2021-2022 WERA1007 - Curly Top virus Biology, Transmission, Ecology, and Management

Annual Meeting Dates: 07/26 – 07/27/2022

Dates of reporting: 07/01/2021-06/30/2022

Report Date 08/30/2022

**Participants at Annual Meeting:**

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**Summary of Meeting Minutes**:

**Punya Nachappa**, host for the meeting welcomed the group to Colorado State University. **Rebecca Creamer**, WERA1007 Secretary, explained a bit about the group and its purpose. Introductions were made, and the agenda was discussed.

The Administrative Advisor for the group, **Mary Burrows**, Montana State University spoke by zoom to the group.

**Punya Nachappa** presented a brief background on curly top virus and the beet leafhopper as it exists in Colorado.

**Rebecca Creamer** presented current disease status in New Mexico in chile and hemp. She discussed the virus strains in chile and hemp and host specificity of certain strains. She discussed how the strains change over time. The most prevalent strain in 2022 is Pepper Yellow Dwarf BCTV-PeYD. She discussed how the warm winter in 2021-2022 influenced the early spring flight of leafhoppers and how that was inconsistent with the current predictive model.

**Avneesh Kumar** presented his work with **Kevin Dorn**, USDA-ARS Sugar Beet Genetics Lab, Fort Collins, CO entitled “**Mapping BCTV resistance gene in sugar beet crop wild relative Beta corolliflora.”** Sugar beet (*Beta vulgaris* ssp. *vulgaris*) is a biennial dicotyledonous plant and a member of the family Amaranthaceae. Sugar beet is one of the only two sugar producing crop. Beet curly top (BCTV) causes one of the major diseases in sugar beet. The virus also infects other important crops such as tomatoes, spinach, pepper, beans and hemp. A low to intermediate resistance is available in the commercial varieties and is difficult to maintain due to its quantitative nature. However, strong resistance was reported in the sugar beet crop wild relative *Beta corolliflora*. Our aim is to study the genetics of the BCTV resistance in *B. corolliflora*. We will use monosomic addition lines of *B. corolliflora* in sugar beet genetic background. We will phenotype MALs of all *B. corolliflora* chromosomes to determine the chromosomal source of resistance. Furthermore, we will perform random mutagenesis on resistant MALs and will determine the locus conferring resistance to BCTV in *B. corolliflora*.

**Lauren Murphy**, CDFA Beet Curly top Control Program, presented their yearly update. The Beet Curly Top Virus Control Program conducts intensive yearround surveys in California to determine where beet leafhopper (BLH) populations are developing. In 2022, the Program's surveys of traditional areas known for high curly top virus (CTV) incidence showed overall relatively low BLH levels, and therefore low CTV damage in susceptible commodity crops. However, the Program confirmed very high CTV damage in several tomato crops in non-traditional areas that do not normally experience CTV damage. The Program will be looking into whether the changing landscape in California may be having an effect on BLH migratory habits and population dynamics.

**Carl Strausbaugh**, USDA-ARS Kimberly, ID, in cooperation with **Eric Wenninger**, University of Idaho, presented their research by zoom on “**Beet curly top virus strains in sugar beets, dry beans, and beet leafhoppers along with vector population dynamics in southern Idaho**.” At the request of a sugar beet industry stakeholder, beet leafhopper (BLH) populations in southern Idaho were tracked in four counties during the 2020 and 2021 growing seasons in desert areas and sugar beet and dry bean fields in southern Idaho. Samples were collected on a weekly basis from mid-April through mid-September to assess all leafhoppers for population levels and the presence of *Beet curly top virus* (BCTV) strains. Crop plants from monitored fields were also assessed for the presence of BCTV strains. Once BLH populations in Elmore Co. began increasing in May, they were present in at least double-digit numbers through most of the summer at all sites both years. However, the BLH numbers at desert sites in other areas were at or near zero. In areas with low BLH desert populations, local weed populations appeared to be the primary source of BLH in crop fields. Preliminary data suggest two haplotypes (based on cytochrome oxidase gene) dominate the BLH population. Over the 22-week collection period, the horizontal card averaged 75% and 51% fewer BLH than the vertical card in 2020 and 2021, respectively. In 2020, 42% of the BLH samples were positive for the BCTV coat protein and Worland was the dominant strain of BCTV. The phytoplasma, morphotyping, and 2021 BCTV strain identifications are currently a work in progress. Once all data are collected, the project will establish the BCTV strains for which host plant resistance is needed and the best time for when control of BLH is necessary.

