separated at the crown and lyophilized (freeze dried), then stored at -80 until further analysis. Root samples from each plot were tested by RT-PCR to confirm BNYVV infection prior to use in metabolome analysis, and remaining roots from the same samples were used for metabolite extractions.

Roots from all three experiments were freeze-dried and stored at -80C so that metabolites could be extracted from all samples at the same time. Upon completion of the last replication, dried root samples were pulverized in liquid nitrogen and sent to the Core Laboratory at Colorado State University (CSU) in Ft. Collins, CO for methanol extraction of metabolites. Metabolome analysis was completed at CSU during the fall, and results of analyses provided to USDA-ARS in late November 2017.

Overall 746 metabolites were found and these were annotated to known compounds or to unknown compounds with a specified mass. These metabolites were examined in all possible combinations of treatments to look for statistically different levels of expression among treatments, including patterns of expression indicating how traditional or RB-BNYVV influence resistant and susceptible sugarbeet during infection, as well as for identification of "interesting" compounds that may play an important role in rhizomania disease development. Metabolite levels were compared among treatments using a 95 percent confidence interval to distinguish compounds with statistically different levels of expression among treatments. Results demonstrated the most important difference in metabolite levels was between healthy sugarbeet plants and sugarbeet plants infected with BNYVV. Results also demonstrated differences between traditional BNYVV and RB-BNYVV. Overall, comparative studies indicated 32% of differences in metabolite levels among treatments were based on the presence or absence of BNYVV (Fig. 1). In contrast, only 3% of variation among treatments could be explained by differences in sugarbeet variety (i.e. the different resistance genes) (Fig. 2). Essentially, results indicate most metabolic differences are caused by the BNYVV infection, and are not influenced much by the presence or absence of either resistance gene. This contrasts with what was observed with our recent proteomics analysis of similar sugarbeet near isogenic lines, in which differences that occurred were influenced by both virus strain and the resistance genes.

In our previous proteomics analysis comparing BNYVV infection of Rz1 and Rz2 sugarbeet with susceptible sugarbeet, we identified a number proteins with differential expression not only between RB- and traditional strains of BNYVV, but also between sugarbeet genotypes (Rz1, Rz2, and susceptible). Results of those studies demonstrated that abundance of select proteins in sugarbeet is significantly altered based on the presence or absence of the two resistance genes (Webb et al., 2015), whereas in the current metabolomics study very limited (3%) differences in the metabolome were determined by the presence or absence of rhizomania resistance genes.
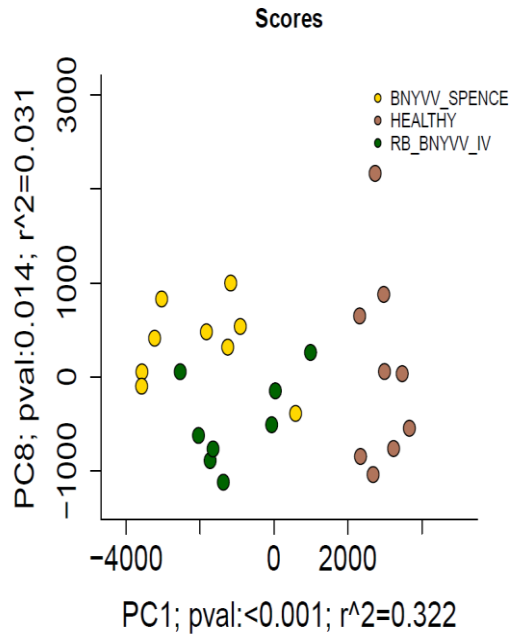
Figure 1. Principle component analysis plot generated from 27 samples derived from 9 treatments showing clear separation by virus type. Yellow: BNYVV-Spence (traditional/wild type BNYVV), Green: BNYVV-IV (Rz1 Resistance breaking BNYVV), Red: Healthy (virus-free sugarbeet).
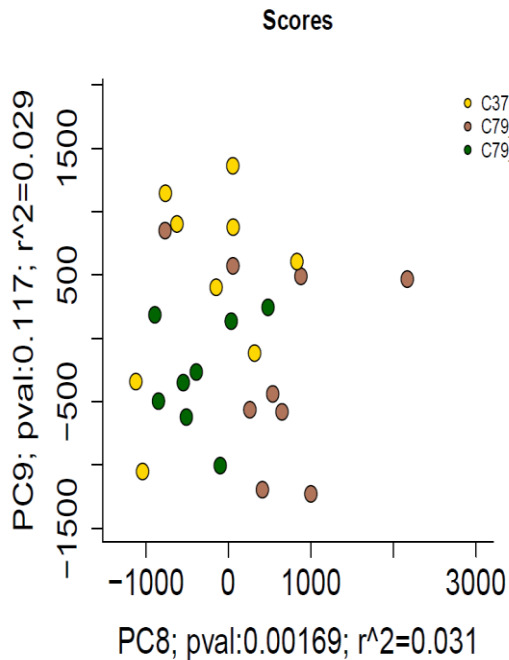
Figure 2. Principle component analysis plot generated from 27 samples derived from 9 treatments showing little separation by sugarbeet genotype (resistance gene or not). Yellow: C37 (susceptible sugarbeet [rz1rz2]), Red: C79-1 (Rz1 resistant sugarbeet [Rz1rz2]), Green: C79-3 (Rz2 resistant sugarbeet [rz1Rz2]).

