Field performance advantage of sugar beet hybrids with *Rz1+Rz2* genes in Minnesota

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ABSTRACT

Beet necrotic yellow vein virus, BNYVV when vectored by the fungus *Polymyxa beta* causes the disease rhizomania of sugar beet. Rhizomania was first identified in the US in 1984 in the Imperial Valley of CA, and today is found in all major US beet growing areas including Minnesota (1996). The disease results in sugar yield loss (SY lbs./acre) greater than 20% under moderate levels of infection, and significant postharvest storage losses in terms of extractable sugar. Several major genes have been identified that confer tolerance to the disease. *Rz1* and *Rz2* are two which have been extensively used in commercial hybrids. Worldwide BNYVV exists as either a four stranded (Type A, B) or five stranded (Type P) member of the *Benyvirus* genus. Type A has variation on RNA3 in the P25 coding region and two motifs (ACHG and VCHG) have been identified in Minnesota and elsewhere. Potential yield trial sites in Minnesota were investigated through soil assay via GH bait test for BNYVV type (RT-PCR), sequenced for motif on RNA 3 and virus concentration levels (ELISA). Hybrids with *Rz1*, *Rz2* and with *Rz1+Rz2* were evaluated under conditions BNYVV Type A ACHG, BNYVV Type A VCHG, and slightly infected soil (ELISA < 0.01) to infected (ELISA > 0.35). Hybrids having both *Rz1+Rz2* genes exhibited greater field tolerance based on virus content and on relative SY than hybrids with either *Rz1* or *Rz2* alone. The use of *Rz1+Rz2* hybrids is an effective means to optimize sugar beet performance with multiple strains of BNYVV found in the upper Midwest.

INTRODUCTION

Beet Necrotic Yellow Vein Virus (BNYVV) is a soil borne virus member of the *Benyvirus* genus. When BNYVV is transmitted to susceptible sugar beets by the zoospores of the fungal vector *Polymyxa betae*, rhizomania occurs. Originally identified in the mid-1950's in Italy, rhizomania has now been identified throughout many of the major sugar beet growing areas of the world including most of Europe, Asia and North America. Worldwide BNYVV associated with rhizomania exists as either a four stranded (Type A, B) or five stranded (Type P, J) member of the *Benyvirus* genus.

In North America, rhizomania was first positively identified in the Imperial Valley of California in 1984. The disease spread to other North American commercial growing areas and was recognized in Minnesota by 1996 in the southern most commercial growing area of the state (Rush, Liu and Lewellen, 2006). The disease is now distributed throughout the major sugar beet growing regions of Minnesota including significant portions of the Red River Valley (Cattanach, 2010). The development of rhizomania is dependent upon environmental conditions that favor zoospore production of the *Polymyxa betae* vector including warm temperatures >72F together with high soil moisture. Once present, rhizomania persists in the soil while the viruliferous resting spores of the *Polymyxa* (cystosori) remain viable for more than 15 years (Abe and Tamada, 1986). Characteristic symptoms include a heavy proliferation of fine lateral roots,

tapering of the tap root (wineglass shape), vascular discoloration, elongated petioles, small leaves, wilting and general chlorosis.

Rhizomania is a potentially devastating disease of sugar beet that causes significant reductions in sugar content and in sugar yield resulting from small and poorly formed roots. Rhizomania has also been associated with significant postharvest storage losses with regards to extractable sugar (Campbell and Klotz, 2008).

Immediately following the identification of rhizomania in Minnesota, seed companies began providing rhizomania tolerant hybrids that relied heavily on one of two major genes that confer resistance: *Rz1* or *Rz2*. While the *Rz1* gene was initially effective, isolates of the BNYVV virus known to break the resistance or diminish the performance benefits of this major gene have since been identified in Minnesota as they had in other parts of the world (Acosta-Leal, Rush, 2007). Several BNYVV types can be identified by variation in the sequence within the P25 coding region of RNA3. At least three of these types have been found in Minnesota include those coded AFHG, ACHG and VCHG. In 2013, variety trial results under very severe rhizomania conditions in southern Minnesota indicated nearly all hybrids with more than one major resistance gene had outperformed those hybrids without multiple major genes for rhizomania resistance (Miller et al, 2014).

A study was conducted to confirm that the combination of the *Rz1+Rz2* genes is more effective than either major gene alone for optimizing sugar beet performance under various strains of rhizomania that have been identified to date in Minnesota. The *Rz2* gene used in this study is derived from a USDA release from Bob Lewellen.

