2023 WERA1007 - Curly Top virus Biology, Transmission, Ecology, and Management

Annual Meeting Dates: 07/25 – 07/26/2023

Report Date 09/1/2023

**Participants at Annual Meeting:**

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**Summary of Meeting Minutes**:

**Rebecca Creamer**, WERA1007 Secretary, explained a bit about the group and its purpose. Introductions were made, and the agenda was discussed.

**Kendra Tapia** – BCTVCP-CDFA- meeting host presented a welcome and **BCTV Control Program 2023 Update.**

CDFA BCTVCP WERA Presentation Summary

* 2022 winter showed sporadic nymph activity and extensive die-off of summer host plant vegetation in November. Heavy rainfall in December facilitated germination of new vegetation growth on the western hillsides
* Early spring activity was limited due to rain and storm events. BLH averages were 0-1 per sweep set. Annual grasses took over most of the host plant vegetation, limiting the ideal conditions for BLH populations.
* By late spring, some Nymph activity started to appear, but overall BLH averages were in the 0-2 per sweep range. BCTV expanded their staff and started surveying more in the Sacramento Valley. Host plant vegetation observed was Filaree, Plantago, Peppergrass, and Mallow.
* The Program performed control operations on the last of May and First of June. 407 acres were controlled in Fresno and Kings Counties.
* Commodity damage has been low in the 0-2% range. The Program has seen a reduction in planted acreage this year due to flooding from the high amount of rainfall we got. Fields that showed above the threshold levels were found to have been near fallow fields or almond orchids that had been recently disked.
* This year has seen low incidences of BLH activity due to the high amount of rainfall we got. Annual grasses essentially seeded out host plant vegetation. Not a typical year, but every year is different.
* The program is expanding further across the state and updating our host plant guide and procedures. The Program continues to evaluate the changes in landscape and environment to see how it will affect BLH migratory patterns.

**Rebecca Creamer** presented a **Curly top overview with New Mexico** **update** on the 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. Pepper curly top BCTV-PCT was found infecting pepper but not hemp. In 2022, both pepper and hemp were found to be infected with Pepper Yellow Dwarf BCTV-PeYD and BCTV-Wor. The warm winter in 2021-2022 influenced the early spring flight of leafhoppers and that was inconsistent with the current predictive model. Beet leafhoppers were shown to prefer CBD hemp and fiber hemp over pepper or grain hemp.

**Batool Alkhatib** presented **Cytopathic effects of curly top virus on infected plants.**

Plant virus infection causes significant impacts on crop productivity due to the diseases they cause and to economics and food security. *Beet Curly top virus* (BCTV) is a highly devastating pathogen affecting a wide range of plant species worldwide and is transmitted only by leafhoppers in the genus *Circulifer*. During the synthesis of the viral components in the host-infected cell, the cell undergoes characteristic biochemical and morphological changes; such morphological changes in cells caused by viral infection are called cytopathic effects (CPE). This study aimed to investigate the CPE induced by BCTV on various plant hosts, including (Tomato, Pepper, Sugarbeet, and Hemp), In such susceptible hosts, BCTV infection resulted in severe stunting, leaf curling, and yellowing, leading to reduced photosynthetic capacity and yield losses. Transmission electron microscopy images of ultrathin sections of infected leaves from these plants revealed that BCTV infection triggered distinct changes in the ultrastructure of some organelles, such as chloroplast, starch grains, vacuoles, and mitochondria along with uneven thickenings of the cell wall. Furthermore, this study showed variations in BCTV strains and their impact on different plant hosts; certain strains showed higher virulence, causing more severe symptoms and physiological disruptions compared to others, suggesting a complex interaction between viral factors and plant host-specific defenses. This is the first study shed light on the diverse ultrastructural impacts of BCTV infection on different plant species; such studies help in understanding the molecular mechanisms underlying the host-virus interactions, which in turn will aid in the development of effective control strategies to mitigate the detrimental impacts of BCTV on agriculture and safeguard global food security.