**Rajtilak Majumdar**, in cooperation with **Paul J. Galewski, Imad Eujayl, Eric Vincill, Carl A. Strausbaugh**, USDA-ARS, Kimberly, ID, presented their work on “**Transcriptional regulation and cross-kingdom RNAi in sugar beet resistance against Beet curly top virus**.”

Sugar beet is highly susceptible to *Beet curly top virus* (BCTV) which significantly reduces yield and sugar content in the semi-arid growing regions. Genetic resistance to BCTV is limited and primarily dependent upon seed treatment with neonicotinoids, the use of which are gradually being restricted. It is not fully understood how sugar beet plants confer resistance against BCTV. Through double haploid production and genetic selection, we have developed BCTV resistant breeding lines in Kimberly, ID. Using BCTV resistant (KDH13, KDH4-9) and susceptible (KDH19-17) lines, beet leafhopper meditated natural infection, mRNA/sRNA sequencing, and targeted metabolite analyses we demonstrate potential mechanisms of resistance against the virus. At early infection stages (within 6 days post inoculation), the differentially expressed micro RNAs (miRNAs) altered expression of their corresponding target genes such as *pyruvate dehydrogenase* (*EL10Ac1g02046*), *serine/threonine protein phosphatase* (*EL10Ac1g01374*), and *leucine-rich repeats (LRR) receptor-like* (*EL10Ac7g17778*), that were highly expressed in the leaves of resistant lines (vs. susceptible). Pathway enrichment analysis showed miRNAs predominantly targeted carbohydrate metabolism related genes. This corroborated well with altered levels of carbohydrates that were generally higher in the resistant lines both under green house and field conditions. We also identified BCTV derived small non-coding RNAs (sncRNAs) targeting critical sugar beet genes such as *LRR* (*EL10Ac1g01206*), and *transmembrane emp24 domain containing* (*EL10Ac6g14074*) and altered their expression. Sugar beet derived miRNAs targeted BCTV capsid protein genes and downregulated their expression which was more pronounced in the resistant lines. Higher C/N ratio in the leaves of resistant lines also indicate a metabolic shift towards C based primary metabolism as evident from the increased levels of related carbohydrates. The data presented here demonstrate the role of cross-kingdom RNAi in sugar beet resistance against BCTV at early stages of infection and identify potential candidate genes for future genetic manipulation to improve host plant resistance.

**Gina Angelella**, USDA-ARS Wapato, WA, presented her work on “**Characterizing beet leafhopper subpopulations within the Columbia Basin**.” We used molecular markers to examine the role of host-plant association and geographical location in beet leafhopper population structure and sequenced 16s rDNA to characterize the microbiome of beet leafhoppers within the Columbia Basin. Beet leafhoppers were collected during 2020 from 20 locations within the Columbia Basin, WA, and from one location in Elmore Co., ID, from crop and non-crop plants. Crops included sugarbeet, potato, and coriander seed, and non-crop plants included flixweed/pinnate tansymustard, tumble mustard, Russian thistle, and kochia. We submitted DNA for genotyping-by-sequencing services after which sequence data were processed using the Stacks *de novo* pipeline to call and clean SNPs, generating a dataset contained sequence data from 231 individuals and 2070 loci. We calculated the fixation index (Fst), analyses of molecular variance, and ran a principal components analysis and STRUCTURE analysis. No population structure was evident among specimens grouped by host plant, nor by collection site within the Columbia Basin; however, there was some differentiation between Elmore Co., Idaho, and Columbia Basin specimens potentially indicating limited movement. Nineteen beet leafhoppers were sequenced for microbiome characterization, including specimens on tumble mustard, kochia, Russian thistle, and sugarbeet from Pasco and Moxee, WA, Elmore Co., ID, and a colony at the Wapato facility. We detected Candidatus *Sulcia muelleri* (obligate endosymbiont) and *Wolbachia*, as well as *Rickettsia* and *Spiroplasma citri* at low frequencies. We screened an additional 448 Columbia Basin specimens for *Wolbachia* and plant pathogens to test for patterns relative to host plant (carrot, carrot seed, coriander seed, lima bean, potato, sugarbeet seed) or geographical location. *Wolbachia* infection was not related to host plant or region, and the proportions of beet leafhoppers carrying BCTV did not vary by *Wolbachia* infection status.