MATERIALS AND METHODS

Field testing sites were grid sampled pre-plant for evaluation for rhizomania via a soil bait test. A rhizomania susceptible genotype (*rzrz*) was grown under greenhouse conditions for seven weeks and roots were pressed, sap was buffered and analyzed by ELISA for BNYVV. Positive samples were subsequently analyzed for BNYVV type using Real-Time PCR. Sites with three BNYVV types were identified by variation in the P25 region of RNA3: AFHG, ACHG and VCHG. A non-infected site was also identified. Six sites were selected for yield trial comparison to include non-infected and all three virus types. The non-disease site was located in the southern part of the state near Randolph, MN (NR3) outside of the commercial beet growing area. Infected sites included one from the Red River Valley in the American Crystal growing area near Felton, MN (FEL) and four from the Southern Minnesota Beet Sugar Cooperative growing area near the towns: Hector, MN (SM1), Maynard, MN (SM3), Prinsburg, MN (SM4), and Montevideo, MN (SM6). Pre-plant descriptions of the locations are described in terms of ELISA values and virus types in Table 01.

Location	Virus type based on P25 sequence	ELISA Values: pre-Plant soil bait test

NR3	NON-rhizomania	0.009
SM6	AFHG	0.350
FEL	ACHG*	Pre-plant info not available
SM3	ACHG	1.400
SM1	VCHG	2.500
SM4	VCHG	0.720

*determined pre-harvest

Table 01. Description of site: virus type and pre-plant BNYVV ELISA values.

A set of four hybrids differing for rhizomania major genes (Table 02) was planted at each of the six sites. Plots consisted of three 56cm rows by 8m in length and were replicated up to six times per location. Trials were managed using standard sugar beet crop production practices.

Variety Description	Genotype Information
Rz1+Rz2*	***Rz1rz*** ***Rz2rz***
Rz1	***Rz1rz***
Rz2	***Rz2rz***
rzrz	***rzrz***

Table 02.
*Varieties with multiple rhizomania resistance sources are marketed as MultiSource® by Betaseed, Inc.

Prior to harvest, hair root and root tip samples were collected from test plots with *Rz*1 and *Rz1+Rz2* genotypes on September 18, 2014. ELISA analysis was conducted to determine additional virus information by site and positive samples were again sequenced for virus type. All plots were harvested between September 20 and 29, 2014 and evaluated for root yield and sugar content. Sugar yield (lbs./acre) was calculated and relative figures were used for the comparison of yield performance.

RESULTS

The results of the pre-plant soil bait test and pre-harvest root samples indicated that no change in virus type had occurred at the testing sites during the growing season. The pre-harvest ELISA values indicate a much lower virus concentration in the *Rz1+Rz2* genotype compared to the *Rz1* genotype. The magnitude of this difference was greater under the ACHG and VCHG types than under type AFHG (Figure 01).

