		1				2.5
17	2			2.2																1.2		
18				1.9		2																
19	2.5			0.9	1.5														1			0.9
20			2																			
21	0.9																					
22	1						0.7								1.9							
23																						
24																						
25										1.5												
26	2.2																					0.7
27																						1.2
28			0.9																	2.1		
29				0.9																		
30								1.8														
31	0.7												0.7									
32				0.9	0.8								0.7									
33	2																					
34			1.5											0.7								
35															0.7							
36				0.7																		
37	2.3							1.1														
38			1.3																			
39																						
40			1.8	0.9		1.9																
41																						
42																						
43																						
44																			1.6		2	
45																			1.4			
46																						
47			1.1																0.3 = neg. control			
48		1																	1078 samples			
49	1.1		0.8																			

Figure 1. Field plot for Rhizomania tests at Glyndon, MN in 2004. Numerals 1->49 represent sampling points at 30 ft. intervals in an east-west direction. Alphabetical letters A->V are directed south to north and are spaced according to alternating 6-row plots where susceptible plants in row 1 of each plot were sampled. Stippled backround designates BNYVV positives in the testing of soil samples, whereas gray background designates BNYVV positive root sample locations on the grid. All positives in the ELISA test were at least 3X higher in absorbance at 405nm than the negative control (0.3OD).