**Tiziana Oppedisano** in cooperation with **Silvia Rondon**, Oregon State University, presented their work on “**Beet leafhopper dynamics in the Lower Columbia Basin of Oregon and transmission efficacy of BCTV in hemp**.” *Circulifer tenellus* Baker (Hemiptera: Cicadellidae) is the only known vector of the beet leafhopper-transmitted virescence agent (BLTVA), that causes purple top disease in potatoes, and *Beet Curly Top Virus* (BCTV), an emergent disease in a relatively new crop like hemp in the lower Columbia Basin of Oregon.

Symptoms of BLTVA infection in potatoes include up-rolling of the leaves, swollen nodes, purplish discoloration, aerial tubers, and early plant decline. Severe BLTVA infestations can cause economic losses. Thus, since 2006, the Oregon State University Irrigated Agricultural Entomology Program has conducted a monitoring beet leafhopper program to help producers decide timing management decisions. Each year, from mid-April until September, 30-37 commercial potato fields were sampled in Umatilla and Morrow counties in eastern Oregon. *Circulifer tenellus* adults were monitored using yellow sticky cards deployed 1.5-3 m outside the field edges next to native weedy vegetation areas. In addition, daily temperatures were obtained from AgriMet weather stations closest to each sampled field. Using the multi-year dataset generated from the pest monitoring and the temperature data, we developed a phenology model based on the accumulated degree-days (DD). Total emergence percentage was modeled as a function of temperature to *C. tenellus* population growth in the field. According to our model, for our region, *C. tenellus* population emergence occurred gradually at 582, 1,440, and 2,823 DD, corresponding to 10, 50, and 90% total emergence, respectively. We hypothesized that besides temperature, other factors such as the availability of a preferred host such as sugar beet are likely to trigger *C. tenellus* to become active early in the season.

BCTV has previously been reported as one of the main constraints affecting hemp production in several US states, including Oregon. In hemp, symptoms of BCTV infections appear in yellowing and stunting, up-curled leaf, twisting, and flat stems. Thus far, little is known about hemp varieties’ susceptibility to the BCTV. In 2021, in a greenhouse experiment, we evaluated ‘Cherry Blossom’ and ‘Cherry Wine’ responses to BCTV transmitted by *C. tenellus*. BCTV-infected *C. tenellus* nymphs and adults were exposed to healthy hemp plants for a week. Preliminary data showed a higher transmission percentage using nymphs in ‘Cherry Blossom’, while the transmission rate was similar with both stages of *C. tenellus* in ‘Cherry Wine’. Currently, our program is testing different BCTV strains in transmission studies.

