WISLER, G.C.*, R. T. LEWELLEN, J. L. SEARS, H.-Y. LIU, and J. E. DUFFUS. USDA-ARS, 1636 E. Alisal St., Salinas, California. **Differences in beet necrotic yellow vein virus (BNYVV) levels among susceptible and resistant sugar beet cultivars grown in the United States.**

Abstract

Beet necrotic yellow vein virus (BNYVV) levels as measured by TAS-ELISA were compared to biological evaluations in representative commercial and experimental sugar beet cultivars that range in reactions to rhizomania from uniformly susceptible to highly resistant and were developed for production in the United States. Differences in absorbance ($A_{405\ nm}$) values measured among the eight cultivars closely corresponded to allelic dosage and to the frequency of the *Rz* allele that conditions resistance to BNYVV. A diploid (*Rzrz*) hybrid had a significantly lower absorbance value than a similar triploid (*Rzrzrz*) hybrid. Cultivars that segregated (*Rzrz:rzrz*) had higher absorbance values than uniformly resistant (*Rzrz*) hybrids as would be expected. For all cultivars, differences were observed among harvest dates, with progressively lower absorbance values measured as the season progressed. Absorbance values were significantly positively correlated with rhizomania disease index scores and negatively correlated with individual root weight, plot root weight, and sugar yield. This information is useful in resistance breeding and evaluation programs, and for the sugar industry in consideration of cultivar choice, inoculum production and rotations for future cropping.

Introduction

Rhizomania of sugar beet (*Beta vulgaris* L.) is an economically important disease caused by the beet necrotic yellow vein furovirus (BNYVV). BNYVV is vectored by the protist-like fungus *Polymyxa betae* (5,16) and survives in infested soil for many years in thick-walled resting structures called cystosori (1,2). Typical symptoms of rhizomania include a constricted taproot referred to as "wineglass" shape, with a proliferation of feeder roots (called "bearding") which appear brown due to the infestation of darkly-colored cystosori and root cell death. In severe infections, taproots show necrosis in the vascular system, or roots can be destroyed which can result in death of the beet. Even in moderate infestations with rhizomania, sugar content and root yields are depressed. Foliar symptoms associated with an impaired root system appear as chlorotic patches in the field which may correspond to the movement of soil by cultivation equipment. The necrotic yellow vein of the leaf, for which the virus is named, is rarely seen in the field.

Control of rhizomania includes avoidance of infested fields by testing soil for BNYVV prior to planting, fumigation or solarization of soil where permitted, and the use of resistant cultivars (15). A wide range of sugar beet cultivars has been developed with varying degrees of resistance, or tolerance to rhizomania. Previous studies in England (3,4) and the Netherlands (21) showed that sugar beet cultivars with different levels of resistance vary in the levels of BNYVV detected in roots. Because infected lateral roots remain in the soil after harvest and viruliferous cystosori survive until the next crop is planted, it is important to plant varieties which do not contribute to increasing levels of BNYVV.

known to be highly susceptible to rhizomania and has frequently been used as a susceptible check. The triploid 'KWS6770' is susceptible to rhizomania and has been grown extensively in the upper midwestern states. 'Beta4776R' is diploid and each plant is supposed to carry one dose of the *Rz* allele (*Rzrz*) derived from crossing a *RzRz* parental line with a *rzrz* line. Presently it is widely grown in California. 'Beta 4038R' is a triploid hybrid with the same homozygous diploid source of resistance to rhizomania as Beta4776R and likewise carries a single dose of the *Rz* allele but genotypically is *Rzrzrz*. It is targeted to beet growing areas in the upper midwestern United States and the eastern slope of the Rocky Mountains. 'HM7072' is being tested for the same areas as 'Beta4038R' and is a diploid hybrid with each plant carrying a single copy of the *Rz* allele. The cultivar 'Rival' has wide adaptation. In addition to carrying the *Rz* allele, it is reputed to also have the rhizomania resistance from the widely grown cultivar 'Rizor'. 'SS-781R' is diploid and each plant originally was thought to carry one copy of the *Rz* allele. It now appears that this hybrid segregates for about 12% susceptible (*rzrz*) plants. SS-781R has been an important variety in California in rhizomania infested areas, particularly in the San Joaquin Valley. '6921H50', an experimental hybrid developed by the USDA-ARS at Salinas and carries less than 50% frequency of both the *Rz* allele and resistance of unknown inheritance from *Beta vulgaris* spp. *maritima* sources (14).