Christian Nansen and Hyoseok Lee presented **Region-wide and real-time mapping of beet leafhopper migration from Coastal Foothills**

Beet leafhoppers (Circulifer tenellus) (Hemiptera: Cicadellidae) are the primary vectors of beet curly top virus (BCTV), a major plant-pathogenic virus in tomato and other crops. In California, beet leafhoppers are known to overwinter in coastal foothills and migrate into the Central Valley during spring. We experimentally manipulated drought regimes of individual plants and acquired plant reflectance profiles to generate standardized indices of greenness (enhanced vegetation index, EVI). Plants were experimentally infested with beet leafhoppers, and we found a significantly negative correlation between EVI and flight propensity for two plant species that serve as common hosts. In related field studies, sticky card trapping at three field locations during two tomato growing seasons showed a significant negative correlation between satellite image-derived EVI and spring migration timing of beet leafhoppers. A predictive model was developed and used to examine annual trends in spring migration timing and relationships with incidence of BCTV symptoms surveyed in the Central Valley. Spring migration timing was negatively correlated with regional incidence of BCTV symptoms. The proposed spring migration model accurately predicted major BCTV outbreaks in 2013 and 2021, years that were characterized by unusually early spring migration from coastal foothills. The spring migration model is freely available as a web-based tool: [https://hyslee.users.earthengine.app/view/beet-leafhopper-migration-in-ca](https://nam10.safelinks.protection.outlook.com/?url=https%3A%2F%2Fhyslee.users.earthengine.app%2Fview%2Fbeet-leafhopper-migration-in-ca&data=05%7C01%7Ccreamer%40nmsu.edu%7C07235b1658434c9e245e08dba293ce64%7Ca3ec87a89fb84158ba8ff11bace1ebaa%7C1%7C0%7C638282527952595638%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=JIuo176iJuBZKXW85gOfDydcJWZzBBSkvQ4kLYtoENA%3D&reserved=0)

**Kylie Swisher Grimm**, USDA-ARS Wapato, WA, presented her work on “**Beet leafhopper testing in the Columbia Basin of Washington**.”

In the Columbia Basin of Washington State, beet leafhopper-transmitted pathogens including Beet curly top virus, Beet leafhopper transmitted virescence agent phytoplasma, and *Spiroplasma citri* cause serious damage to vegetable and seed crops each year. Since 2021, researchers with the USDA Agricultural Research service and Washington State University have sought to obtain near real-time pathogen prevalence data in beet leafhopper specimens captured near potato and other vegetable or seed fields across the region. Through improving extraction and diagnostic protocols, researchers were able to acquire pathogen data on a weekly basis, providing this data to growers through the Washington State University Potato Decision Aid System online tool. This data will allow important seasonal or regional trends to be identified. Ultimately, this data could be incorporated each year into grower IPM programs, improving beet leafhopper management by either decreasing the cost and hazard of unnecessary insecticide sprays or increasing crop yield and quality by preventing disease outbreaks through well-time insecticide applications.

**Carl Strausbaugh**, USDA-ARS Kimberly, ID, in cooperation with **Eric Wenninger**, University of Idaho, **L. Jackson,** and **E. Vincill** presented their research by zoom on “**Beet curly top viruses and phytoplasmas in sugar beets, dry beans, and beet leafhoppers along with vector population dynamics in southern Idaho**.”