Continuing studies are focusing on identification of specific compounds that differ among treatments. Although these detailed studies are just beginning, some interesting results have already been identified, including compound $C_{40}H_{107}N_{17}OS_4$ (Fig. 3). This compound had low expression in the absence of virus in both susceptible (rz1rz2) and resistant varieties (both Rz1 and Rz2), but higher expression with virus infection when either traditional or Rz1-resistance-breaking BNYVV strains were present. In general, the expression of this compound mimics what would be "expected" in a traditional gene-for-gene type of resistance. The highest expression of compound $C_{40}H_{107}N_{17}OS_4$ was observed in the susceptible line (C37) with the traditional BNYVV strain (Spence), but expression differences were also significant with the RB BNYVV strain (which we believe is generally less fit overall than traditional BNYVV based on its performance in field situations). The fact that this compound is expressed at elevated levels in all varieties indicates its expression is a response to infection, but not necessarily associated with ability of the plant to resist infection (no strong differential effect with resistant beets).
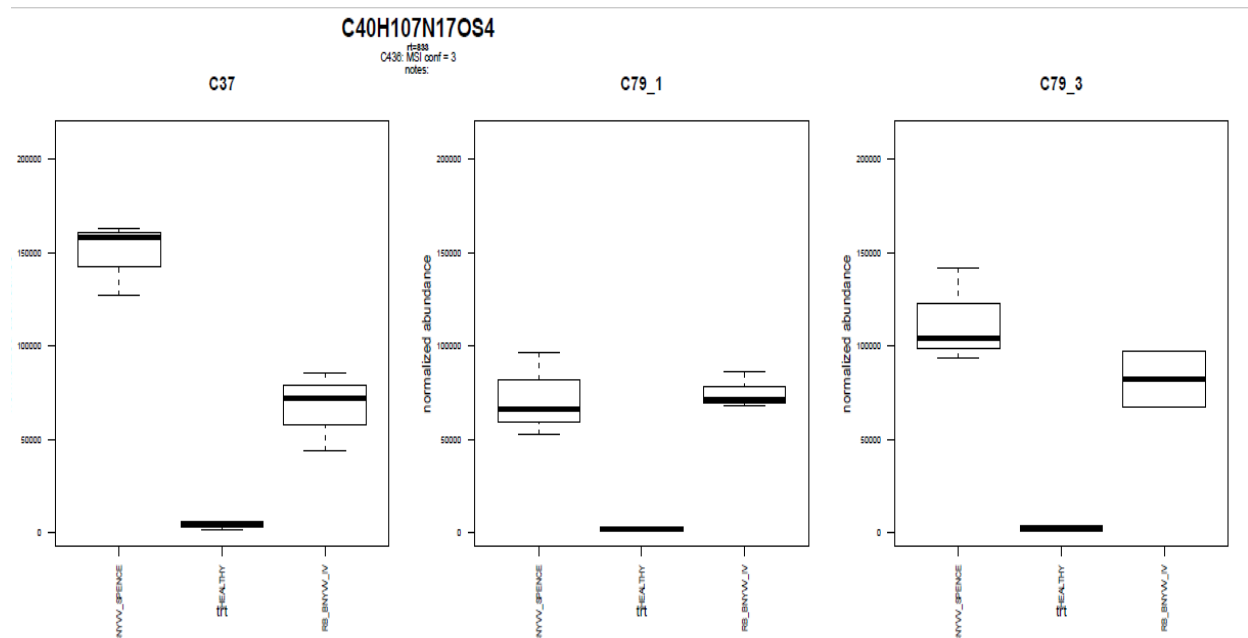
Figure 3. Abundance of compound $C_{40}H_{107}N_{17}OS_4$. C37 = susceptible sugarbeet (rz1rz2), C79-1 = Rz1 resistant sugarbeet (Rz1rz2), C79-3 = Rz2 resistant sugarbeet (rz1Rz2).