Table 1. Sugar Beet Hybrids Used in Virus Titer Experiments
Salinas, California, 1997 Growing Season

Identification	Source	Description	Genotype
USH11	USDA-ARS	diploid susceptible	*rzrz*
KWS6770	Betaseed	triploid susceptible	*rzrzrz*
Beta4776R	Betaseed	diploid resistant	*Rzrz*
SS-781R	Spreckels	diploid segregating	*Rzrz:rzrz*
Rival	Holly	diploid resistant	*Rzrz*
HM7072	Novartis	diploid resistant	*Rzrz*
Beta4038R	Betaseed	triploid resistant	*Rzrzrz*
6921H50	USDA-ARS	diploid segregating	*B. maritima*

Serological Analysis of BNYVV: Previous studies have shown that polyclonal antisera to BNYVV cross-react slightly with beet soil-borne mosaic virus (BSBMV), another furovirus infecting sugar beet in ELISA tests and in western blot analyses (22,23). This cross-reactivity is seen whether antiserum is prepared to the purified virions or to the capsid protein (CP) which has been expressed *in vitro* (25). The different molecular mass of the BNYVV CP (ca. 22 kDa) compared to that of BSBMV (ca. 24 kDa), however, allows for definitive differentiation of the two viruses in western blot assays. Monoclonal antibodies produced to BNYVV (courtesy of L. Torrance and G. Grassi) and antiserum prepared to the C-terminal one third of BNVYY CP (courtesy of K. Richards) show complete specificity to BNYVV in both ELISA and western blot assays (Table 2).

Although western blot analysis provides conclusive distinction between BNYVV and BSBMV, the large numbers of samples to be assayed in three harvests, in addition to the need for quantitation of BNYVV content in sugar beet cultivars with different levels of resistance, necessitated the use of ELISA tests for these studies. A TAS-ELISA was developed in collaboration with Agdia, Inc. that would provide specificity to BNYVV, with no cross-reactions with BSBMV isolates (Table 2), in addition to the ability to obtain a wide range of absorbance values for BNYVV. Serial dilutions of BNYVV-infected leaf and root tissues showed a decrease in absorbance readings that corresponded to decreased concentrations of expressed plant sap (data not shown). Previous studies showed a relationship between virus concentrations in BNYVV-infected plants and absorbance values obtained in ELISA (17,21). Preliminary TAS-ELISA tests were made to confirm the specificity of this test for BNYVV (Table 2).

Table 2. TAS-ELISA readings for BNYVV and BSBMV Using BNYVV Antisera[a]

Test Sample	Absorbance(A_{405})[b]
BNYVV beet roots	2.227
BNYVV *B. macrocarpa*	2.770
BSBMV-TX *B. macrocarpa*	0.127
BSBMV-MN *B. macrocarpa*	0.132
Healthy beet roots	0.153
Healthy *B. macrocarpa*	0.127

[a] TAS-ELISA using polyclonal (trapping) and monoclonal (detecting) antibodies to BNYVV. Preliminary tests for specificity to BNYVV.

[b] Absorbance at A_{405} represents the average of at least two wells.

Polyclonal antiserum used as the trapping antibody was made from the BNYVV CP which was expressed *in vitro* (the clone for the BNYVV CP was kindly provided by K. Richards). The pETH plasmid expressing the CP was identified by western blot assays and was used to transform the appropriate host for expression, *E. coli* strain BL21DE3pLysS, according to Studier et al. (20). An insoluble fusion protein of ca. 22 kDa was overexpressed and purified by SDS-PAGE as previously described (25). Antiserum was prepared in rabbits by Berkeley Antibodies (Richmond, California). This antiserum was used to coat microtiter plates (Immulon I; Chantilly, VA) at a 1/1000 dilution in coating buffer (0.05 M sodium carbonate, pH 9.6).