Beet curly top in sugar beet and common bean is a major yield limiting disease which is caused by *Beet curly top virus* (BCTV) and is vectored by the beet leafhopper (BLH; *Circulifer tenellus*). BLH populations in southern Idaho were tracked during the 2020 and 2021 growing seasons in desert areas and sugar beet and common bean fields with yellow sticky cards to assess BLH population levels and identify the curly top virus species/strains and phytoplasmas present. Plants from monitored crop fields were also assessed for the same pathogens. Once BLH populations in Elmore Co. began increasing in May, they were present in double-digit numbers per card through the summer at all sites both years. However, the BLH numbers at other desert sites were at or near zero; local weed populations and not desert areas appeared to be the primary source of BLH in crop fields. Based on cytochrome oxidase gene, two haplotypes dominated the BLH population both years. Both years, BCTV strains Worland (Wor) and Colorado (CO) were the primary strains in BLH and plant samples. The CA/Logan, Pepper curly top (PeCT), and Severe strains of BCTV were also detected in BLH along with *Spinach curly top Arizona virus* (SpCTAV). Phytoplasmas were detected in 1% of BLH samples both years. Phytoplasmas, SpCTAV, and PeCT were not detected in plant samples. This project established the curly top species/strains for which host plant resistance is needed as well as the time and areas when crops are at highest risk for infection.

**Max Schmidtbauer** in cooperation with **Laine Hackenberg**, Colorado State University, presented his work “**Occurrence of beet curly top virus in hemp in Colorado and insights into the potential seed transmission.**

Beet curly top virus (BCTV) is vectored solely by the beet leafhopper (*Circulifer tenellus*) and is the most serious disease affecting sugar beet production in the United States. Hemp is characterized as *Cannabis sativa L.* which expresses THC at or under a federally set limit of 0.3% and has become a crop of interest in many states. While BCTV has been studied in sugar beet for over one hundred years, in 2015, BCTV was first reported to infect hemp in western Colorado. Starting in 2021, Laine Hackenberg conducted a virome survey of hemp in different counties in Colorado. Using next generation sequencing, she hopes to provide understanding around the occurrence and distribution of BCTV and other viruses of interest in hemp in Colorado. Beet curly top virus is also not known to be seed transmitted in sugar beet, tomatoes, and a handful of other crops. With hemp emerging a new host of BCTV, it is important to also determine if seed transmission of this virus is possible in hemp. Through a variety of serological assays, we hope to give answers to our growers who rely on a secure, pathogen free hemp seed stock for their livelihoods.

**Jordan Withycombe** in coordination with **Jinlong Han, Punya Nachappa, Vamsi Nalam,** presented her talk on “**Sweet Surprise: The search for genes conferring curly top resistance in sugar beet**.”

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 unknown. 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-Sequencing. There was no significant difference in adult survival and reproduction on either variety suggesting that plant resistance mechanism was not antibiosis. In preference assays, BCTV-infected leafhoppers showed preference to feed on the susceptible variety compared to resistant variety; however, non-infected leafhoppers did not show a preference. RNA-seq experiments were conducted using a factorial experiment design: 2 varieties × 3 treatments (non-infected leafhoppers, BCTV-infected leafhoppers, control) × 3 timepoints (1, 7, 14 dpi) ×3 biological replicates). Following sequencing, the bioinformatic analysis was conducted using CLC genomics workbench where differentially expressed transcripts (DETs) were identified for each variety and timepoint. Final analysis is still in progress, but genes associated with plant hormonal pathways (ABA and JA pathways) and protein synthesis and ubiquitination are of interest.

**Rajtilak Majumdar** presented his work in cooperation with **Paul Galewski, Matthew Lebar, Carl Strausbaugh, Rakesh Minocha, Stephanie Long, Imad Eujayl, Christopher** **Rogers** on “**Untargeted metabolome and targeted analyses of C and N metabolites, and degradome reveal distinct mechanisms associated with resistance and susceptibility during sugar beet interactions with Beet curly top virus”**