Further Research:

Although we have not requested additional funding for this project we will be continuing data analysis and interpretation of results. Through characterization of differential abundance of compounds and identification of these compounds, we expect to improve our knowledge of what is happening biochemically in sugarbeet during BNYVV infection and development of rhizomania disease. We will also examine results of this metabolome analysis in comparison to to those of our previous studies on proteomics (Larson et al., 2008; Webb et al., 2014, 2015), and studies by others on gene expression and protein interactions (Fan et al., 2014, 2015; Thiel and Varrelmann, 2009). This should allow us to begin to piece together how BNYVV causes disease in plants by determining changes that occur in infected vs. healthy sugarbeet. Ultimately we anticipate gaining insight into how resistance genes are able to suppress BNYVV levels by identifying differences in biochemicals produced (this study) along with changes in gene expression (previous studies). This information will be useful toward application of marker-based selection of traits that may enhance performance of resistance genes, as well as for identification of targets for use of new biotechnology-based methods that should lead to novel methods to prevent rhizomania disease in sugarbeet.

Literature Cited:

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

Aliferis, K. A. and S. Jabaji. 2010a. H NMR and GC-MS metabolic fingerprinting of developmental stages of *Rhizoctonia solani* sclerotia. *Metabolomics* 6:96-108.

Aliferis, K. A. and S. Jabaji. 2010b. Metabolite composition and bioactivity of *Rhizoctonia solani* sclerotial exudates. *Journal of Agricultural and Food Chemistry* 58:7604-7615.

Aliferis, K. A. and S. Jabaji. 2012a. Deciphering plant-pathogen interactions applying metabolomics: principles and applications. *Canadian Journal of Plant Pathology* 34:29-33.

Aliferis, K. A. and S. Jabaji. 2012b. FT-ICR/MS and GC-EI/MS metabolomics networking unravels global potato sprout's responses to *Rhizoctonia solani* infection. *PLOS one* 7, no. 8:1-8.

Fan H, Sun H, Wang Y, Zhang Y, Wang X, Li D, et al. (2014) Deep sequencing–based transcriptome profiling reveals comprehensive insights into the responses of *Nicotiana benthamiana* to *Beet necrotic yellow vein virus* infections containing or lacking RNA4. PloS One 9: e85284. doi: 10.1371/journal.pone.0085284. pmid:24416380

Fan, H., Zhang, Y., Sun, H., Liu, J., Wang, Y., Wang, X., Li, D., Yu, J., and Han, C. 2015. Transcriptome analysis of Beta macrocarpa and identification of differentially expressed transcripts in response to Beet necrotic yellow vein virus Infection. PLoS One DOI: 10.1371/journal.pone.0132277

Fiehn, Oliver, Joachim Kopka, Peter Dormann, Thomas Altmann, Richard N. Trethewey, and Lothar Willmitzer. 2000. Metabolite profiling for plant functional genomics. *Nat Biotech* 18, no. 11:1157-1161.

Gidner, S., B. L. Lennefors, N. O. Nilsson, J. Bensefelt, E. Johansson, U. Gyllenspetz, and T. Kraft. 2005. QTL mapping of BNYVV resistance from the WB41 source in sugar beet. *Genome* 48:279-285.

Heuberger, Adam L., Corey D. Broeckling, Kaylyn R. Kirkpatrick, and Jessica E. Prenni. 2013. Application of nontargeted metabolite profiling to discover novel markers of quality traits in an advanced population of malting barley. *Plant Biotechnol J*:n/a.

Heuberger, Adam L., Faith M. Robison, Sarah Marie A. Lyons, Corey D. Broeckling, and Jessica E. Prenni. 2014. Evaluating plant immunity using mass spectrometry-based metabolomics workflows. *Frontiers in Plant Science* 5:1-11.

Larson, R. L., W. M. Wintermantel, A. L. Hill, L. Fortis, and A. Nunez. 2008. Proteome changes in sugar beet in response to *Beet necrotic yellow vein virus*. *Physiol. and Mol. Plant Pathol.* 72:62-72.