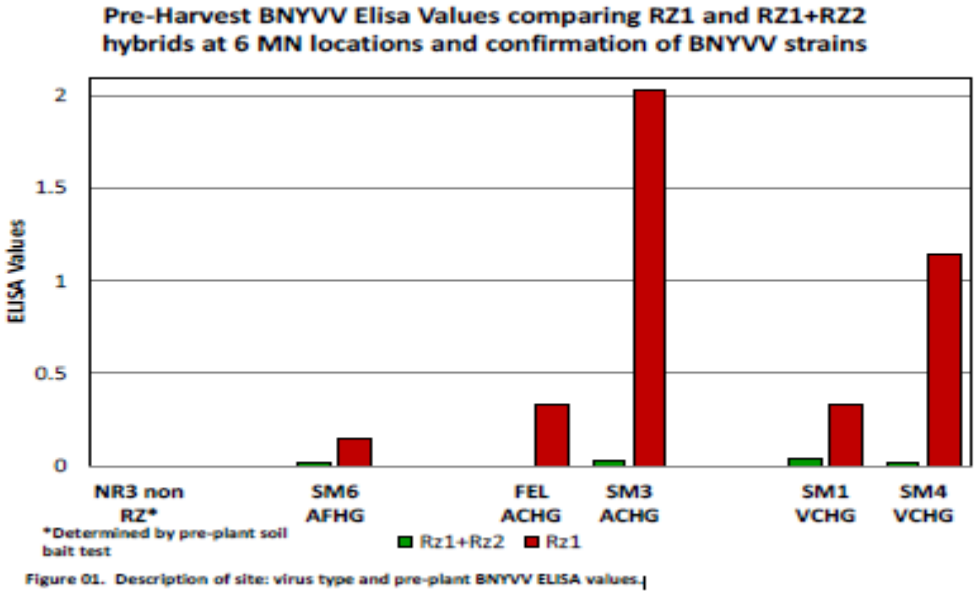
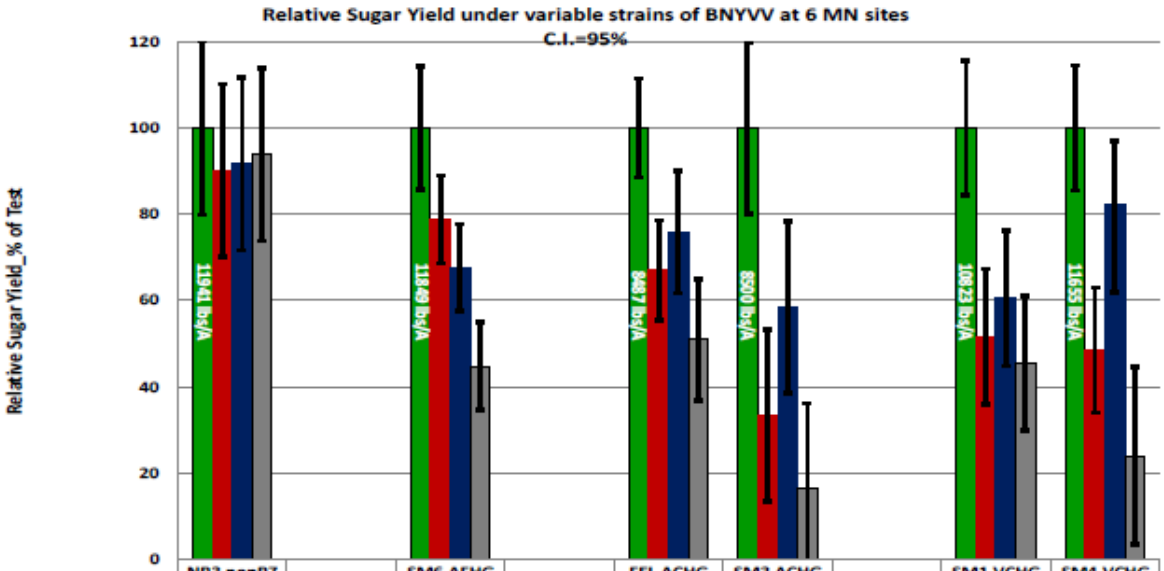


Figure 01. Description of site: virus type and pre-plant BNYVV ELISA values.

The four genotypes, rhizomania susceptible (*rzrz*), *Rz1*, *Rz2* and *Rz1+Rz2*, had similar performance potential for sugar yield under non-infected conditions at NR3 for sugar yield. At rhizomania infected sites with all three BNYVV types, the *Rz1+Rz2* genotypes outperformed the *Rz1*, *Rz2* and *rzrz* genotypes for sugar yield. The rhizomania susceptible genotype (*rzrz*) consistently had the lowest performance.

The greatest maximum difference for sugar yield between the *Rz1+Rz2* genotype and others occurred at SM3 where it was >60% greater than the *Rz1* genotype; >35% greater than the *Rz2* genotype and >80% than the susceptible genotype. Overall, differences were greater in sites infected with BNYVV infection ACHG and VCHG than with the AFHG type (Figure 02).



	NR3 nonRZ		SM6 AFHG		FEL ACHG	SM3 ACHG		SM1 VCHG	SM4 VCHG
Rz1+Rz2MS	100.0		100.0		100.0	100.0		100.0	100.0
Rz1	90.1		78.8		67.0	33.3		51.6	48.5
Rz2	91.7		67.6		75.9	58.5		60.4	82.4
rzrz(susceptible)	93.9		44.8		50.9	16.3		45.4	24.0

Figure 02. Relative sugar yield.

Additionally, the sugar content of the *Rz1+Rz2* hybrid, while nearly identical to the *Rz1* hybrid at most locations was significantly greater at both sites SM1 and SM4 where the VCHG type had been identified(Figure 03).