Plant samples consisted of fibrous lateral roots which had been scraped from each beet, and added to 2 ml of sample extraction buffer (phosphate-buffered saline, pH 7.2 with 0.5% Tween 20 and 0.4% dry milk powder). Root tissues were macerated in sample extraction bags using a hand held roller press (Agdia, Inc.). Expressed sap was added as paired wells to plates at 150 µl per well. A list of computer generated random numbers was used to determine the placement of the 576 test samples per harvest on 23 microtiter plates. Each plate also contained paired wells with (i) sample buffer only (ii) a rhizomania diseased root and healthy root tissues in

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sugar beet (*Beta vulgaris* L.), (iii) a non-inoculated, and (iv) a BNYVV-systemically infected *B. macrocarpa* (*B. vulgaris* spp. *maritima* var. *macrocarpa*) leaf (Table 3).

The BNYVV monoclonal antibody which was used as the detecting antibody, and the goat-anti-mouse IgG-alkaline phosphatase conjugate were provided by Agdia and used according to their instructions. Absorbance readings (A_{405} nm) were made at 15 minute intervals up to 2 hr using a Bio-Tek EL312e microplate reader (Winooski, VT).

Table 3. TAS-ELISA Readings (A_{405}) of BNYVV for Varieties, Dates of Harvest, Varieties X Dates; test 4197

Variety	Genotype	July 14	August 18	October 22	Mean
USH11	*rzrz*	0.947[a]a[b]	0.365c	0.226efg	0.513b
KWS6770	*rzrzrz*	1.024a	0.414c	0.341cd	0.593a
Beta4776R	*Rzrz*	0.257def	0.150ghi	0.117hi	0.175de
SS-781R	*Rzrz:rzrz*	0.343cd	0.164fghi	0.140ghi	0.216de
Rival	*Rzrz*	0.316cde	0.138ghi	0.128ghi	0.195de
HM7072	*Rzrz*	0.218efg	0.111i	0.138ghi	0.156e
Beta4038R	*Rzrzrz*	0.562b	0.220efg	0.212fgh	0.332c
6921H50	experimental	0.356cd	0.192fghi	0.155ghi	0.234d
Mean		0.503a	0.219b	0.182b	0.302
Healthy beet root		0.105	0.096	0.102	0.101
BNYVV beet root		0.513	0.372	0.482	0.456
Healthy B.mac.		0.106	0.098	0.103	0.103
BNYVV B. mac.		1.654	1.031	2.345	1.677

[a]Values represent an average of two wells from eight replications of nine beets each.
[b]Within each set of means, those with a letter in common are not significantly different (p=0.05).

Field Trials: Field trials were conducted at the USDA-ARS, U.S. Agricultural Research Station, Salinas, California, where rhizomania tests have been conducted on infested land since 1984. The primary test in this study, 4197, was planted 1 May 1997 in a split-plot design, where dates were the main plot, with eight cultivars (subplots) randomized into three harvest dates (July 14, August 18, October 20), and eight replications. The plots were over-seeded and plants at the two-leaf stage were thinned to a spacing of 16 cm between single plants. Standard best cultural practices were used including weed, insect and disease control. Sprinkler irrigation was used throughout the season at weekly intervals to field capacity in order to enhance rhizomania development. Irrigation and harvests were timed so that each harvest was made 3 days after the most recent irrigation. For the first two harvests, plots were 2.3 m long with 0.6 m alleys. Excluding end plants, nine beets were randomly harvested within each plot.

In each of the three harvests, the 9 randomly selected beets from each plot (72 plants per cultivar; 576 plants per harvest date) were dug by hand, topped just above the lowest leaf scar, and washed free of soil particles. Fibrous roots were scraped from each beet, 0.5 g of which was taken for the ELISA test. In the first harvest, only the TAS-ELISA was done. In the second and third harvest, ELISA tests were done, tap roots were individually weighed, and each beet root was scored according to a rhizomania disease index (DI). This root score index was adapted using a scale of 0 to 9 to comply with the international scale for sugar beet germplasm evaluation where 0 = no visual symptoms, 1 = very resistant (nearly normal taproot and minor bearding), 3 = resistant (taproot slightly to moderately constricted, moderate bearding and taproot discoloration), 5 = intermediate (taproot wineglass shaped, feeder roots bearded, taproot discolored), 7 = susceptible (severe bearding and stunting, taproot destroyed) and 9 = highly susceptible (death of beet). For the third harvest, plots were longer than for the first two harvests, at 5.2 m long, to accommodate both laboratory and yield evaluations. Plots were adjusted to 3.6 m following consecutive individual plant harvests from 1.6 m near one end of each plot. Remaining beets were harvested mechanically at the end of the third harvest, weighed and run through a standard sugar laboratory to measure sucrose concentration. Sugar yield was calculated from plot weight and sucrose concentration.

