IDENTIFICATION AND WHOLE EXTRACTION OF *HOMOLADISCA COAGULATA-VIRUS01 (HoCV-01* FROM TEXAS GLASSY-WINGED SHARPSHOOTER POPULATIONS

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Reporting Period: The results reported here are from work conducted November 2008 through September 2009.

ABSTRACT

The glassy-winged sharpshooter (GWSS) is an invasive pest and important vector of *Xylella fastidiosa (Xf)*, a xylem-limiting bacteria that causes Pierce's disease (PD) in grapes as well as other agricultural diseases. The primary method of managing the spread of *Xf* is controlling its insect vector populations. Methods such as chemical control are not target specific and lead to problems such as residue contamination, injury to non-target organisms, and insecticide resistance. Identifying agents that can impact GWSS populations is the goal of a biological control strategy. In this study, we have identified and extracted whole GWSS *Virus 01 (HoCV-01)* from populations of GWSS collected in Texas. *HoCV-01* is a novel virus that harbors pathogenic potential with regard to GWSS. Future plans for *HoCV-01* include reintroduction into GWSS populations through feeding. Increased amounts of *HoCV-01* ingestion may lead to weakened populations of GWSS that are more susceptible to control methods such as insecticides. This would decrease the amount of insecticide needed to produce a desired mortality rate in insect populations.

LAYPERSON SUMMARY

The glassy-winged sharpshooter (GWSS) is the most economically important insect with respect to the spread of *Xylella fastidiosa (Xf)*, the causal agent of Pierce's disease (PD). Therefore control of this insect is of paramount importance to the management of the disease. While insecticides have been used successfully to reduce the economic impact of this disease system, alternate methods of population insect control are needed to continue management in the future. Biological control offers alternatives to chemical control that can be effective in negatively impacting insect population without harmful environmental effects or concern for insecticide resistance. In this work, we molecularly describe a virus that shows promise as a tool for biological control. While this virus does not cause significant acute mortality, it may reduce the fitness of insects to a point where other control methods would be more effective. We suggest that viral infection will make insects more sensitive to insecticide treatment, resulting in lower LD50 rates for achievement of significant control. This means that lower levels of insecticide can be used effectively.

INTRODUCTION

The glassy-winged sharpshooter (GWSS) is the major vector of *Xylella fastidiosa (Xf)* Wells in the southern USA (Adlerz 1980; Blua et al., 1999). The plant pathogenic bacterium, *Xf*, has caused economic losses to several agricultural industries in North America and is associated with many plant diseases such as Pierce's disease (PD), and oleander leaf scorch. PD of grapevine has become a well recognized *Xylella*-related disease; the vector profile is well known and the epidemiology of the disease is well documented (Hopkins et al., 2002). The introduction of GWSS into new areas is directly related to increased occurrence of PD in vineyards (Perring et al., 2001). Therefore, the management of PD depends heavily on the ability to control its vectors, especially GWSS.

Methods of vector manipulation such as chemical control with the use of insecticides are not target specific and lead to problems such as residue contamination, injury to non-target organisms, and insecticide resistance. The search for more benevolent pest management strategies has led to the use of biocontrol agents such as fungi and parasitoids. However, by utilizing viruses that currently reside in GWSS populations,a viral bio-control that is even more precise may be developed (Hunnicutt et al., 2006).

HoCV-01 is a member of the genus *Cripavirus* and family *Dicistroviridae (*Hunnicutt et al., 2006). It is a novel virus that harbors pathogenic potential with regard to GWSS. The focus of this study was the identification and extraction of whole *HoCV-01* found in populations of GWSS collected in Texas. Once identification was complete, the genome was sequenced and checked for variation which may produce an increase in virulence. Following sequencing, whole *HoCV-01* was extracted in order to reintroduce it into GWSS populations.

OBJECTIVES

1. Identify *HoCV-01* in populations of GWSS collected in Texas.
2. Sequence viral capsid protein and check for variation between strain found in Texas and strain found in California.
3. Extract and purify whole *HoCV-01* with intent to reintroduce into uninfected populations of GWSS.

MATERIALS AND METHODS

RNA Extraction. GWSS bodies were collected in microcentrifuge tubes and homogenized. GWSS RNA was separated from the solution and purified using a Qiagen RNeasy kit (Qiagen™, Germantown, MD).