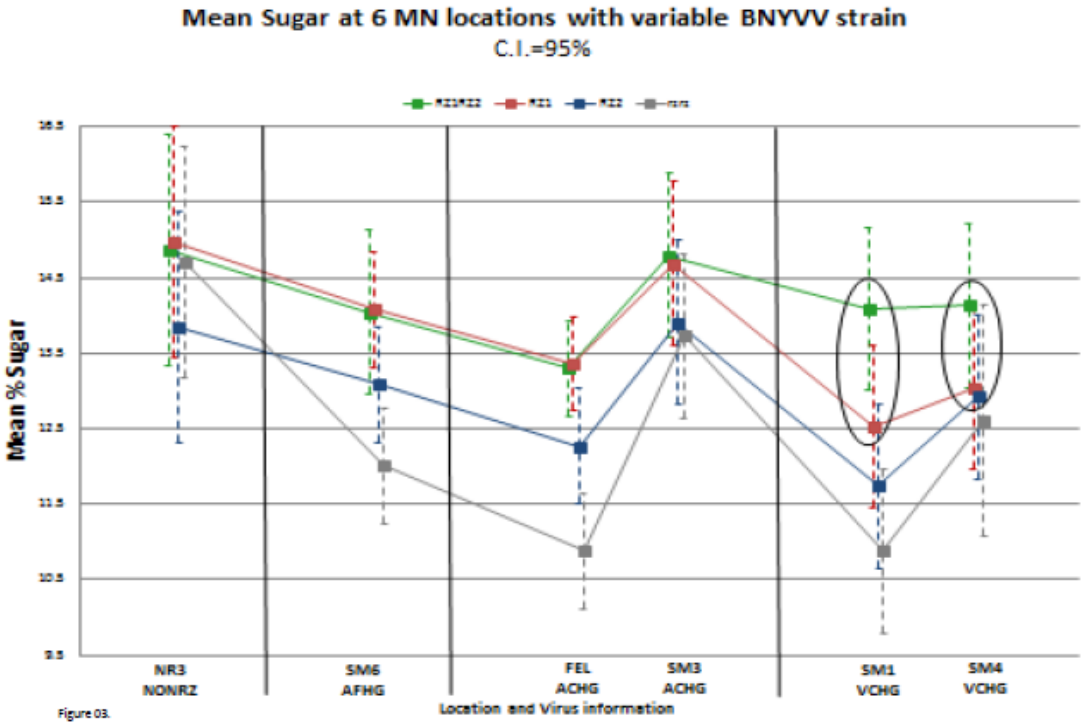


Figure 03.

DISCUSSION

Based on the results, there are several positive benefits from utilizing *Rz1+Rz2* hybrids in commercial sugar beet production in Minnesota. The *Rz1+Rz2* genotypes such as the commercial MultiSource® variety (Betaseed, Inc.) used in the study can realize improved sugar yield performance in fields infected with various BNYVV types, whereas under non-infected conditions there is no apparent risk in terms of performance disadvantage compared to fully susceptible hybrids or those with either the *Rz1* or *Rz2* major gene alone. Data also indicate that the use of *Rz1+Rz2* hybrids safeguards sugar content more than *Rz1* hybrids in the presence of variable strains of BNYVV. Finally, results indicate that there is less virus proliferation in the roots of *Rz1+Rz2* genotypes than with *Rz1* genotypes, implying that less buildup of rhizomania inoculum occurs. 2014 official variety trial data (Figure 04, Bloomquist 2014) demonstrate that variation within both *Rz1+Rz2* hybrids and within single source hybrids exists due to genetic performance potential, local adaptation and tolerance to other pathogens. Hybrids with multiple rhizomania resistance sources provide the best opportunity for both optimizing sugar content and sugar yield in growing areas of Minnesota where several strains of BNYVV can occur.