Laurent V, Devaux P, Thiel T, Viard F, Mielordt S, Touzet P, et al. (2007) Comparative effectiveness of sugar beet microsatellite markers isolated from genomic libraries and GenBank ESTs to map the sugar beet genome. Theor Appl Genet 115: 793–805. pmid:17646961 doi: 10.1007/s00122-007-0609-y

Liu, H., J. L. Sears, and R. T. Lewellen. 2005. Occurrence of resistance breaking *Beet necrotic yellow vein virus* of sugar beet. *Plant Disease* 89:464-468.

Liu, H. Y. and R. T. Lewellen. 2007. Distribution and molecular characterization of resistance-breaking isolates of Beet necrotic yellow vein virus in the United States. *Plant Dis.* 91:847-851.

Rellan-Alvarez, R., S. Andaluz, J. Rodriguez-Celma, G. Wohlgemuth, G. Zocchi, A. Alvarez-Fernandez, O. Fiehn, A. F. Lopez-Millan, and J. Abadia. 2010. Changes in the proteomic and metabolomic profiles of *Beta vulgaris* root tips in response to iron deficiency and resupply. *BMC Plant Biology* 10:120.

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

Scholten, O. E., T. S. De Bock, R. M. Klein-Lankhorst, and W. Lange. 1999. Inheritance of resistance to *Beet necrotic yellow vein virus* in *Beta vulgaris* conferred by a second gene for resistance. *TAG* 99:740-746.

Scholten, O. E., R. M. Klein-Lankhorst, D. G. Esselink, T. S. De Bock, and W. Lange. 1997. Identification and mapping of random amplified polymorphic DNA (RAPD) markers linked to resistance against *Beet necrotic yellow vein virus* (BNYVV) in *Beta* accessions. *TAG* 94:123-130.

Thiel, H., and Varrelmann, N. 2009. Identification of Beet necrotic yellow vein virus P25 pathogenicity factor-interacting sugar beet proteins that represent putative virus targets or components of plant resistance. Mol Plant-Microbe Interact. 22(8): 999-1010.

Webb, K. M., C. J. Broccardo, J. E. Prenni, and W. M. Wintermantel. 2014. Proteomic profiling of sugar beet (*Beta vulgaris*) leaves during rhizomania compatible interactions. *Proteomes* 2:208-223.

Webb, K. M., W. M. Wintermantel, N. Kaur, J. E. Prenni, C. J. Broccardo, L. M. Wolfe, and L. L. Hladky. 2015. Differential abundance of proteins in response to *Beet necrotic yellow vein virus* during compatible and incompatible interactions in sugar beet containing *Rz1* or *Rz2*. *Physiological and Molecular Plant Pathology* 91:96-105.

Wisler, G. C., R. T. Lewellen, J. L. Sears, H. Liu, and J. E. Duffus. 1999. Specificity of TAS-ELISA for *Beet necrotic yellow vein virus* and its application for determining rhizomania resistance in field-grown sugar beets. *Plant Disease* 83:864-870.

Budget Justification: Funds for general laboratory supplies, as well as kits and reagents necessary for metabolite extraction, and other metabolome analyses were provided through a combination of BSDF funds and USDA-ARS in-house funds (Not SBREB) during 2016. These charges were covered with 2016 funds. Plant growth work at Salinas is nearly completed, and all samples will be sent for analysis once the current and final experiment is completed this month (Dec. 2016).

A GS-11 USDA-ARS postdoctoral research associate (Dr. Navneet Kaur, ARS Salinas) will conduct data analysis, with guidance and assistance from Drs. Broekling (CSU) and Webb (USDA) in Ft. Collins. Dr. Kaur's salary for sample preparation and research on this project was provided by SBREB in 2016. We are only requesting $6,000 from SBREB in 2017 to support Dr. Kaur's salary (additional salary funds were requested from BSDF). Dr. Kaur cannot be paid with USDA in-house funds due to her nationality (India). Therefore we are requesting limited funds from BSDF to assist with Dr. Kaur's salary to finish out the project involving data analysis and interpretation of results. An existing agreement is in place between USDA-ARS and BSDF to utilize BSDF funds for ARS salaries.

Budget:	**USDA**	**BSDF**	**SBREB**
Labor	$25,000	$10,000	$6,000
Equipment (over $250.00)	$0	$0	$0
Supplies	$800	$0	$0
Service (metabolomics analysis)	$9,000	$0	$0
Travel	$0	$0	$0
TOTALS:	$34,800	$10,000	$6,000