RT-PCR & Gel Electrophoresis. Each 1µL GWSS RNA sample was combined with 10µL 2X Reaction Mix (Invitrogen Molecular Probes™, Eugene, OR), 0.4µL forward primer, 0.4µL reverse primer, 0.4µL Platinum® Taq DNA Polymerase (Invitrogen Molecular Probes™, Eugene, OR), and 8µL DEPC H_2O in solution. Samples were then subjected to a Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) using an iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). 2µL of each GWSS DNA sample was subjected to gel electrophoresis using 2µL ladder, 2µL loading dye per sample, and a 1% agarose gel containing 100mL TAE buffer and 1g agarose gel. Gels were subjected to 100V and 400A for 50 min. and checked under Ultraviolet light in a Bio Doc-It Imaging System (Cole-Parmer™, Hanwell, London).

Sequencing PCR & Ethanol Precipitation. Sequencing was done on sight in the Bextine Molecular Biology Laboratory at The University of Texas at Tyler. An amount of 2µL of each GWSS DNA sample was combined with 2µL Nano-pure H_2O, 2µL primer (only the forward or the reverse primer were used in this step), 4µL GenomeLab™ DTCS Quick Start Mix (GenomeLab™, Fullerton, CA), and taken through a Sequencing Polymerase Chain Reaction, or Sequencing PCR in an iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). Each sample was then combined with 2µL 3M NaOAc, 2µL 100nM EDTA, 1µL 20mg/mL Glycogen, vortexed thoroughly, and subjected to an ethanol precipitation. During the ethanol precipitation, sample DNA was purified for sequencing with separate washes of ice cold 95% and 70% ethanol. The resulting pellet of purified DNA was mixed with 40µL Sample Loading Solution, vortexed, and transferred to a sequencing plate.

DNA Sequencing. DNA samples were sequenced using a CEQ 8000 Genetic Analysis System (Beckman Coulter Inc., Fullerton, CA). Prior to loading the samples into the sequencer, each sample was combined with one drop of Mineral Oil. Data was analyzed using Bioedit® Sequence Alignment Editor (Ibis Biosciences, Carlsbad, CA).

Whole Virus Extraction. Infected GWSS bodies were placed in a mortar and pestle and homogenized in 100mL of phosphate buffer containing 0.02mg DETCA. The homogenate was then transferred to 50mL centrifuge tubes and centrifuged at 1600rpm for 20 minutes in an Eppendorf 5804R Centrifuge (Eppendorf , Hamburg, Germany). The resulting supernatant was split into two ultra-centrifuge tubes, combined with more phosphate buffer with DETCA, vortexed, and ultra-centrifuged at 22,000rpm for 16 hours in a Sorvall® RC-5B Refrigerated Superspeed Centrifuge (DuPont Instruments, Wilmington, DE). Following ultra-centrifugation, the supernatant was discarded, and the pellet was dissolved with 5mL phosphate buffer with 0.4% Na-deoxycholic acid and 4% Brij 52. The resulting solution was centrifuged and 1600rpm for 15 minutes, passed through a 0.45µm filter, and collected into large Eppindorf tubes. The unrefined *HoCV-01* solution was placed in a dialysis membrane, placed in a large beaker containing a stir-bar and ddH_20, and placed in a refrigerator at 4°C. The ddH_20 was changed out ever five-six hours until a white precipitate could be seen in the dialysis membrane. The purified *HoCV-01* solution was collected into micro-centrifuge tubes and stored at -80°C.

RESULTS AND DISCUSSION

HoCV-01 was detected in GWSS populations collected in Texas. Sequence comparison of the Texas strain of *HoCV-01* against the sequenced California strain (Hunnicutt et al., 2006) shows some variation. The percent similarity between the strains is **98.8%.**

Base pair 828, Cytosine in the California strain, is a Thymine in the consensus strain (**Figure 1**). This changes the amino acid translation from Serine (polar side chain) into Leucine (non-polar side chain). Also, due to a Guanine insertion in the consensus strain at base pair 904 (possibly a deletion in the California strain), variation downstream in the amino acid chain was observed (**Figure 1**).

The presence of variation between the Texas *HoCV-01* sequence and the California *HoCV-01* sequence is a possible indication that the Texas strain may exhibit increased virulence. The Guanine insertion at base pair 904 caused variation in all downstream amino acid translation. This could lead to changes in protein folding and ultimately changes in protein function. Altered protein functions may cause an increase in virulence in the Texas *HoCV-01* strain.