In adjacent duplicated field trials, the eight cultivars were evaluated for yield under similar disease pressure and cultural practices. These trials, numbered 1697, 1997, 3597, and 3997 were randomized complete block designs with eight replications. One-row plots were 72 cm wide and 6.1 or 6.4 m long. Test 3597 was hand harvested and topped, and roots were scored for rhizomania on the 0-9 DI scale. Classes 0-3 were considered resistant and 4-9 susceptible. Following root scoring, all beets were bulked by plot, washed, weighed, and run through the sugar analysis laboratory. The other field trials were mechanically harvested for yield and sugar analysis so individual beets were not scored for reactions to rhizomania.

Data analysis: Data obtained from individual plants within each plot of test 4197 were averaged and used for statistical analyses. These data were collected at three harvest dates (576 plants per harvest) and consisted of ELISA values, DI (root score), root yield, per cent sucrose, and sugar yield. Initially all data were analyzed for the split-plot analysis at Salinas using MSTAT, where dates were the main plots. Heterogeneity of variances occurred for optical densities as measured by TAS-ELISA and individual root weights. Analyses of these traits were done with SAS PROC MIXED (SAS Institute Inc., Cary, N.C.). The data were transformed by natural logs which alleviated the heterogeneity for root weights and greatly reduced the heterogeneity for optical densities. For the optical densities and root weights, the means and confidence intervals were transformed back to the original scale. For correlations among absorbance (A_{405nm}), absorbance of test sample/absorbance of healthy roots (abs/H), root score, root weight, per cent sucrose, and sugar yield, the date X variety means were used (Table 4). Data obtained from the individual randomized complete block tests 1697, 1997, 3597, and 3997 to evaluate performance of the eight varieties were also analyzed using MSTAT.

Table 4. Coefficients of correlation among treatment means from two harvest dates.[a]				
	Absorbance	Absorbance/H[b]	Root Score	Root Weight (g)
Absorbance	---------	0.99**	0.87**	-0.89**
Abs/Healthy	0.99**	---------	0.87**	-0.89**
Root Score	0.95**	0.95**	---------	-0.89**
Root Weight	-0.76*	-0.76*	-0.87**	---------

[a] The correlations for harvest date two (August 18) are above the diagonal and those for date three (October 22) are below. [b]Absorbance at (A_{405nm}) for test samples divided by the absorbance for healthy root samples. * significant at the 0.05 level of probability. ** significant at the 0.01 level of probability.

Results

Serological analysis: The TAS-ELISA test modified for this study gave no background cross-reactions with other furoviruses of sugar beet, in particular, isolates of BSBMV (Table 2). One isolate each of BSBMV from Texas and Minnesota gave reactions equivalent to those of healthy sugar beet roots and healthy leaf tissues of *B. macrocarpa*. In addition, a wide range of readings were observed with different BNYVV samples of varying serial dilutions, thus providing for the ability to measure differences in BNYVV content among resistant and susceptible sugar beet varieties.

Differences in absorbance (A_{405nm}) values for BNYVV measured by TAS-ELISA among the eight cultivars closely corresponded to dosage and frequency of the *Rz* allele that conditions resistance to BNYVV (Table 3). The diploid *Rzrz* hybrid Beta4776R had a significantly lower value than the similar triploid *Rzrzrz* hybrid Beta4038R. Cultivars that segregated *Rzrz:rzrz* (i.e., SS-781R and 6921H50) had higher absorbance values than the uniformly resistant *Rzrz* hybrids Beta4776R and HM7072.