**Jordan Withycombe** in cooperation with **Avneesh Kumar, Kevin Dorn, Punya Nachappa** and **Vamsi Nalam**, Colorado State University, presented her talk on “**Enhancing sugar beet breeding efforts through identifying the mechanisms and genes conferring curly top resistance**.” Curly top disease is caused by the beet curly top virus (BCTV) which is exclusively vectored by *Circulifer tenellus*, the beet leafhopper. Current management strategies for BCTV include the use of BCTV-resistant or tolerant varieties, but the underlying genetic mechanism is not known. It is critical to determine the mechanism of resistance and identify resistant gene(s) that can be implemented in breeding programs. The objectives of my project were: 1) classify the nature of curly top disease resistance in resistant (EL10) and susceptible (FC-709-2) sugar beet varieties using insect preference and performance assays and 2) characterize the transcriptional response to BCTV infection and BLH feeding in both varieties using RNA-Seqencing. There was no significant difference in adult survival and reproduction on resistant and susceptible varieties suggesting that plant resistance mechanism was not antibiosis. In preference assays, BCTV-infected leafhoppers showed preference to susceptible variety compared to resistant variety at all timepoints; however, non-infected leafhoppers did not show a feeding preference. Studies are underway to perform RNASeq experiments with a factorial experiment design: 2 varieties × 3 treatments (non-infected leafhoppers, BCTV-infected leafhoppers, control) × 3 timepoints (1, 7, 14 dpi) ×3 biological replicates). Total RNA will be subjected to stand specific RNA-Seq. The bioinformatic analysis will focus on identifying differences in overall gene expression between EL-10 and FC-709-2 and looking for unique plant response genes expressed by either variety. Overall, outcomes of this study help to identify more durable and broad-spectrum viral resistance in sugar beets.

**Max Schmidtbauer** in cooperation with the **Punya Nachappa** laboratory group, Colorado State University, presented his work “**A little help from my friend: virus infection reduces susceptibility of beet leafhoppers to neonicotinoid insecticides**.” Sugar beets are grown across the western United States for their lucrative taproot and are an economically important crop generating 1.16 billion dollars in 2021. However, production is threatened by beet curly top virus (BCTV), a viral pathogen that severely reduces yield. The virus is exclusively transmitted by the beet leafhopper, *Circulifer tenellus*. Current management options for BCTV rely on neonicotinoid seed treatments and pyrethroid foliar sprays. However, there are reports of neonicotinoid resistance in numerous insect pests and this insecticide group may have harmful effects on non-target insects. Hence, the goal of this research is to examine susceptibility of beet leafhoppers to three different application rates (2x, 1x, 0.1x) of insecticides and determine their ability to lower virus transmission. We hypothesize that applying insecticide at a higher label rate will result in an increased mortality of beet leafhoppers, and therefore reduced virus transmission. At the 1x rate, neonicotinoids were more effective than pyrethroids, yielding 100% mortality of BCTV-infected and non-infected insects. However, at the 0.1x rate BCTV-infected leafhoppers had a higher survival rate than non-infected insects. This result was observed in only the neonicotinoid trials and not the pyrethroid trials. Although experiments are ongoing, confirming this potential insecticide resistance in BCTV-infected insects would be a key piece of information to aid in tracking the epidemiology of BCTV and make predictions about leafhopper populations. With this knowledge, farmers would be able to adjust their management strategies to achieve more sustainable and profitable sugar beet production practices.

Various participants presented field perspectives from different states.

Idaho – Carl, Eric, Raj – There was a slow late start to initial leafhopper flights because of the cool, wet spring. Some age-related resistance in sugarbeets is present and beans can grow out of symptoms.

* Thomas, Tiffany – Commercial sugarbeets have some tolerance. There was higher BCTV incidence in Idaho, which might be due to the mild winter, which could have promoted the growth of weed hosts

Colorado – Punya – BCTV symptoms have been found in early stage hemp in Southern Colorado (Alamosa area). Neonicotinoids were banned in Boulder County, so the rest of the state may follow. BCTV has been found in tomatoes, peppers, and sugarbeets.

New Mexico – Rebecca - An unusually warm winter allowed the beet leafhopper to overwinter in weeds in pecans, which has not been noted before. Leafhopper were collected by sweep net in late December and early January, which has also never been found before.

California – Lauren, Zach – There was historically high BCTV only in southern valleys. They had low virus this year due to the malathion treatments and early dry weeds. In contrast more northern and eastern California, including the Sacramento Valley and northern San Joaquin Valley, had highly unusual high BCTV disease pressure. This might be influenced by cultural practices such as growing more tree crops and more fallow fields because of low water availability. Possibly the beet leafhoppers are living year round in the valleys and not migrating from the foothills.