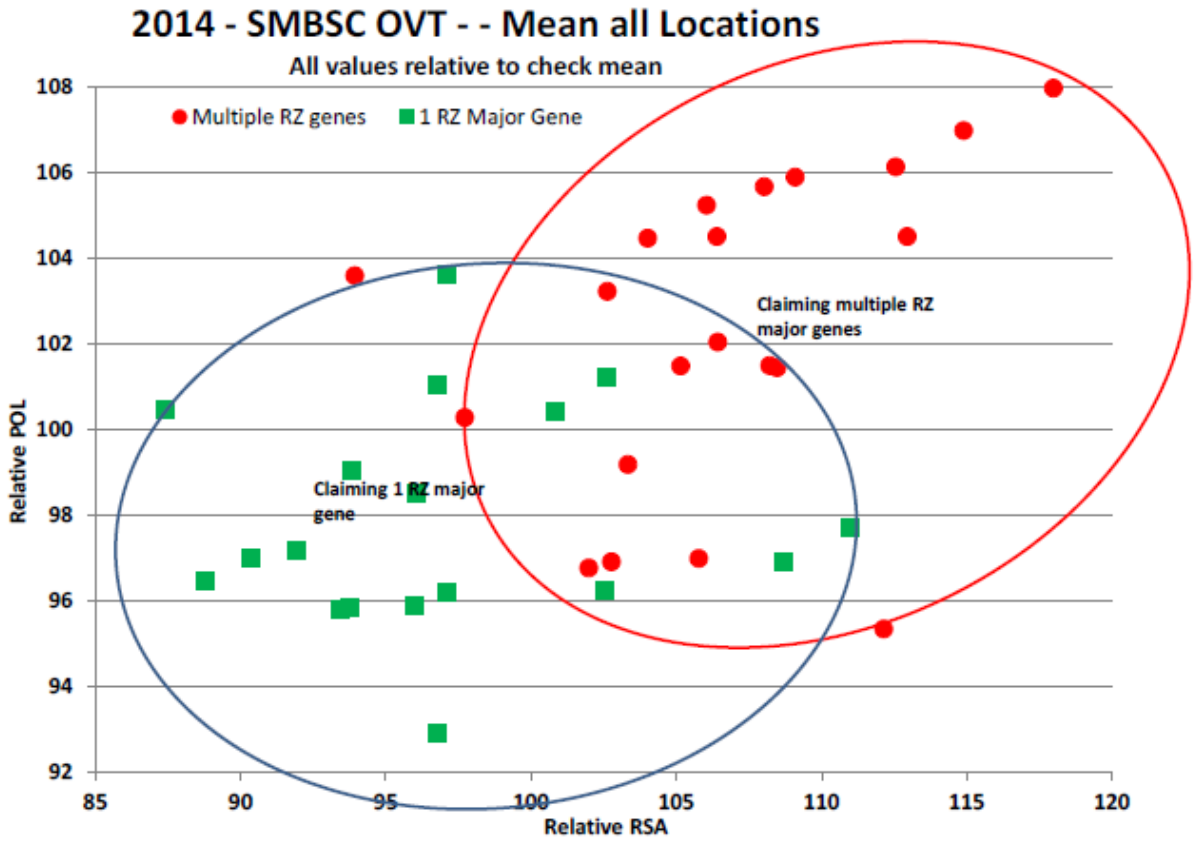


Figure 04. 2014 Southern Minnesota Beet Sugar Cooperative Official Variety Trials.
Mark Bloomquist, Production Agronomist, Southern Minnesota Beet Sugar Cooperative.

References

Abe, H. and T. Tamada. 1986. Association of Beet necrotic yellow vein virus with isolates of *Polymyxa betae*. Ann. Phytopathol. Soc. Jpn. 52: 235-247.

Acosta-Leal, R. & Rush, C. M. (2007). Mutations associated with resistance-breaking isolates of Beet necrotic yellow vein virus and their allelic discrimination using TaqMan technology. Phytopathology 97, 325–330.

Bloomquist, M. 2014. 2014 Southern Minnesota Beet Sugar Cooperative Official Variety Trials Results.

Campbell, L.G., and K. L. Klotz. 2008. Postharvest Storage Losses Associated with Rhizomania in Sugar Beet. Plant Dis. 92: 75-580.

Cattanach, A. 2010. Disease Management by Variety Selection. Rhizomania Map. Prepared and distributed by American Crystal Sugar Agricultural Department.

Miller, J. P., M. Rekoske, and E. Lindroos. 2014. Impact of American Germplasm for Resistance Breeding in Sugar Beet. Poster program 1.6, 74th IIRB Congress.

Rush, C. M., Liu, H.-Y., Lewellen, R. T., and Acosta-Leal, R. 2006. The continuing saga of rhizomania of sugar beets in the United States. Plant Dis. 90:4-15.

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