Field Trials: For all cultivars, differences were observed among harvest dates, with progressively lower absorbance values measured as the season progressed, particularly from July 14 to August 18. A highly significant cultivar X date of harvest interaction occurred. This interaction can largely be explained by rate and magnitude of decrease in absorbance values for the susceptible cultivars compared to the resistant ones. Absorbance readings for the July 14 harvest clearly discriminated differences in varietal reactions more distinctly than did the subsequent harvests (Table 3). Differences in varieties based only upon the results from the third harvest date would not have shown allelic dosage and frequency effects. These results suggest that because of the effects of plant age, environmental factors and/or sampling techniques (it may be more difficult to sample recently infected lateral roots in larger, older beets than in younger, smaller ones). Timeliness is an important consideration in use of ELISA to evaluate varietal reactions to BNYVV when testing directly from field-grown beets.

The correlations in Table 4 show that there are close associations between the variables used to evaluate reactions to rhizomania, including, absorbance (A_{405nm}), absorbance/healthy, root score and root weight. There was nearly a perfect correlation between absorbance readings of test samples and absorbance of test samples divided by those of healthy roots grown in pasteurized soil (absorbance/healthy). This suggests that very little plate-to-plate variability and

experimental error occurred. Likewise, the absorbance/healthy values that represented the adjusted absorbance values due to plate differences were very small. The highly significant positive correlations between absorbance/healthy values and root scores (r = 0.87, 0.95 for dates 2 and 3, respectively) showed that visual disease reaction scores of these roots were highly associated with virus concentration.

The correlations between absorbance/healthy and root weight (r=-0.89, -0.76 for harvest dates 2 and 3, respectively) were negative as would be expected (Table 4). These inverse correlations suggested that high virus concentration or rhizomania disease reactions could be predicted by tap root weight. Root weights and disease scores also were highly inversely correlated (r = -0.91, -0.87 at p = 0.01 for harvest dates 2 and 3, respectively). Also, as shown by the harvest date results, virus levels decreased through the course of the season. Observations at Salinas over many years has suggested that virus levels as measured by ELISA varied depending on timing of irrigation (wetting-drying periods). For this reason, we felt that it was necessary to measure virus content from a field trial with controlled irrigation and with plant samples at each harvest three days after irrigation.

In addition to the primary test, 4197, in which the roots were evaluated three times during the growing season for reactions to BNYVV by ELISA, the rhizomania disease index, root yield, per cent sucrose, and sugar yield, four additional replicated tests (1697, 1997, 3597, and 3997) were grown at Salinas under moderate and severe incidences of rhizomania, as measured by the above parameters (Table 5). Tests 1697 and 1997 were intended to be rhizomania-free, but at harvest it was obvious that these fields were moderately infested. Thus, no rhizomania-free test was available for comparison. The identical seed lots of all eight cultivars were grown in these five tests. In all tests, the two susceptible checks had significantly lower yields than the more resistant entries (Table 5). Comparison of sugar yield between Beta4776R (*Rzrz*) and Beta4038R (*Rzrzrz*) under the two moderate tests (1697 and 1997) and the two severe tests (3597 and 3997) (Table 5) again suggested that the level of resistance conditioned by allelic dosage was reflected in root yield, per cent sucrose, and sugar yield. Under moderate rhizomania conditions, the yield difference was small (ca. 4%) between these two cultivars and not significantly different, whereas under the severe conditions the difference was larger (ca. 13%) and was significantly different. In all tests, under severe disease pressure, the advanced hybrid Beta4776R tended to have the highest root and sugar yields. Roots from test 3597 were individually scored for reaction to rhizomania at harvest. There was a good association and comparable ranking of the root score means for varieties across tests 3597 and 4197 for the corresponding harvest date (date 3 for test 4197). These tests support the data and interpretations made for test 4197. The agronomic data for test 4197 appear to be valid and, under the conditions of these tests, consistently measured and differentiated varietal reactions to BNYVV.