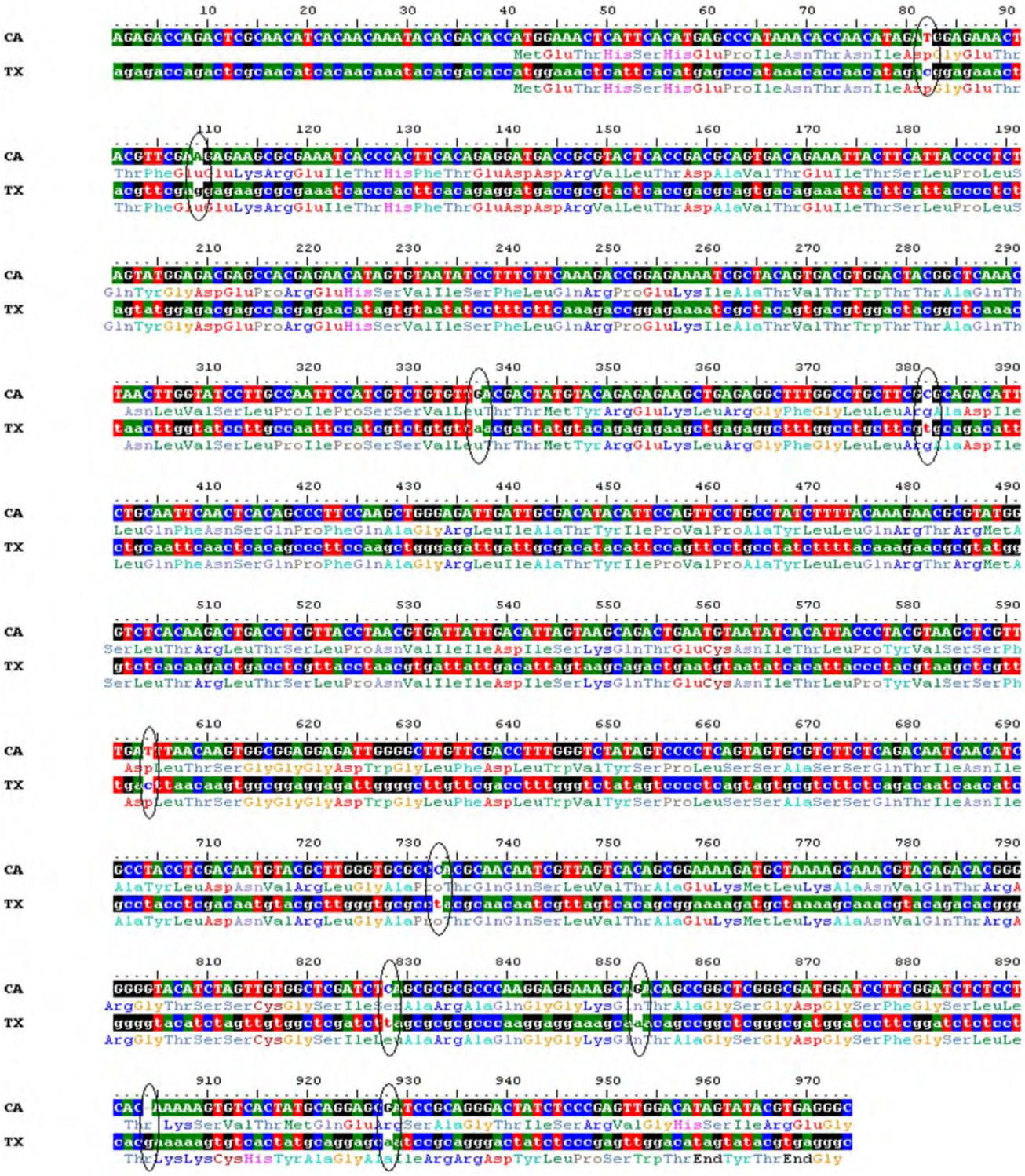

Figure 1. Sequence comparison with protein translation of the California *HoCV-01* strain and the Texas HoCV-1 strain. Note the circled areas which indicate variation between the two strains.

CONCLUSIONS

The presence of *HoCV-01* in populations of GWSS collected in Texas is crucial in developing an ideal viral bio-control and pest management strategy. The possible increase in virulence in the Texas strain is also an indication of the pathogenic potential of *HoCV-01*. This novel virus has been extracted and purified. In a following study, purified *HoCV-01* will be reintroduced to a population of uninfected GWSS in order to determine increases in population weakness or mortality that may result. The results of this experiment are crucial in further understanding the insect vector, GWSS. The management of PD depends heavily upon the ability to control its insect vectors.

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FUNDING AGENCIES

Funding for this project was provided by the Texas Pierce's Disease Research and Education Program, and the USDA Animal and Plant Health Inspection Service.