Washington – Gina, Kylie – The trapping network is designed to collect around potatoes, but has included vegetables and seed crops. Last year there were around 40,000 leafhoppers collected. The peak in leafhoppers numbers was delayed until the end of June. For the leafhoppers tested, there was higher amounts of BCTV followed by BLTVA, followed by *Spiroplasma citri*.

Research questions and priorities

California would like to follow the migratory habits of the beet leafhopper, perhaps by testing for virus strains on the valley floor.

Kevin Dorn would like help with a public sugarbeet database the includes genotype and phenotype data. Wants others to add information.

What makes a good host for the beet leafhopper? What is the nature of non-host resistance, especially that found in weeds?

Barley is a non-host; does the virus replicate in the plant?

There is a Kochia panel at Colorado State University with more than 100 curated specimens. This could be helpful in determining beet leafhopper preferences.

The 2023 WERA 1007 meeting will be held in California, with Lauren Murphy hosting. Possible dates are July 10-11 or the first week of August.

**Project Objectives:**

1. **Assess the current status of curly top and set priorities for integrated research on curly top disease.**
2. **Characterization of curtovirus strains including virus genetic diversity, new virus strains and virus in new hosts.**
3. **Organize research on the biology and ecology of the leafhopper, virus transmission, and the role of weed hosts in curly top in the western US.**
4. **Organize research to improve virus and vector management.**
5. **Provide a national platform for education on curly top disease, virus/insect/plant ecology and management, collaboration among scientists involved in these activities, and extension of research-based information for producers.**

**Objective 1:**  Accomplished through annual meeting presentations and goal setting. See above minutes.

**Objective 2:** Worked toward this goal in individual research programs. See above minutes.

**Objective 3:** Made progress toward the goal. See above minutes.

**Objective 4:** Made progress toward goal. See above minutes.

**Objective 5:** Collaborative curly top projects for 2021-22 season were carried out between Carl Strausbaugh and Rebecca Creamer and between Carl Strausbaugh and Punya Nachappa. Rebecca Creamer is currently conducting a cooperative project with Punya Nachappa.

**Impact Statement**

Curly top is an economically important disease in many states in the western U.S. Members of the WERA1007 group increased the knowledge of the virus biology, its transmission, and the management of the disease. The range of virus strains was expanded in California, Colorado, Washington, and New Mexico. The resistance to curly top in sugarbeets was assessed, aspects of the curly top virus transmission were characterized, and the use of foliar insecticides for vector control was tested. These findings should help improve the management of curly top in sugar beet and other affected crops in the western U.S.

**Publications**

The group did not publish a report together. The following curly top related publications were published during the last year:

Strausbaugh, C.A. and Wenninger, E.J. (2022) [*Foliar insecticides for the control of curly top in Idaho sugar beet, 2021.*](https://eprints.nwisrl.ars.usda.gov/id/eprint/1762/) Plant Disease Management Reports. 16:1. 17 March 2022.

Majumdar, R. and Strausbaugh, C.A. and Vincill, E.D. and Eujayl, Imad A. and Galewski, Paul J. (2022) [*Leaf bacteriome in sugar beet show differential response against beet curly top virus during resistant and susceptible interactions.*](https://eprints.nwisrl.ars.usda.gov/id/eprint/1770/) International Journal of Molecular Sciences. 22 July 2022.

Majumdar, R. and Galewski, Paul J. and Eujayl, Imad A. and Minocha, Rakesh and Vincill, E.D. and Strausbaugh, C.A. (2022) [*Regulatory roles of small non-coding RNAs in sugar beet resistance against beet curly top virus.*](https://eprints.nwisrl.ars.usda.gov/id/eprint/1757/) Frontiers in Plant Science. 12:1-22. 10 January 2022. Available: <https://doi.org/10.3389/fpls.2021.780877>