Table 5. Performance of sugarbeet cultivars under differing severities of rhizomania.						
	Test 1697[a]			Test 1997[b]		
	Sugar Yield (kg/ha)	Root Yield (t/ha)	Sucrose (%)	Sugar Yield (kg/ha)	Root Yield (t/ha)	Sucrose (%)
Susceptible checks						
USH11	4938	51.6	9.5	5824	56.6	10.3
KWS6770	5406	50.7	10.6	8356	64.0	13.1
Resistant hybrids						
Beta4776R	10848	81.2	13.4	13669	93.7	14.6
SS-781R	8692	72.5	11.9	10948	81.1	13.5
Rival	8789	66.1	13.3	11533	80.9	14.3
HM7072	10294	69.4	14.8	12955	82.1	15.8
Beta4038R	10180	72.5	14.1	13310	84.6	15.7
USDA expt. hybrid						
6921H50	9524	76.9	12.3	11520	84.8	13.6
LSD (P=.05)	946	6.0	0.8	1017	6.1	0.6

T. 5,cont'd.	Test 3997[c]			Test 3597[d]				
	Sugar Yield (kg/ha)	Root Yield (t/ha)	Sucrose (%)	Sugar Yield (kg/ha)	Root Yield (t/ha)	Sucrose (%)	Rhizomania Reaction DI[e]	%R[f]
Susceptible checks								
USH11	5449	47.6	11.0	3528	30.3	11.7	4.6	36.0
KWS6770	6947	53.7	13.0	4735	33.3	14.3	4.5	38.9
Resistant hybrids								
Beta4776R	12333	84.9	14.5	11146	68.0	16.4	2.4	94.9
SS-781R	8838	65.3	13.6	6692	48.1	14.0	3.1	77.1
Rival	8943	63.4	14.2	8413	54.1	15.6	2.8	83.7
HM7072	10820	68.6	15.7	8989	53.5	16.8	3.2	76.9
Beta4038R	10961	68.0	16.2	9454	54.7	17.3	3.5	64.9
USDA expt. hybrid								
6921H50	10478	79.0	13.3	9032	64.8	14.0	2.9	81.7
LSD (P=.05)	1117	7.0	0.8	762	5.0	0.7	0.4	10.3

[a] Test 1697 grown at Salinas under moderate rhizomania conditions. Planted 10 April 97; Harvested 2 October 97. One-row plots 6.4m long. Eight replications; randomized complete block (RCB) design.

[b] Test 1997 grown at Salinas under moderate rhizomania. Planted 10 April 97; Harvested 29 September 97; 1-row plots, 6.4m long. Eight replications; RCB.

[c] Test 3997 grown at Salinas under severe rhizomania. Adjacent to Test 4197. Planted 1 May 97; Harvested 20 October 97. One-row plots 6.1m long. Eight replications, RCB.

[d] Test 3597 grown at Salinas under severe rhizomania conditions. Planted 30 April 97; Harvested 29 October 97; One-row plots, 6.1m long. Eight replications, RCB.

[e] DI = disease index where individual roots scored at harvest on a scale of 0 (no symptoms) to 9; test 3597.

[f] %R = % resistant where classes 0-3 were considered resistant (test 3597).

Discussion

Three cultivars which range from uniformly susceptible (*rzrzrz*; KWS6770) to diploid resistant (*Rzrz*; Beta4776R) to triploid resistant (*Rzrzrz*; Beta4038R) were chosen as the best representatives of distinct allelic dosages to illustrate the association between the *Rz* allele and the three variables which were measured in this study, including absorbance, root score and root weight. Those cultivars for which the genetics is well documented show a strong positive correlation with regard to allelic dosage and root score, whereas they were highly negatively correlated with root weight. For sugar beet cultivars with unknown inheritance this information can be useful to predict their genetic background.

Selection for resistance to rhizomania in sugar beet in California has been based on evaluation of roots for rhizomania symptoms, yield and sugar analysis from field grown beets. This was due, in part, to access to the presence of highly infested fields on the research stations, an amenable climate, and the ease of this procedure. In contrast, in Europe where BNYVV infested fields have not usually been available near breeding stations and the growing season is more restrictive, selection for resistance to rhizomania have usually been based upon ELISA tests from greenhouse and growth chamber grown plants.

Our studies have shown that the current field evaluation system used in the U.S. by industry and public agencies is equally suitable to the more laborious and expensive evaluation by ELISA assays. Using varieties that are currently important to the U.S. beet production, we showed that the ELISA readings are significantly correlated with root score, and negatively with root weight and % sucrose. These readings and evaluations, when compared against a range of Rhizomania susceptible and resistant cultivars indicate these data can be useful for prediction of the genetic background of cultivars about which less is known. Root weights and visual scoring are usually made much more easily in a breeding or testing program than absorbance measurements from ELISA tests.

