

Entomopathogenic virus entry and replication site in the Glassy-winged Sharpshooter, *Homalodisca vitripennis*. (Hemiptera: Cicadellidae).



Wayne Hunter^{1*}, Xiomara Sinisterra², Diann Achor³, Laura Hunnicutt¹

*1. USDA, ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Rd., Ft. Pierce, FL 34945.

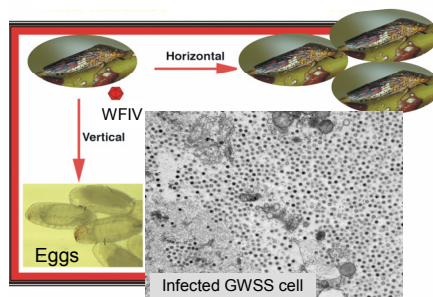
2. University of Florida-IFAS, Fort Pierce, FL. 3. University of Florida-IFAS, Lake Alfred, FL

Wayne.hunter@ars.usda.gov

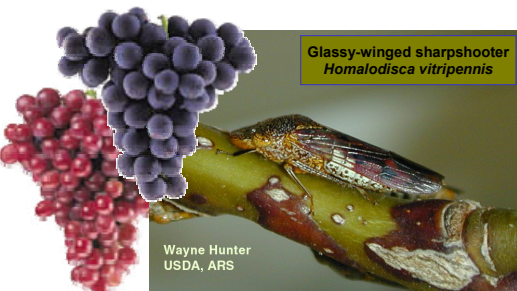
Primary Investigations:

1. Discovery of new viruses infecting leafhoppers which may be used in the management leafhopper pests.
2. Identification of the genetic basis of GWSS immunity and stress responses during virus infection.

Virus transmission in GWSS



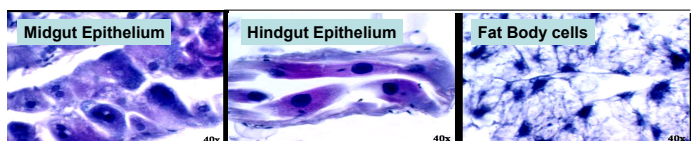
While other viruses, such as Insect Iridovirus can cause infection through contact with infected individuals such as during mating, or through oviposition, HoCV-1 appears to be spread through an oral mode of transmission, possibly as leafhoppers daub the tip of the stylets on contaminated plant surfaces.



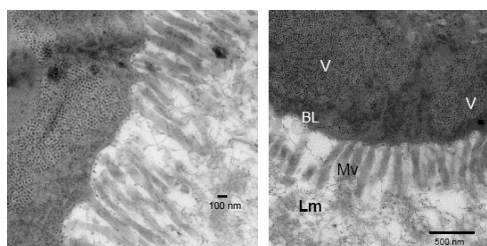
Pierce's disease of grapes is caused by the bacterial pathogen *Xylella fastidiosa*, which limits the U.S. viticulture industries. The glassy-winged sharpshooter, GWSS, is the primary disease vector spreading Pierce's disease. Discovery and application of insect viral pathogens of leafhoppers have yet to be thoroughly exploited as potential biological control agents. Herein we examined the infection site in sharpshooters exposed to HoCV-1. The newly discovered leafhopper virus, HoCV-1, is a ssRNA virus, that belongs to the family *Dicistroviridae*. Sharpshooters in southern California as well as in North Florida have been determined to be infected with the virus.

RESULTS:

- GWSS adults were determined to be infected using primers specific to HoCV-1, in rtPCR analysis.
- The midgut was dissected from field collected sharpshooters, prepared for transmission electron microscopy, and examined. GWSS which were rtPCR positive for virus also had virus in the midgut tissues.
- Viral transcripts were abundant (hundreds) in the cDNA library made from midgut tissues, while only two transcripts were found to from the cDNA library made from salivary glands.



Example of another insect infecting virus, 'Whitefly Iridovirus', WFIV, (above) which is a dsDNA virus that infects various tissues such as the midgut, fat body, muscle, and nerve tissues within GWSS. In contrast, HoCV-1, which is a ssRNA virus, appears to cause infection primarily in the midgut Basal Laminae.



(Above) The recently identified leafhopper viral pathogen HoCV-1, was isolated from field collected adult glassy-winged sharpshooter leafhoppers. The virus was observed in midgut tissues using transmission electron microscopy, TEM. Gut lumen, Lm, showing Microvilli, Mv; Virus, V, was observed inside the microvilli, Mv, the basal lamellae, BL, with large amounts of virions. Analysis using rtPCR of midguts which were dissected from virus exposed leafhoppers tested positive for virus. Transmission electron micrographs, HV60kV, Direct 44000x, AMT Camera System. Image by Wayne Hunter, Agricultural Research Service.

Summary:

- *Leafhopper vectors of Pierce's Disease, such as the GWSS, *Homalodisca vitripennis*, are susceptible to insect infecting viruses.
- Infection caused increased mortality (~ 20-60%) of 5th instar nymphs.
- Currently known and newly discovered insect infecting viruses may have potential for the management of leafhoppers when combined with other measures, by increased infection within the GWSS population, or through improving natural virus infectivity.
- Virus infection causes increased stress on the GWSS and may increase susceptibility to insecticides, abiotic and biotic stresses.

Research needed:

Cost of mass production of viruses still remains one of the major obstacles to the widespread use of insect infecting viruses as biological control agents.

Emerging Solutions:

Discovery of new leafhopper viruses combined with emerging technologies, such as molecular methods, may permit some insect viruses to be mass produced more cost effectively thereby opening a niche for the commercialization and use of viruses in area-wide suppression programs against leafhopper and other insect pests.