  Sugar beet is highly susceptible to Beet curly top virus (BCTV) which significantly reduces yield and sugar production in the semi-arid growing regions worldwide. Sources of genetic resistance to BCTV is highly limited and primarily dependent upon seed treatment with neonicotinoids, the use of which is gradually being restricted. Through double haploid production and genetic selection, we have developed BCTV resistant sugar beet breeding lines. Using BCTV resistant (R) [KDH13 and KDH4-9] and susceptible (S) [KDH19-17] lines, beet leafhopper meditated natural infection, and combined omics approaches we demonstrate potential mechanisms of resistance against the virus. Untargeted metabolome analysis revealed higher accumulation of specific isoflavonoid O-glycosides, flavonoid 8-C glycosides, triterpenoid, iridoid-O-glycosides in the leaves of the ‘R’ lines (vs. ‘S’). In general, majority of the amino acids were higher in the ‘S’ line except for GABA that was significantly higher in the ‘R’ lines with infection. The ‘S’ line accumulated higher amounts of polyamines (PAs) especially Spm at early [2- and 6- days post inoculation (dpi)] infection stages (vs. ‘R’ lines) with no visible disease symptoms. Higher PA and amino acid (AA) contents in the leaves of ‘S’ line corroborated well with the higher expression of genes involved in the biosynthesis of ornithine (Orn), arginine (Arg), and PAs during early stages of infection. The trend in PAs and AAs stated earlier under greenhouse conditions was similar in plants exhibiting disease symptoms under field conditions, and ‘R’ lines (vs. ‘S’) maintained higher leaf sucrose, glucose + galactose, fructose contents, and C/N ratio. The overall findings suggest a combination of transcriptional regulation and production of putative antiviral metabolites might contribute to BCTV resistance. In addition, genome divergence among BCTV strains differentially affects the production of small non-coding RNAs (sncRNAs) which may potentially affect pathogenicity and disease symptom development.

Various participants presented field perspectives from different states.

Idaho – There was earlier infection and hoppers were found even during heat waves. There has been a decrease in curly top in Idaho, with only very low levels of disease. Used to be highest in Treasure Valley. Neonicotinoids have nearly eliminated the disease. Now only find single infected plants. Working toward breeding for frost tolerant high sugar varieties.

Montana and Wyoming had very low levels of curly top.

Colorado – Have found curly top on hemp and on leafhoppers on sticky traps.

California - There were low levels of insect-transmitted pathogens this year due to the high rainfall. Sacramento Valley had curly top in 2021 and 2022 and the presence of spinach curly top. Those years were warm and dry with increased fallowing of fields due to water conservation.

Washington – The trapping network is designed to collect around potatoes, but has resulted in very good prediction for curly top.

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.

How long after neonicotinoids are banned before the leafhoppers and curly top take explode?

Can we bridge the gap between planting and when disease kicks in with other insecticides?

How beneficial is a leafhopper trapping system program for control of curly top?

What are alternative pesticides that could help with curly top?

Can new technologies such as CRISPR or RNAi help with BCTV?

The 2024 WERA 1007 meeting will be held in Salinas, CA, with Bill Wintermantel hosting. Possible dates are July 22-23 or August. 6-7.

**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 Kylie Swisher Grimm and between Rebecca Creamer and 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., Majumdar, R., Wenninger, E.J. 2023. Foliar insecticides for the control of curly top in Idaho sugar beet, 2022. Plant Disease Management Reports. 17. Article ST004.

Strausbaugh, C.A., Eric, W., Jackson, L.K., Vincill, E.D. 2023. Beet leafhopper and BCTV strain survey 2022 progress report. The Sugarbeet. p.50-52.

Gorman, C.J., Crowder, D.W., Swisher Grimm, K.D. 2023. A high-throughput plate method for nucleic acid extraction from beet leafhopper (Hemiptera: Cicadellidae) and potato psyllid (Hemiptera: Triozidae) for pathogen detection. J. Econ. Entomol. Doi:10.1093/jee/toad153

Swisher Grimm, K.D., Gorman, C. Crosslin, J. 2023. New Assays for rapid detection of beet leafhopper associated plant pathogens, Candidatus *Phytoplasma* *trifolii*, *Beet curly top virus*, and *Spiroplasma citri*. Plant Disease doi: 10.1094/PDIS-04-23-0769-RE

Creamer, R., Simpson, A., Rheay, H.T., Brewer, C.E. 2023. Interactions fo beet leafhopper (Hemiptera:Cicadellidae) vector of beet curly top virus and hemp in New Mexico. Environmental Entomology doi.org/10.1093/ee/nvad069