There was a close association between the dosage and frequency of the *Rz* allele and BNYVV levels in lateral roots, as measured by TAS-ELISA. It would be expected, and it was shown, that within hybrids such as SS-781R, that fully susceptible (*rzrz*) segregants in the hybrid would increase the mean virus content. When individual plant ELISA, visual, and yield

ratings were examined and taken into account for hybrids such as SS-781R, the plants that were probably *Rzrz* have values similar to Beta4776R and the putative *rzrz* plants were similar to USH11 or KWS6770. Of more interest was the relationship between allelic dosage and virus levels. It was clear that in terms of virus levels, *Rzrz* < *Rzrzrz* < *rzrz* ≅ *rzrzrz*. Incomplete dominance (gene dosage) is a common phenomenon for host-plant resistance to viruses. Fraser (1987) found that many virus resistances inherited at a single locus were expressed in an incompletely dominant manner. Pelsey and Merdinoglu (1996) showed that *Rz* was inherited as incompletely dominant when measured by virus content of greenhouse grown plants in standardized inoculum tests. Our results suggest that a further increment of resistance may be achievable in sugar beet hybrids. It is likely that the *RzRz* genotype would then produce less virus than the currently employed *Rzrz* or *Rzrzrz* genotypes. As time and resources permit, it will likely behoove breeders to develop homozygous *RzRz* parental lines for all of the components of commercial hybrid cultivars. These more resistant *RzRz* cultivars could give a higher level of protection against rhizomania and could certainly be important in limiting inoculum buildup in soils. This lag in the use of only *RzRz* parental lines components reflect the time and efforts necessary to incorporate and fix a single gene into all component lines and advanced breeding material. Experimental, homozygous resistant lines are available and will be included in future research along the lines of this study.

Correct diagnosis of BNYVV can be confounded by low levels of cross-reactivity with other furoviruses, in particular BSBMV, as has been previously demonstrated. In addition, levels of BNYVV, which is dependent on the production of viruliferous *P. betae* zoospores, can vary in sugar beets during the growing season in Rhizomania-infested fields. This study shows what has been observed over the years by researchers, that levels of BNYVV can change during a growing season. As the season progressed in this study, levels of BNYVV continued to decline as measured by TAS-ELISA, in spite of the presence of well developed Rhizomania symptoms, regardless of the cultivar. This could be due to several factors which are unknown at this time but could include plant susceptibility as it declines with age, where younger plants are more susceptible than older ones, or climatic conditions during the season. These results confirm observations over many years at Salinas that late summer BNYVV titer values do not seem to reflect varietal reactions. Because sugar beet is not considered to be a good systemic host for BNYVV due to the extremely low occurrence of systemic symptoms and restriction of BNYVV primarily to the area of proliferated roots (10) the level of BNYVV in sugar beet roots is dependent on the activity of the vector which itself is dependent on soil temperature, soil moisture content, and beet root exudates.

This study shows that the breeder or agronomist can be fairly confident of measuring varietal reactions to rhizomania by either scoring or weighing field grown material. Root weights and visual scoring are usually made more easily in a breeding or testing program than absorbance measurements from ELISA tests. This information is useful in resistance breeding and evaluation programs and for the sugar industry in consideration of cultivar choice, inoculum production and rotations for future cropping.

In addition to the *Rz* (Holly source) resistance factor, other sources of resistance to rhizomania have been found (11). Some of these sources appear to be the *Rz* allele, but others appear to be different from *Rz* (19). At least one of the sources, when tested under severe

rhizomania conditions provides better protection than *Rz* (11,15). Tests are underway to map each of these sources of resistance, determine their allelism, and identify molecular markers (8,18,19). If additional major genes at different loci are discriminated, these may reduce the vulnerability of *Rz*. In addition, preliminary evidence suggests that one or more of these genes condition lower levels of BNYVV content than *Rz*. With marker assisted selection, it may become feasible to combine multiple resistance factors into individual cultivars to obtain improved resistance to rhizomania, further decrease BNYVV inoculum production (17), and provide more durable resistance.

The mention of firm names or trade products does not imply endorsement or recommendation by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Acknowledgment: We thank Tim Gottwald and Bruce Mackey for their advice and assistance regarding statistical analyses.

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